

POSTER SESSION ABSTRACTS

CFTR

1★

CFTR: EARLY, COTRANSLATIONAL QUALITY CONTROL

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Mutations in CFTR (cystic fibrosis transmembrane conductance regulator) cause cystic fibrosis. Many of the disease causing mutations disrupt the folding, transport, or function of the protein. CFTR is folded cotranslationally and multiple interactions with many partners are involved in proper folding and transport to the plasma membrane. The disease-causing folding mutations disrupt these processes leading to an inability of CFTR to reach the later stages in the secretory pathway and to degradation of the immature protein. These early interactions are critical decision points as interference with the later quality control, for example at the terminal stage of degradation, does not lead to improved folding. To test the hypothesis that altered interactions of the mutant CFTR with the partner proteins reflect early and exploitable quality control mechanisms, site-specific photocrosslinking, involving tRNA-mediated incorporation of photocrosslinking probes into the CFTR nascent chains during in vitro translation, was utilized to identify the factors that preferentially interact with mutant CFTR. Proteins that preferentially recognized the mutant chains were identified by utilizing biochemical reconstitution and the crosslinking assay in combination with mass spectrometry. The first set of proteins identified reveal a previously unappreciated mechanism of quality control and include those implicated in regulating mRNA stability, translational elongation, and targeting of the nascent chain for degradation - providing a system for regulating the expression of the mutant chains at an early step. Understanding the process of selectivity of this quality control system may uncover novel targets for intervention in the pathogenesis of CF.

(Supported by the CFF and NIH NIDDK.)

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UNDERSTANDING RESCUE OF F508DEL-CFTR VARIANTS WITHOUT THE REGULATORY EXTENSION (ΔRE) AND REGULATORY INSERTION (ΔRI) BY GENETIC REVERTANTS AND CHEMICAL MODULATORS

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Structural high-resolution knowledge of CFTR is crucial to understand how disease-associated mutations compromise its maturation and activity. Crystal structures of wt- and F508del-NBD1 indicate that they do not substantially differ, except for two segments designated regulatory extension (RE, Ser654-Gly673) and regulatory insertion (RI, Gly404-Leu435), both suggested to be conformationally dynamic, namely upon phosphorylation [1,2].

Here, we hypothesize that the enhanced dynamic flexibility of these regions in F508del-CFTR may also result in exposure of hydrophobic surfaces which are more hidden in wt-CFTR contributing to the low folding efficiency of the mutant protein.

To test this concept, we firstly assessed the impact of removing RE and RI (separately and jointly) on the in vivo processing of full-length wt- and F508del-CFTR. Secondly, we evaluated the effect of known F508del-revertant mutations (R1070W, G550E) on maturation of ΔRE/ΔRI variants. Finally, we aimed to assess the effects of small molecule compounds VX-809 (lumacaftor) and VX-770 (ivacaftor), on processing and function of these CFTR variants.

Functional characterization by iodide efflux assays shows that RE removal (ΔRE) does not affect processing efficiency of either wt-, or F508del-CFTR. In contrast, removal of RI alone (ΔRI), or together with RE (ΔRI/ΔRE), from F508del-CFTR restores both maturation and function of the mutant. In fact, F508del-ΔRI-CFTR function is restored to 100% levels

of wt-CFTR. These data are consistent with previous studies [3] and suggest that these variants acquire a native (fully folded) conformation in vivo.

Interestingly, in contrast to their effects on F508del-CFTR, both R1070W and G550E revertants fail to rescue F508del-ΔRE-CFTR processing, suggesting that when present, RE helps stabilizing both sites. In contrast, both revertants further increase (16% and 26% respectively) F508del-ΔRI-CFTR processing and their respective activity (by iodide efflux) is ~67% and ~100% of wt-CFTR levels, suggesting that R1070W impairs F508del-ΔRI-CFTR channel opening (but not maturation). Remarkably, this impairment is not rescued by VX-770 (vs 50μM genistein) but the peak response is faster. On the other hand, introduction of G550E on ΔRI-CFTR (wt background) almost completely abolishes channel activity (~90% reduction), without affecting maturation.

Additional studies on maturation efficiency and functional characterization of these CFTR variants with CFTR modulators are underway to help elucidate their mechanism of action.

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3★

SMALL MOLECULE CORRECTORS OF ΔF508 CFTR RESTORE GLOBAL STRUCTURAL ASSEMBLY BUT NOT LIFETIME OR FUNCTIONAL THERMAL STABILITY

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Most cystic fibrosis results from a single mutation (deletion of phenylalanine 508, ΔF508) in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, causing the ion channel protein to be misfolded, thermally unstable and degraded by cellular quality control systems. High throughput screenings for small molecules to overcome this defect have yielded several partially effective "correctors." However none of the compounds yet discovered restore cell surface function of the mutant to more than a few percent of the wild-type level. Neither the mechanism by which this degree of correction is achieved nor why it is so limited is understood. However, recent studies of both the first nucleotide domain (NBD1) from which F508 is deleted and full-length ΔF508 CFTR suggest that the limitation may be due to the need to both thermally stabilize NBD1 and repair ("patch") its interface with the second membrane-spanning domain (MSD2). In this study, we found that several of the more effective correctors including VX-809 did not restore channel thermal stability or the metabolic life of ΔF508 CFTR although they did promote the global assembly of the multi-domain protein in which formation of the NBD1-MSD2 interface is a crucial step. The effect of VX-809 was much more potent when combined with second site mutations in NBD1 that promote ΔF508 CFTR maturation and thermally stabilize channel activity, but to a lesser extent when combined with NBD1/MSD2 patching mutants. VX-809 was not effective in promoting other misprocessing mutants present in NBD1, but it did correct disease-associated mutations in various cytoplasmic loops (CL1-CL4). These findings indicate that a primary short-coming of existing correctors discovered in cell-based screens is their inability to restore thermodynamic stability to ΔF508 CFTR and emphasize the need to identify compounds with this ability. The combination of existing correctors ("assembly promoters") with NBD1 thermal stabilizers may provide a more complete therapeutic strategy for CF patients with the ΔF508 mutation. (Supported by the NIH and CFF.)

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AVAILABILITY OF F508DEL CFTR FOR SMALL MOLECULE CORRECTION

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Small molecules that correct F508del CFTR folding defects and enhance surface localization comprise an important therapeutic strategy for cystic fibrosis lung disease. However, compounds that correct the F508del mutation and confer $\geq 20\%$ wild type activity in primary airway epithelial cells have not been readily identified. The inability to obtain robust and therapeutically relevant levels of F508del CFTR correction by a single molecule may in some cases reflect diminished pools of Band B substrate (i.e. from blunted F508del synthesis and/or increased degradation). In the present experiments, we observed markedly diminished steady state levels of F508del (Band B) compared to WT CFTR protein in HeLa and bronchial epithelial cells, even when massive levels of F508del mRNA were overexpressed. In human primary airway cells, the paucity of Band B F508del was even more pronounced (in some cases below levels of detection) although F508del and WT mRNA were comparable. These results suggest an approach to improving F508del CFTR maturation that augments the pool of mutant CFTR suitable for folding repair. In this regard, we show that the proteasome inhibitor epoxomicin induces a large increase in F508del Band B, but (as expected) does not enhance CFTR rescue by C18 (a VX-809 analog), indicating accumulation of an irreversibly misfolded F508del intermediate. In an effort to identify novel compounds that augment "repairable" F508del protein, we developed a rapid throughput compound library screen. Cell-based imaging was used to semi-quantitatively estimate distribution of F508del CFTR by analysis of two-dimensional images. Cells were permeabilized, labeled with antibodies that recognize internal CFTR epitopes, and monitored using Evotec Opera™, an automated confocal microscopy system. We evaluated ~2000 known bioactive compounds from the NIH Roadmap Molecular Libraries Small Molecule Repository. A total of 25 primary hits were identified and one compound, 865022, was found to 1) increase CFTR levels in a dose dependent fashion, 2) augment F508del CFTR Band B compared to control, 3) increase cAMP dependent anion efflux in HeLa cells expressing I539T/F508del CFTR, and 4) stimulate CFTR dependent short circuit current in human bronchial epithelial monolayers. Co-treatment with 865022 and C18 synergistically enhanced F508del CFTR dependent ion transport following activation by forskolin or genistein. These results suggest an approach to increase efficiency of pharmacologic intervention by enlarging the corrector-restorable pool of F508del CFTR.

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DEVELOPING ANTIBODIES CAPABLE OF MONITORING CONFORMATION AND MATURATION OF CFTR

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Antibody reagents that distinguish among various CFTR conformational states could provide a valuable research tool and elucidate mechanisms that underlie defective biogenesis. Based on evidence that CFTR NBD2 requires an NBD1 scaffold for proper folding, we pursued experiments using the second NBD as an antigen in CFTR $-/-$ mice. Over 300 hybridomas were obtained and screened by protein ELISA. Seventy-four positive supernatants were studied by 1) immunoprecipitation under mild denaturing conditions (designed to preserve peptide structure), 2) cell based CFTR binding assays, 3) Western blotting, and 4) epitope mapping. Several robust antibodies to CFTR NBD2 were identified and are being distributed by the CFTR Protein Folding Consortium. One of the new reagents was shown by numerous laboratories to exhibit conformational sensitivity, and discriminate properly folded (versus unfolded) full length CFTR. In another study, because the interaction between cytoplasmic loop 4 (CL4) and the F508 segment of NBD1 is essential for CFTR maturation, 384 hybridomas were tested against these regions. Interestingly, two monoclonal antibodies preferentially recognize wild type versus the mutant full length NBD1 by ELISA. Thirty-one hybridomas against CL4 are currently being evaluated by Western blot and immunoprecipitation (WT versus F508del CFTR grown at 37°C or 27°C). Finally, we are developing probes specific for CFTR at the cell surface. Antibodies against extracellular loop 4 (ECL4) have not been previously available, perhaps in part due to bulky polysaccharide attachments that blunt immunogenicity. Accordingly, we devised a strategy to monitor processing of WT or F508del CFTR expressed in a glycan deficient cell line (GnTI- HEK293S). In preliminary studies, we show this model allows WT CFTR to traffic properly to the cell surface (as judged by immunohistochemistry), exhibit surface biotinylation, and retain Cl⁻ channel activity. The model also faithfully reproduces the F508del processing defect. Antibodies against ECL4 are being tested as a means to optimize F508del CFTR corrector molecules (by quantitative surface localization) and probe extracellular ("inward" versus "outward") configurations of CFTR during channel gating. Monoclonal antibodies that discriminate specific CFTR conformations will serve as useful tools for investigating protein biogenesis and drug discovery in the future.

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6★

RDR-1: CORRECTION AND INTERACTION WITH DELTAF508-NBD1

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The most common mutation in CFTR, deletion of phenylalanine 508 (deltaF508), destabilizes folding of the NBD1 domain, resulting in impaired trafficking of the mutant channel to the cell surface, poor gating kinetics, and reduced metabolic stability. Stabilization of the deltaF508-NBD1 domain by small molecule binding or facilitation of folding via a chaperone-like mechanism is expected to enhance CFTR trafficking. Recent results (Rabeh et al., Cell 148, 150-163 and Mendoza et al., Cell 148, 164-174) with deltaF508-NBD1-stabilizing mutants, however, suggest that NBD1

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stabilization alone is not sufficient for appreciable correction of deltaF508-CFTR, but that additional downstream points in the folding pathway, including proper folding of the NBD1-ICL4 interface are necessary to restore significant levels of CFTR cell surface expression. RDR-1 is a small molecule corrector of deltaF508-CFTR reported to act via binding to NBD1 (Sampson et al., *Chemistry and Biology* 18,321-242). If this compound stabilized NBD1, we reasoned that this compound would have little activity by itself, but could act in synergy with correctors working downstream of the NBD1 folding. We therefore characterized the corrector activity of RDR-1 alone and in combination with VX-809 in FRT cells by Western blot and in primary human bronchial epithelial cells by short circuit current measurements. RDR-1 corrector activity in hBE cells is not detectable, but RDR-1 significantly enhances the corrector activity of VX-809 when applied to cells for 24 h (but not acutely). Western blots measuring "C-band" maturation also indicate a strong synergistic effect of co-correction with RDR-1 and VX-809 compared to either compound alone. We further measured stabilization of NBD1 by RDR-1, comparing differential scanning fluorimetry (DSF) with the label-free differential scanning calorimetry (DSC) method, using murine and human constructs of wild-type and deltaF508 NBD1 proteins. While our DSF results concur with published results, DSC measurements detected no change in the melting temperature of deltaF508-NBD1. These results suggest that RDR-1 exerts its effects through an alternate mechanism, nevertheless complementary to that of VX-809, but independent of any interaction with NBD1.

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SEEING ATP HYDROLYSIS IN REAL TIME: WHAT DOES IT TELL US?

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CFTR is a chloride channel evolved from the exporter member of the ATP binding cassette (ABC) Protein Superfamily. Recent structural/functional studies of CFTR suggest that CFTR employs the evolutionarily conserved structural motifs in its nucleotide binding domains (NBDs) as well as transmembrane domains (TMDs) to enact the function of a gated pore for anion permeation. CFTR is gated by association and dissociation of its two NBDs that are governed by ATP binding and hydrolysis respectively. Single-channel kinetic data of CFTR have led to a gating model with one-to-one stoichiometry between the gating cycle and the ATP hydrolysis cycle. This strict coupling between NBDs and TMDs fits nicely with the popular alternating access model proposed for ABC transporters at large. However, in a recent report (Jih et al., 2012), by identifying a transient post-hydrolytic state, we raised the possibility that the ATP hydrolysis cycle and the gating cycle may not be strictly coupled. A critical piece of evidence needed to support the non-strict coupling idea is to demonstrate a direct transition from a post-hydrolytic open state to the original ATP-bound pre-hydrolytic open state. Lately, we accidentally found a CFTR mutant (R352C) that exhibits two distinguishable single-channel conductance levels (O1 and O2). Interestingly, the transition of the two open states follows a preferred order (C→O1→O2→C), indicating an input of free energy that drives the predominant O1→O2 transition over the opposite O2→O1 transition. Considering the known mechanism of CFTR gating, the most likely source of such free energy input is ATP-hydrolysis. This idea is supported by the observation that only C→O1→C was seen in the presence of ATP when introducing mutations (e.g., E1371S) that abolish ATP hydrolysis into the R352C channels. However, in the absence of ATP, R352C/E1371S channels exhibit only C→O2→C transitions, indicating that without ATP-induced NBD dimerization, the pore conformation must be different from that opened by ATP-induced NBD dimerization. Statistical analysis of all single-channel gating events revealed a considerable amount of opening events containing more than one O1→O2 (i.e. C→(O1→O2)_n→C) transition. If we accept the idea that the O1→O2 transition represents the hydrolysis of 1 ATP molecule, these surprising gating transitions would reflect openings embedded with hydrolysis of more than one ATP molecule—a violation of one-to-one stoichiometry. We thus argue that the gating cycle in CFTR is not strictly coupled to ATP hydrolysis cycle. Furthermore, the fact that the post-hydrolytic O2 state is capable of "re-entering" the pre-hydrolytic O1 state indicates that the gate remains open for a finite period after NBD dimer separation and the release of hydrolytic products. In other words, there is a delay for the closing signal transduction from the NBDs to the gate. These new results lead us to propose a new gating model that fea-

tures energetically coupled NBDs and TMDs: both NBDs and TMDs hold a certain degree of autonomy to function on their own but conformational changes in each domain are energetically coupled. Importantly, this new model offers a new target for the action of CFTR potentiators.

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STUDYING THE UNFOLDING OF THE PURIFIED FULL LENGTH CFTR MEMBRANE PROTEIN

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The major cystic fibrosis causing mutation, the deletion of phenylalanine at position 508 (F508del-) of CFTR, leads to misfolding of the protein during biosynthesis and its retention in the endoplasmic reticulum of the cell. To date, the primary defect in protein misfolding has been studied in cell-based assays employing post-translational modification as a biochemical read-out of folding and biosynthetic rescue. We hypothesize that the identification of targeted pharmacological interventions which can effectively ameliorate the misfolding of F508del-CFTR will benefit from studies which directly monitor the folding-unfolding transitions of the purified, full length mutant protein. Our laboratory has developed methods in efficiently purifying full length wildtype (WT-) and F508del- CFTR from a Sf9 cell expression system. The purified CFTR protein is then used for developing biophysical methods to enable future identification of small molecules which directly bind to CFTR to modify folding and unfolding. Toward this goal we have adapted fluorescence based methods described in recent studies of the purified ABC importer of *Escherichia coli*, BtuCD. These approaches induced *in vitro* unfolding of the protein by chemical denaturants, guanidine hydrochloride (GnHCl) and urea, and the subsequent conformational changes were monitored through changes in intrinsic tryptophan fluorescence. Similarly to BtuCD, n-dodecyl β-D-maltoside detergent solubilized CFTR is susceptible to unfolding by increasing concentrations of both denaturants. Upon unfolding of native WT- CFTR with increasing concentrations of both denaturants, the peak intrinsic tryptophan fluorescence intensity decreases and the wavelength emission maxima shifts from 323 nm to longer wavelengths of 332 nm which is also commonly known as a red shift. The susceptibility of CFTR to GnHCl is much greater than with urea with 80% of its unfolding occurred at 1 M GnHCl whereas 80% unfolding occurred at 4 M urea. In the case of BtuCD, unfolding at 4 M urea is associated with de-stabilization of the interface between the nucleotide binding domains and the coupling helices presented by the membrane domains. Since this interface is proposed to be disrupted by F508del mutation, our future studies will probe the susceptibility of purified F508del-CFTR protein to urea and predict whether the mutant will have enhanced susceptibility to urea denaturation. Future studies using this approach will test whether certain pharmacological small molecules can significantly decrease the susceptibility to unfolding of F508del-CFTR induced by urea. These studies constitute the first biophysical approaches towards investigating the unfolding of purified full length CFTR which promise to provide insight into the structural basis for F508del-CFTR misfolding as well as to provide an assay for small molecules which act directly to modify such unfolding. Supported by CIHR and CF Canada Operating Grant to C.E.B., CF Canada Fellowship award to PDWE and Studentship Award to S. Chin.

9★

CELLULAR BIOSYNTHETIC MACHINERY CONTROLS COTRANSLATIONAL NBD1 FOLDING BY BOTH POSITIVELY AND NEGATIVELY INFLUENCING SUBDOMAIN COMPACTION

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The efficiency of NBD1 folding is a limiting factor in CFTR biogenesis and trafficking that is further compromised by the Phe508 deletion. NBD1 is difficult to fold *in vivo* and does not refold efficiently *in vitro*, suggesting that critical folding steps are required to achieve its native conformation. In cells, protein folding is coupled to translation, occurs vectorially, and takes place in a crowded environment that is influenced by chaperones and interacting partners. Thus, understanding how NBD1 folds *in vivo* requires a

very different approach than in vitro refolding assays. To address this, we analyzed de novo NBD1 folding as the nascent chain is synthesized and emerges from the ribosome using fluorescence energy transfer (FRET) between an N-terminal donor (CFP) and a cotranslationally incorporated fluorescent acceptor dye, 7-nitrobenz-2-oxa-1,3-diazole. Previous studies using an acceptor probe located at residue 450 in NBD1 revealed that folding is initiated by abrupt compaction of an N-terminal ATP-binding subdomain as residues 515-550 are synthesized. To define the complete NBD1 folding pathway, acceptor probes were incorporated at residues 487 and 567.

FRET for the acceptor at residue 487, N-terminal to beta-strand 6 (S6), reached a maximum only after the entire alpha-subdomain had emerged from the ribosome (truncation 624). In contrast, when the nascent chain was released from the ribosome, optimal positioning of S6 was achieved at a much shorter length (truncation 500-550). Intriguingly, the discrepancy between folding on the ribosome and off the ribosome was not observed in alpha-subdomain-deleted chains, indicating that the ribosome attachment inhibits NBD1 folding during alpha-subdomain synthesis. For the acceptor at residue 567, located at the N-terminus of S7, maximal FRET was observed after S8 had emerged from the ribosome (truncation 644). Introduction of proline into S8 (I601P) substantially decreased FRET for residue 567, indicating that S7 positioning requires a cooperative movement of S8 to form the four-stranded beta-sheet core (S3, S6, S7, and S8). In addition, D529F, a mutation that enhances NBD1 folding in cells (Mendoza et al., 2012) positioned S7 earlier during synthesis. This suggests that alpha-subdomain folding possibly involving interaction of D529 with R555 impairs the beta-sheet core formation. It is plausible that keeping the alpha-subdomain more flexible and less rigid can assist S7 intercalation to the core, which might be the structural basis for suppressor mutations in alpha-subdomain. In a stark contrast to S6, S7 positioning occurred only for nascent chains that remained attached to the ribosome. Thus, biosynthetic machinery (ribosome and/or cytosolic factors) delays nascent chain folding during alpha-subdomain synthesis but is required to facilitate intercalation of beta-strands within the core. Our results suggest that the core formation may represent a limiting step during in vitro refolding, and indicate that NBD1 cotranslational folding requires a series of subdomain compactations that are dynamically regulated by cellular biosynthetic machinery. Supported by NIH, CFFTI and CFF.

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PHOSPHO-DEPENDENT INTERACTIONS OF THE REGULATORY R REGION OF CFTR AND STRUCTURAL MODELS OF DYNAMIC R REGION INTERACTIONS WITHIN FULL-LENGTH CFTR AND WITH 14-3-3

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The complex regulation of CFTR channel trafficking and gating is a phosphorylation dependent process that is modulated by the ~200 residue long cytoplasmic regulatory (R) region, which is intrinsically disordered in isolation and contains nine of the ten controlling PKA phosphorylation sites. The exact mechanisms by which this intrinsically disordered part of the protein regulates the channel and the specific segments important in this regulation are currently unknown. Building on our previous work, here we perform NMR and fluorescence binding studies on non-phosphorylated and highly PKA-phosphorylated states of the isolated human CFTR R region and demonstrate binding of the R region to a number of intramolecular and intermolecular partners. These include human CFTR NBD1, NBD2, and a 42-residue peptide from the C-terminus of CFTR, as well as the STAS domain of the SLC26A3 chloride-bicarbonate exchanger that is a co-regulator of CFTR and the 14-3-3β protein implicated in CFTR processing. Analysis of NMR resonance broadening and chemical shift changes upon

binding coupled with fluorescence binding measurements provides evidence for multi-site interactions of various segments of the R region and also shows that the same segments interact with different partners. Our data reveal that NBD binding is much stronger to the non-phosphorylated R region while binding to the C-terminus peptide, STAS domain and 14-3-3β is stronger in the PKA phosphorylated state. Similar to many other disordered proteins, the R region is a “hub” for regulatory protein interactions, which integrates and mediates multiple regulatory signals for CFTR. Based on these binding data, structural models for the dynamic interactions of the R region within full-length CFTR and with 14-3-3 are presented that provide a foundation for further refinement of our understanding of the structural basis of phospho-regulation of CFTR. This work was supported by the CF Foundation Therapeutics and Cystic Fibrosis Canada.

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INVESTIGATION OF THE ROLE OF THE CFTR C-TERMINAL REGION AND NHERF1 IN CONTROLLING CHLORIDE CHANNEL ACTIVITY

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Introduction: The extreme C-terminal region of CFTR is unique to this member of the ATP-binding cassette family, and it contains a PDZ motif that is recognized by NHERF1. The NHERF1 protein is thought to mediate an interaction with the cytoskeleton and hence influence CFTR recycling and CFTR localization (Guggino & Stanton, 2006).

We have separately expressed and purified full-length CFTR, CFTR C-terminal polypeptide (C-term 1440-1481) and NHERF1 PDZ domain. We have also co-expressed and co-purified a NHERF1 PDZ domain – C-term 1440-1481 complex.

Results: The C-terminal region of CFTR was expressed in *E. coli* with a better expression at lower growth temperatures. The purified protein showed a CD spectrum indicative of a relatively unstructured polypeptide, as expected and as confirmed separately by NMR (Bozoky et al., 2012). Co-expression and co-purification of NHERF1 and the C-terminal CFTR polypeptide yielded a complex of the two proteins, as determined by SDS-PAGE and mass-spectrometry. Surprisingly the complex is detected even under the harsh conditions employed for electrophoresis and mass spectrometry, implying a very tight association. The mass spectrometry data could also be interpreted as showing that both NHERF1 and the C-terminal polypeptide in the complex are phosphorylated. Intrinsic *E. coli* kinases such as CK2 may be involved in this processing.

Purified NHERF1 PDZ domain was incubated with full-length CFTR protein. Despite the presence of a deca-His tag immediately downstream of the normal CFTR C-terminus as well as detergent (dodecyl maltoside), the NHERF1 was able to bind to the Ni-NTA sepharose – immobilized CFTR protein, and co-eluted with it. We studied the CFTR-NHERF1 complex using electron microscopy and single particle averaging. Surprisingly, the complex displayed an open, inward-facing configuration in contrast to the closed, outward-facing conformation previously reported for isolated CFTR. Whether NHERF1 has a role in stabilizing the inward-facing conformation of CFTR in vivo remains to be investigated. It is possible that NHERF1 is able to prevent close approach of the NBDs via a steric hindrance. Alternatively, the NHERF1 may mask the C-terminal regions interactions with other domains of CFTR (e.g. R-region, NBD1) which might stabilize the closed, outward-facing configuration (Bozoky et al 2012).

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IDENTIFICATION OF EFFICACIOUS F508DEL CFTR CORRECTORS USING A NOVEL HOMOLGY MODEL

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New CFTR correctors are needed to improve the cellular processing and function of F508del CFTR. We explore the hypothesis that a homology model of CFTR based on existing structural information serves as a useful

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template for structure-based screening and design of correctors specific for the F508del defect. A homology model was created with emphasis on the region of the missing phenylalanine residue (F508del) using crystal structures from isolated NBD1-F508del. This molecular position was placed in the context of intracellular loop 4 (ICL4) using P-glycoprotein structure as a scaffold. In silico docking of 1.5 million commercially available compounds and subsequent constraints of distance and F508 mimetic potential allowed a much smaller subset of compounds (n<200) to be evaluated directly in CF cells.

As an initial screen, CFBE41o- cells stably transduced with the F508del transgene were incubated 24 hrs with each test compound (10 μ M) in comparison to C18 (3 μ M) and vehicle control, then studied under voltage clamp conditions in modified Ussing chambers. CFTR was sequentially stimulated with forskolin (20 μ M) and genistein (50 μ M) followed by CFTRInh-172 (10 μ M) to calculate CFTR dependent currents. Of 92 compounds prioritized for evaluation, 28 (30%) produced cAMP stimulated currents 2 SEM greater than control. Subsequently, a subset of these compounds was evaluated in primary human bronchial epithelial cells (HBE) derived from a single F508del CFTR homozygote; CFTR dependent currents were calculated following addition of forskolin and CFTRInh-172. Of 9 compounds tested in HBE to date, 4 (44%) exhibited cAMP stimulated I_{sc} significantly greater than vehicle control, with 7.6 – 19.5% of WT CFTR activity ($P<0.05$ – $P<0.005$, $N\geq 4$ /condition) observed compared to 30.4% of WT CFTR activity with C18 alone ($P<0.0005$).

These studies indicate successful identification of novel F508del CFTR folding correctors using a homology model of CFTR. The relatively high hit rate of active molecules based on in silico screening suggests this homology model could assist with characterization and optimization of new classes of CFTR modulators. Further efforts to advance the model are ongoing, and may provide an alternative strategy to develop CFTR correctors and furnish new insight regarding the F508del molecular defect.

13★

REDUCED AIRWAY SURFACE PH IMPAIRS BACTERIAL KILLING IN THE PORCINE CYSTIC FIBROSIS LUNG

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Cystic fibrosis (CF) is a life-shortening disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Although bacterial lung infection and the resulting inflammation cause most of the morbidity and mortality, how loss of CFTR first disrupts airway host defense has remained uncertain. We asked what abnormalities impair eradication when a bacterium lands on the pristine surface of a newborn CF airway. To investigate these defects, we interrogated the viability of individual bacteria immobilized on solid grids and placed on the airway surface. As a model we studied CF pigs, which spontaneously develop hallmark features of CF lung disease. At birth, their lungs lack infection and inflammation, but have a reduced ability to eradicate bacteria. We found that in newborn wild-type pigs, the thin layer of airway surface liquid (ASL) rapidly killed bacteria *in vivo*, when removed from the lung, and in primary epithelial cultures. Lack of CFTR reduced bacterial killing. We also found that ASL pH was more acidic in CF, and reducing pH inhibited the antimicrobial activity of ASL. Reducing ASL pH diminished bacterial killing in wild-type pigs, and increasing ASL pH rescued killing in CF pigs. These results directly link the initial host defense defect to loss of CFTR, an anion channel that facilitates HCO_3^- transport. Without CFTR, airway epithelial HCO_3^- secretion is defective, ASL pH falls and inhibits antimicrobial function, and thereby impairs killing of bacteria that enter the newborn lung. These findings suggest that increasing ASL pH might prevent the initial infection in patients with CF and that assaying ASL pH or bacterial killing could report on the benefit of therapeutic interventions.

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14★

THE PSEUDOMONAS AERUGINOSA PROTEASE LASB DEGRADES CFTR AND REGULATES CYTOKINE PRODUCTION IN THE LUNG

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Objective of the study: We have recently shown that host neutrophil elastase (NE) is partly responsible for cystic fibrosis transmembrane conductance regulator (CFTR) degradation and loss of function in epithelial cells and in a *Pseudomonas aeruginosa* (P.a) lung infection murine model (Le Gars et al, submitted).

We hypothesized that in addition to NE, other P.a virulence factors may also be implicated in CFTR degradation and loss of function. We focused our study on LasB, a P.a metalloprotease virulence factor known to disable several important innate immune molecules.

Methods: In vitro: Bacterial strains used were either WT PAO1 (a wild-type P.a strain), or a Δ LasB deletion mutant. Purified LasB protein was obtained from Nagase Bicochemicals Ltd, Fukuchiyama, Japan (batch 6763139, activity 3040 units per mL).

Epithelial cell lines A549 and NCI-H292 were transfected with non-replicative Adenovirus (Ad)-d170/3 “null,” Ad-GFP-WT-CFTR or Ad-GFP- Δ F508-CFTR (gift of Pr. Boulanger, Univ. of Lyon) during 48hrs, to allow for expression of CFTR. After, cells were either treated with purified LasB (5-10 nM) for 20hr or infected with WT PAO1 or its Δ LasB mutant (10^{-3} to 10^{-9} moi) for 20 hrs. Cells were then lysed and lysates were analysed for either CFTR RNA expression or for GFP-CFTR protein by Western blot analysis.

In vivo: C57Bl/6 mice were instilled intra-nasally with 10^9 plaque forming units (pfu) of either Ad “null” or Ad-GFP-CFTR-WT. Four days after instillation, mice were instilled through the same route with either PBS, WT PAO1 or the Δ LasB mutant (5×10^6 cfu/mouse). Twenty hrs post-infection, mice were sacrificed, broncho-alveolar lavage (BAL) was performed and inflammatory differential cell count and cytokines were measured.

Results: 1) We showed in vitro that purified LasB degrades, in a dose-dependent fashion, GFP-WT CFTR band C and GFP- Δ F508-CFTR band B up to 60 and 55%, respectively. This was caused by neither a change in RNA CFTR turn-over (measured by Q-PCR) nor by cytotoxicity (measured by LDH activity).

2) Similarly, WT PAO1, at a dose of 10^{-9} moi, diminishes GFP-WT CFTR expression by 60% in NCI-H292 cells, compared to 40% in presence of 10^{-9} moi Δ LasB mutant. In A549 cells, GFP-WT-CFTR expression was reduced by 25% and 10%, respectively.

3) In vivo, after infection with both strains, we showed no difference in lung inflammatory cell counts (macrophages, neutrophils, lymphocytes), nor in KC, IL-6, TNF- α , or IL-12 levels. By contrast, secretion of IL-1 β and IL-10 were drastically reduced in mice infected with Δ LasB PAO1, compared to mice infected with WT PAO1.

Conclusions: We demonstrate for the first time that P.a LasB metalloprotease degrades both WT and Δ F508-CFTR in epithelial cells. This new finding may have implications in exacerbations of genetically-sufficient CFTR individuals infected with P.a (eg COPD/bronchiectasis patients) as well as for Δ F508 CF patients.

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15★

REGULATION OF CFTR EXPRESSION AND FUNCTION BY MICRORNAS

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MicroRNAs (miRNAs) are increasingly recognized as important post-transcriptional regulators of gene expression and changes in their actions can contribute to disease states. The effector functions of miRNAs in several facets of pulmonary biology have been identified, but their actions in airway epithelia are just emerging. There is no established role for miRNAs in the regulation of fluid and electrolyte transport in the airways, yet this process is vital to homeostasis and often perturbed in disease states. We profiled miRNA expression in well-differentiated primary cultures of human cystic fibrosis (CF) and non-CF airway epithelia, and discovered miR-509-3p and miR-494 abundance to be increased in CF cells. We hypothesized that miRs-494 and -509-3p influence CFTR expression via interactions with predicted binding sites in the 3' UTR of CFTR. We validated the predicted interactions for both miRNAs with the 3' UTR of CFTR. Human airway epithelia transfected with the mimics of miR-494 or -509-3p showed decreased CFTR expression and reduced anion transport, while their respective anti-miRs produced the opposite effect. Interestingly, the two miRNAs acted cooperatively in regulating CFTR expression. Upon infecting airway epithelial cells with *S. aureus* or stimulating cells with the pro-inflammatory cytokines TNF α or IL-1 β , we observed an increase in the expression of both miRNAs and a concurrent decrease in CFTR expression and function, suggesting that inflammatory mediators may regulate these miRNAs. Transfecting airway epithelia with miRs-509-3p and -494 anti-miRs or blocking NF- κ B signaling before stimulating cells with TNF α or IL-1 β inhibited these responses, suggesting that expression of both miRNAs is regulated in part by NF- κ B signaling. This study highlights the emerging role of miRNAs in regulating CFTR expression and function. In addition, our findings suggest that disease states associated with airway infection and inflammation may influence CFTR expression and function via miRNA networks.

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A NOVEL MICRORNA NETWORK REGULATES CFTR BIOSYNTHESIS: IMPLICATIONS FOR CYSTIC FIBROSIS THERAPEUTICS

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Cystic fibrosis transmembrane conductance regulator (CFTR) is an anion channel that when mutated causes the disease cystic fibrosis (CF). Many obstacles hinder the understanding of CF disease pathogenesis, impeding advancements in understanding how mutations cause disease, and slowing the progress towards new treatments. miRNAs comprise a large family of ~21-nt long non-coding RNAs that function as key post-transcriptional regulators of gene expression in metazoans and plants. While CFTR structure and function is well studied our knowledge of the transcriptional and post-transcriptional regulation of CFTR expression is incompletely understood. To investigate the role that miRNAs play in regulating CFTR expression, we first profiled miRNA expression in well-differentiated primary cultures of human airway epithelia. We hypothesized that miR-138 regulates CFTR expression through its interactions with the transcriptional repressor SIN3A. We validated SIN3A as a miR-138 target. Using ChIP we

show that SIN3A interacts with CTCF on the CFTR promoter at the -20.9 kb DHS. Repressing SIN3A with a miR-138 mimic or SIN3A siRNA significantly increased CFTR expression and function in epithelia. Interestingly, transcript profiling analysis demonstrates that miR-138 influences the expression of many proteins known to associate with CFTR, thereby influencing CFTR biosynthesis. Both miR-138 and SIN3A are highly conserved across species, and we replicated these findings in pig airway epithelia, suggesting that the miR-138/SIN3A mediated regulation of CFTR is evolutionarily conserved. The most common CFTR mutation, Δ F508, causes protein misfolding, degradation, and CF. Remarkably, manipulating the miR-138/SIN3A regulatory network improved the biosynthesis of CFTR- Δ F508, restoring Cl⁻ transport to human CF airway epithelia. To our knowledge, this is the first example of an individual microRNA having such broad regulatory functions. This discovery provides new therapeutic avenues for restoring CFTR function to cells affected by the most common cystic fibrosis mutation.

17★

UDP-GLUCOSE IS A POTENT AND STABLE PRO-INFLAMMATORY MEDIATOR PRESENT IN CF LUNG SECRETIONS

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Chronic airway inflammation is a pathological feature of CF lung disease. Neutrophils are key players in fighting infections, but they also are major contributors to the inflammatory response in CF lungs. Thus, elucidating the mechanisms involved in CF airway neutrophil inflammation is relevant to the development of CF therapies. The most recently discovered purinergic receptor subtype, the P2Y₁₄ receptor (P2Y₁₄-R), is not expressed in airway epithelia, but P2Y₁₄-R transcripts are abundantly expressed in peripheral neutrophils. We discovered that UDP-glucose (UDP-Glc), the most potent naturally occurring P2Y₁₄-R agonist, is released with mucins from goblet cell-rich epithelia in vitro. Importantly, UDP-Glc accumulates in vivo in CF lung secretions at concentrations (100 -1000 nM) previously reported to promote P2Y₁₄-R-mediated signaling in model cell lines. However, the actions of UDP-Glc on neutrophil responses remain undefined. We tested the hypothesis that UDP-Glc acts as an extracellular signalling molecule for neutrophils by assessing its effect on cell shape and actin cytoskeleton reorganization, chemotaxis, and Rho activation. Fluorescence microscopy analysis indicated that, in the absence of external stimulus, most neutrophils displayed round shape and phalloidin uniformly stained the cortical actin fibres. Incubation of neutrophils with UDP-Glc resulted in changes in cell shape that were accompanied by a sharp increase in the intensity of the cortical actin staining and the formation of a broad ruffle-like structure in one cellular pole. Using a modified Boyden chamber, we demonstrate that UDP-Glc promoted neutrophil chemotaxis (0.1-100 μ M; EC₅₀ = 1.3 μ M), which was blocked by Rho kinase inhibitors. In line with these results, UDP-Glc evoked rapid and robust activation of RhoA in these cells (pulldown assay). Using the HL60 human promyelocytic leukemia cells, we demonstrate that UDP-Glc-evoked responses occurred in a P2Y₁₄-R expression-dependent manner. Ecto-nucleotidases expressed on airway epithelial cells and neutrophils rapidly hydrolyze extracellular ATP, but incubation of radiotracer UDP-[³H]Glc for up to 1h with human bronchial epithelial cells, isolated human neutrophils, or purulent CF sputum resulted in negligible metabolism of the UDP-sugar, as assessed by HPLC. These results support the notion that UDP-Glc is a stable and potent pro-inflammatory mediator that promotes P2Y₁₄-R-regulated RhoA activation, cytoskeleton rearrangements, and chemotaxis in human neutrophils. Given the high levels of UDP-Glc found in CF lung secretions, our study suggests a role of the neutrophil P2Y₁₄-R as a contributor to lung inflammation in CF.

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18★

INTRINSIC CHOLESTEROL DEPLETION DISRUPTS LIPID RAFT STRUCTURE IN NEUTROPHILS OF INDIVIDUALS WITH CYSTIC FIBROSIS

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Considerable debate exists as to the underlying cause of dysregulated neutrophil activity in cystic fibrosis (CF). The focus of this study was to resolve this incongruity and to enlighten us on whether an intrinsic CF-related defect modifies neutrophil activity or alternatively, whether the observed neutrophil changes are allied to inflammation. Lipid rafts play critical roles in many aspects of neutrophil activity and function. Cholesterol is a structural component of lipid rafts, and depletion of cholesterol leads to disorganization of lipid raft microdomains. The aim of this study was to investigate the potential of altered membrane cholesterol levels as a possible cause for dysregulated neutrophil activity in CF.

Neutrophils were isolated from stable patients with CF homozygous for the $\Delta F508$ mutation or during an exacerbation (n=13), healthy controls (n=13) and stable non-CF bronchiectasis patients (NCFB; inflammatory control, n=6). Cell plasma membranes were isolated by sucrose-gradient ultracentrifugation. Neutrophil cholesterol content was measured employing the Invitrogen Amplex® Red Cholesterol Assay Kit. Altered raft protein expression was assessed by 2D-DIGE followed by LC-MS/MS and confirmed by western blot analysis. Statistics were analysed using GraphPad Prism 4.0 with significance determined at $p \leq 0.05$.

Here, we show that whole cell lysates prepared from neutrophils of stable patients with CF contained $25.6\% \pm 1.8\%$ less cholesterol than control cells. Although no significant difference in levels of cytoplasmic cholesterol were detected, CF neutrophil membrane cholesterol content was significantly lower than the control donor cells ($1.16 \pm 0.7 \mu\text{g}$ cholesterol per mg membrane protein compared to $2.57 \pm 0.52 \mu\text{g}/\text{mg}$, respectively, $P < 0.05$). We also provide evidence that the reduction in plasma membrane cholesterol content perturbed formation of lipid rafts. To this end, lower protein expression levels of lipid raft structural proteins, flotillin-1 and flotillin-2, were detected in neutrophil membranes of individuals with CF during an infective exacerbation or when stable, compared to healthy or NCFB control samples (n=6 for each cohort, $P < 0.05$ by ANOVA). An alteration in the function of caveolin-1 which transports cholesterol to the plasma membrane is explored as a cause for the observed defect.

Our data has identified major changes to membrane cholesterol and lipid raft structure intrinsic to the CF neutrophil and not present in the inflammatory control group. Results suggest that therapeutics targeting cellular cholesterol content may positively impact on neutrophil activity in CF.

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MACROPHAGE ACTIVATION PATTERNS AND DEVELOPMENT OF AIRWAY INFLAMMATION IN SCNN1B TRANSGENIC MICE

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Chronic bronchitis, defined as airway inflammation associated with muco-obstruction, is a characteristic manifestation of cystic fibrosis (CF) and other serious pulmonary diseases, such as chronic obstructive pulmonary disease (COPD). Scnn1b transgenic mice overexpress the Scnn1b gene (coding for the epithelial Na^+ channel subunit, βENaC) in airway Clara cells, resulting in increased Na^+ absorption, airway surface liquid dehydration, mucus adhesion to airway surfaces, and, finally, airway inflammation. These mice provide an excellent animal model to investigate the pathogenesis of chronic bronchitis. The characterization of pathophysiology of lung disease in Scnn1b transgenic mice has revealed increased macrophage number, alterations in macrophage morphology, and persistent neutrophilic and transient eosinophilic infiltrates. Macrophages are thought to play a pivotal role in regulating the initiation, maintenance, and resolution of inflammation. However, it is unknown whether macrophages and their activation patterns are involved in the initiation and/or maintenance of airway inflammation in Scnn1b transgenic mice. Therefore, our hypothesis in the study was

that the initiation and the course of airway inflammation in Scnn1b transgenic mouse is determined by the macrophage activation patterns. To address this hypothesis, we performed gene expression analysis on granulocytes-depleted BAL macrophages (95.86 ± 0.25 (SEM) % pure populations of macrophages). The gene expression data analyses in control littermates at different age points (1 day, 3 days, 10 days and 6 weeks) revealed age-associated shift in macrophage activation patterns. The comparison of gene expression patterns in whole lung tissue and pure macrophages from Scnn1b transgenic mice at different age points revealed disease stage-associated macrophage activation patterns (predominantly M1 i.e Classical activation, at 3 days of age and predominantly M2 i.e Alternative activation, at 6 weeks of age). Taken together, the current data suggest that macrophages play a critical role in the development and progression of lung disease.

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RELATIVE MOVEMENT OF THE SIXTH AND ELEVENTH TRANSMEMBRANE SEGMENTS DURING OPENING AND CLOSING OF THE CFTR CHANNEL

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The outer mouth of the CFTR channel pore is lined by amino acid side chains coming from transmembrane segments (TMs) 6, 11 and 12. However, the relative alignment of these TMs, as well as their relative movements during channel opening and closing are still unknown. To gain three-dimensional structural information on the outer pore, we have studied cross-linking of introduced cysteine residues in the open and closed state. To do this, we mutated pore-lining residues from TM6 (R334, K335 and T338) and TM11/12 (S1118, T1121, T1122, G1127 and T1134) to create 15 cysteine pairs. Proximity of introduced cysteine pairs was determined by sensitivity to externally applied copper (II)-o-phenanthroline (CuPhe, which induces disulfide bond formation between cysteines) and dithiothreitol (DTT), and inhibition by external cadmium ions, by using whole cell patch clamp recording. We found that when applied to channels activated by forskolin, CuPhe led to a reduction in current amplitude in R334C/T1122C, R334C/G1127C and T338C/S1118C whereas it did not affect the other 12 cysteine pairs, suggesting that these three cysteine pairs are close together and able to form disulfide bonds rapidly in activated channels. We also observed that cadmium coordination occurred between R334C and G1127C and between R334C and T1122C, resulting in strong inhibition relative to single cysteine mutants. Since cross-linking or cadmium coordination by some pairs of cysteines may be possible only in the closed state or in the open state, we examined the state-dependence of CuPhe and cadmium inhibition of R334C/T1122C, R334C/G1127C and T338C/S1118C. In order to isolate the open state, we manipulated NBD function by introducing the E1371Q mutation (which increases channel open probability and slows channel closure). We observed that E1371Q prevented both spontaneous and CuPhe-induced cross-links as well as cadmium coordination between R334C and G1127C, suggesting that R334C and G1127C are close together only in closed channels. E1371Q did not prevent cross-linking or cadmium coordination between R334C and T1122C. Surprisingly, R334C/T1122C/E1371Q formed disulfide cross-links spontaneously, which indicates that R334C and T1122C are close together and able to form spontaneous cross-links in open channels. In the case of T338C/S1118C, E1371Q facilitated cross-linking and also strengthened cadmium inhibition, suggesting that cross-links and cadmium coordination occur preferentially in open channels, implying that T338C and S1118C are closer together in open channels. These results provide us new information on the three-dimensional structure of outer mouth of the CFTR channel pore. R334 (TM6) is close to G1127 (TM11) in closed channels, and to T1122 (TM11) in open channels, while T338 (TM6) is close to S1118 (TM11) in open channels. Moreover, the altered relative location of residues in open and in closed channels that we infer allows us to propose that channel opening and closing may be associated with a relative translational movement of TMs 6 and 11, with TM6 moving "down" during channel opening.

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HSC70 INHIBITS ENAC FUNCTION BY PROMOTING ITS RETRIEVAL FROM THE APICAL SURFACE OF EPITHELIA

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Relative hyperfunction of the epithelial sodium channel (ENaC) in the CF airway is hypothesized to result in the airway surface liquid depletion, decreased mucociliary clearance and increased bacterial colonization of the airway seen in CF patients (Matsui et al., Cell, 1998). Regulation of CFTR and ENaC trafficking may involve similar mechanisms (e.g., Lang et al., Cell Physiol Biochem, 2003; Dieter et al., Obes Res, 2004; Wagner et al., Cell Physiol Biochem, 2001; Sato et al., Cell Physiol Biochem, 2001), and we are particularly interested in the influence of Δ F508-CFTR correctors on ENaC trafficking and functional expression. Our group's recent work suggests that the prototype Δ F508-CFTR corrector, 4-phenylbutyrate (4PBA), can differentially alter the expression of highly homologous 70kDa heat shock proteins Hsc70 and Hsp70 (Suaud, et al, 2011). Furthermore, the 4PBA-mediated decrease in Hsc70 expression, and increase in Hsp70 expression could both promote correction of Δ F508-CFTR trafficking (Rubenstein and Zeitlin, Am J Physiol Lung, 2000; Choo-Kang and Zeitlin, Am J Physiol Lung, 2001). Because of the similarities in trafficking between ENaC and CFTR, we hypothesized that ENaC trafficking is also regulated by Hsc70 and Hsp70.

Our group's previous studies of ENaC regulation in *Xenopus* oocytes demonstrated that Hsc70 and Hsp70 had opposite effects on ENaC function and surface expression. Hsc70 overexpression decreased ENaC expression, while Hsp70 had the opposite effect (Goldfarb et al., PNAS, 2006). Here, we probed the mechanism underlying these differential effects in epithelial cells. We selected MDCK epithelial cells that stably express $\alpha\beta\gamma$ -ENaC and have tetracycline-inducible overexpression of Hsc70, or an ATPase-deficient Hsc70 as a negative control. We found that Hsc70 overexpression decreased ENaC functional expression (amiloride-sensitive Isc) when these cells were grown as polarized monolayers and tested in Ussing chambers. This decrease in functional expression correlated with decreased surface expression of ENaC in surface biotinylation experiments, and likely resulted from an increase in the rate of functional ENaC retrieval from the cell surface. In addition, in co-immunoprecipitation experiments, Hsc70 overexpression increased ENaC's association with Rab4 and Rab7, the Rab GTPases that colocalize with early and late endosomes, respectively. In contrast, Hsc70 overexpression decreased the association of ENaC with Rab11, the Rab GTPase associated with recycling endosomes. These data suggest that Hsc70 promotes the endocytosis and inhibits the recycling of ENaC channels. These data are in distinct contrast to our recent data that Hsp70 promotes the association of ENaC with Coat Complex II (COP II) and does not alter the rate at which ENaC is retrieved from the apical surface of epithelia (Chanoux, et al., JBC, 2012). These data therefore support the hypothesis that Hsc70 decreases ENaC functional and surface expression in epithelial cells by promoting ENaC's internalization and degradation.

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FOLDING, TRAFFIC AND KINETIC DEFECTS ACCOUNT FOR F508DEL-CFTR RETENTION IN THE ENDOPLASMIC RETICULUM

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CFTR bearing F508del, the most common CF-causing mutation, is retained at the endoplasmic reticulum (ER) due to misfolding and sent for premature degradation, failing to reach its proper location, the apical membrane of epithelial cells. Several approaches have been used to rescue

F508del-CFTR misfolding and trafficking defects, namely: 1) low temperature incubation (26°C); 2) genetic revertants (second-site mutations); and 3) small-molecule correctors, favouring correct folding and/or promote traffic. Understanding the mechanism of action (MoA) of these distinct agents allows the development of novel and multiple therapeutic tools for enhanced results.

Our aim is to assess the synergistic/additive effects of correctors, genetic revertants and 26°C incubation to learn more about their MoA. We used different genetic revertants in cis with F508del, either alone or in combination, and two previously described small-molecule correctors - VRT-325 and Corrector 4a and the under testing drug, VX-809.

Results from Western blotting (assessing processing of CFTR) and iodide efflux (assessing channel function) show that genetic revertants at different CFTR sites are additive in promoting the rescue of F508del-CFTR protein and also with low temperature. Furthermore, 26°C incubation promotes the accumulation of F508del-CFTR in an unstable conformation that is rapidly degraded at 37°C, suggesting that this accumulation is mainly due to attenuated proteasomal degradation. Finally, our data show that combinations of small-molecule correctors are additive with 4RK (the simultaneous mutation of four arginines into lysines at the arginine framed tripeptides - R29K, R516K, R555K, R766K) and low temperature to rescue F508del-CFTR but not all the above three correctors tested are additive with G550E or R1070W, suggesting different possible MoAs.

Altogether, our results contribute to a better understanding of F508del-CFTR rescuing mechanisms. This is crucial to the development of novel combinatory therapeutic approaches aimed at correcting the mutant protein.

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RESCUE OF TGF-BETA DOWN-REGULATED CL⁻ TRANSPORT BY A CFTR POTENTIATOR

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Background: TGF- β is a pleiotropic signaling molecule with important roles in tissue injury, inflammation and repair, and is a modifier of the CF phenotype. The mechanism linking TGF- β activity to disease modification is unknown, but results from our laboratory and others suggest that TGF- β downregulates both CFTR and TMEM16A expression and function in T84 colonocytes through a p38 MAPK pathway. In the current studies, we examined whether the negative effects of TGF- β on Cl⁻ transport via CFTR and TMEM16A in T84 cells could be reversed by CFTR potentiators. For the purposes of this study, we focused on flavonoids that have previously been demonstrated to activate F508del CFTR (following corrected trafficking to the cell surface – Pyle LC, et al. Am J Respir. Cell Mol. Biol. 2010;43:607).

Methods: T84 cells were grown in culture using standard methodology, and seeded onto Costar 0.3 μ M permeable supports 5-7 days prior to studies in Ussing chambers. When monolayers achieved a resistance of 1000-1500 ohm.cm², cells were serum starved for 24 hours (0.1% FBS), and then exposed to experimental conditions for 48 hrs (control, 10 ng TGF- β). Inserts were then mounted and studied under voltage clamp conditions, warmed to 37°C and continuously gassed with 95%O₂/5%CO₂ in RPMI 1640 media + 25 mM HCO₃⁻. Cells were treated with 10 μ M indomethacin (20 min), 100 μ M amiloride (10 min), 10 μ M forskolin + 100 μ M IBMX to activate CFTR or 2 μ M ionomycin to activate TMEM16A (10 min), 100 μ M carbachol (10 min), and 10 μ M CFTRinh172 (10 min). For studies screening flavonoid potentiation of CFTR, T84 monolayers were treated with 0.1 μ M forskolin (to minimally activate CFTR) followed by 10 and 100 μ M flavonoids (10 min each), and blocked by 10 μ M CFTRinh172 (10 min). Paired T tests were used to compare Isc responses across conditions.

Results: Flavonoid screening identified several molecules that activated CFTR-dependent currents at 10 μ M (genistein, apigenin, quercetin, biochanin, narigenin), but all except apigenin inhibited Cl⁻ secretion at higher doses (100 μ M). Based upon these results, we examined apigenin's capacity to restore TGF- β -downregulated Cl⁻ transport. TGF- β downregulated CFTR currents were increased by co-treatment with apigenin [mean val-

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ues in $\mu\text{A}/\text{cm}^2$ - Control = 69.2, TGF- β = 24.69, TGF- β + apigenin (25 μM) = 40.04, TGF- β + apigenin (100 μM) = 59.73; $p = 0.015$ for apigenin conditions compared with TGF- β alone]. TGF- β downregulated TMEM16A currents were also increased by co-treatment with apigenin [mean values in $\mu\text{A}/\text{cm}^2$ - Control = 63.68, TGF- β = 30.97, TGF- β + apigenin (25 μM) = 77.1, TGF- β + apigenin (100 μM) = 120.3; $p = 0.01$ for apigenin conditions compared with TGF- β alone].

Conclusions: The flavonoid CFTR potentiator apigenin rescues TGF- β -downregulated Cl^- transport in T84 monolayers, demonstrating synergistic effects with cAMP and calcium-dependent Cl^- transport signaling. CFTR potentiation may represent a feasible strategy to restore Cl^- transport produced by excessive TGF- β activity.

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STABILITY PREDICTION OF NUCLEOTIDE BINDING DOMAIN 2 OF CFTR USING MD SIMULATIONS

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Cystic fibrosis (CF) is a genetic disease caused by mutations to the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. CFTR is comprised of two membrane spanning domains (MSDs) connected through four intra-cellular domains (ICDs) to two nucleotide binding domains (NBDs). The role of the NBDs is to control the gating of the channel, which is located at the interface between the two MSDs through a dimerization/dissociation cycle brought about by the binding of two and hydrolysis of one ATP molecule(s). The development of new CF therapies will be largely aided by the availability of structural and dynamic information of full-length CFTR and its domains.

Multiple CFTR mutations compromise the gating of CFTR. Stabilizing the NBD1:NBD2 dimer increases the open probability of the channel. Thus, the NBD1:NBD2 interface is a relevant target for CFTR modulators. Unlike NBD1, only a single crystal structure is available for NBD2 solved by fusing the C-terminus of this domain to MalK. This structure features the canonical NBD fold with small, albeit significant, deviations in subdomain geometry which render it dimer-incompatible (DI). However, whether these changes are inherent to the NBD2 sequence or result from the MalK fusion or crystallization conditions is an open question.

To answer these questions we have performed Replica Exchange MD (REMD) simulations on a series of NBD2 constructs in DC and DI conformations, the former generated by homology modeling, and evaluated the thermodynamic properties and dynamic behavior of these constructs from their averaged potential energies and Root Mean Squared Fluctuations (RMSF) profiles, respectively. In all cases, the fluctuations and the potential energy of the DI form were found to be lower than those of the DC form suggesting that the preference to the DI form is inherent to NBD2 and that the presence of additional CFTR domains is required to force it into a dimer compatible form.

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REPLICA EXCHANGE MD SIMULATIONS OF WT- AND ΔF508 NBD1 FROM CFTR

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Cystic fibrosis (CF) is a lethal genetic disease with an estimated worldwide patient population of 70,000. Available treatments for CF are still largely symptomatic and the median survival age of CF patients is in the mid-30's. CF is caused by mutations to the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. The most common mutation, occurring in >90% of CF alleles, is the deletion of F508 from NBD1 (ΔF508 -NBD1), leading to a thermally unstable domain and a misfolded, non-functioning CFTR. Studying the effect of ΔF508 on NBD1 and CFTR structure and function is key to the development of new CF therapies.

Available crystal structures of wt- and ΔF508 NBD1 are structurally similar, with differences primarily localized to the ΔF508 region, suggesting that disparities in their properties may result from their dynamic profiles. However, previous MD simulations of these structures led to conflicting

results, potentially due to non-converged sampling of the potential energy surface.

To resolve this discrepancy, we repeatedly simulated wt- and ΔF508 -NBD1 using Replica Exchange MD (REMD) simulations and evaluated their dynamic behavior from their respective Root Mean Squared Fluctuations (RMSF) profiles. REMD simulations allow more frequent crosses of energy barriers, compared with standard MD, and thus better sampling of the conformational space. Our REMD simulations show that the fluctuations of ΔF508 -NBD1, especially in few regions, are consistently and significantly higher than those of wt-NBD1 in agreement with its lower thermal stability. Furthermore, in contrast with the highly localized differences between wt- and ΔF508 NBD1 observed by analyzing their crystal structures, REMD simulations suggest that differences in their biochemical properties result from correlated local and non-local differences in their dynamic profile.

We applied similar simulations and analysis to NBD1 and its ΔF508 mutant in the presence of suppressor mutations, V510D and the Teem 3M mutations (G550E, R553Q and R555K), which were found to rescue maturation and function of ΔF508 -CFTR to near wt levels. Similar to the above described results, we found that the fluctuations of ΔF508 -constructs are consistently, and in a few regions significantly higher than those of the wt constructs, again in agreement with their lower thermal stability. However, these regions are not necessarily identical to those observed in the absence of the suppressor mutations.

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[ΔF508]CFTR AND [WILDTYPE]CFTR INTERACT FUNCTIONALLY WITH THE PROINFLAMMATORY MEDIATOR TNFR1 IN THE ENDOPLASMIC RETICULUM

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Background: Cystic fibrosis (CF) is an autosomal recessive genetic disease, caused by mutations such as [ΔF508] in the CFTR gene, which has a proinflammatory phenotype in the CF lung. This proinflammatory phenotype is due to intrinsic activation of the TNF α /NF κ B signaling pathway in infection-free CF lung epithelial cells, which drives expression of the neutrophil chemokine IL-8 (1-3). However, the mechanism(s) by which mutations in CFTR are accompanied by activation of this pathway are poorly understood. Independent of the CF condition, it is known that TNF α binds to the TNFR1 in the plasma membrane, where it can initiate a sequence of phosphorylation-dependent activation steps, first involving the TNFR1 complex, then the IKK'some, and then the NF κ B complex. Finally, when I κ B α is phosphorylated and degraded, NF κ B, p65 is free to localize into the nucleus, where it binds to the κ B site on the IL-8 promoter. This activates synthesis of IL-8 mRNA, and downstream translation into IL-8 protein. However, where (or whether) CFTR might interact with proteins in any of these activation steps remains a matter of conjecture.

Hypothesis: Initial studies in both HEK293 cells, and in IB3-1 CF lung epithelial cells revealed that the endoplasmic reticulum (ER) and Golgi expressed the highest levels of TNFR1, as well as the other members of the TNFR1 complex. Inasmuch as [ΔF508]CFTR fails to traffic out of the ER, we hypothesized that a functional interaction might occur between CFTR and TNFR1.

Results: To test this hypothesis we examined the possibility of this interaction in HEK293 cells, expressing either [wt] or [ΔF508] CFTR, and in [ΔF508]CFTR IB3-1 and AAV-repaired [wt]CFTR IB3-1/S9 cells and CFPAC cells. Using a confocal immunohistochemistry approach with CF cells, we found that both mutant and wt forms of CFTR co-localized with TNFR1, as well as other components of TNFR1 complex, in the ER. This interaction appeared to have functional consequences. For example, using a NF κ B-luciferase reporter construct in the HEK293 cell system, we found that both [wt] and [ΔF508] CFTR could enhance TNF α -activated NF κ B activity. Consistently, we found that hyper-expression of either recombinant [ΔF508] or recombinant [wt]CFTR delayed recovery of I κ B α after degradation induced by TNF α , thus leaving NF κ B, p65 available for further activation of inflammation. We also found that recombinant forms of both [ΔF508] and [wt] CFTR could enhance the NF κ B luciferase activity that had been induced by any of the four canonical components of the TNFR1 Complex (viz, TNFR1, TRADD, TRAF2 and RIP). Finally, in the co-immuno-

precipitation experiments, we were also able to detect interactions between CFTR and TNFR1, TRADD, TRAF2, and RIP.

Conclusions: It is therefore possible that the proinflammatory CF phenotype may have its origins in aberrant ER chemistry, driven in part by an interaction between $\Delta F508$ CFTR and the TNFR1 complex proteins in the endoplasmic reticulum.

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UNCOVERING AND REDESIGNING ALLOSTERIC COUPLING NETWORKS IN THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

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Many fundamental cellular processes including gene expression are tightly regulated by protein allostery. The molecular mechanism of allosteric signal propagation from the effector site to the active site remains unclear. Here we present a graph-theoretical approach to uncover the underlying networks of residues involved in allosteric signal propagation. We apply our methodology to rescue the function of the disease causing deletion mutant cystic fibrosis transmembrane conductance regulator (ΔF508-CFTR). We previously reported that perturbation of dynamic coupling within the first nucleotide-binding domain (NBD1) of ΔF508-CFTR results in thermal instability of the protein. Using our methodology, we determined critical nodes in the allosteric networks within NBD1 and rationally redesigned the same to modulate dynamic coupling between distant regions. Our results indicate that the residue S492 is a critical node in the network of interacting residues within NBD1 that primarily mediates dynamic coupling between structurally distant regions. Our predicted mutations substituting S492 not only improved thermostability of ΔF508 CFTR, but also enhanced surface expression and function of the mutant in controlled in vitro experiments. Overall, our results present a widely applicable computational methodology to not only identify, but also modulate allosteric pathways in proteins.

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ANALYSIS OF THE EFFECTS OF TEMPERATURE ON F508DEL-CFTR CHANNEL GATING AND THE ACTION OF CFTR POTENTIATORS

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Low temperature treatment partially rescues misfolded F508del-CFTR onto the cell surface. However, the open probability (P_o) of F508del-CFTR is less than one third that of wild-type (wt) CFTR and the channel rundown occurs rapidly at physiological temperatures. Here, we investigate the temperature-dependence of F508del-CFTR channel activity. To address our aim, we studied C127 cells expressing wt- and F508del-CFTR using excised inside-out membrane patches. Experiments were performed at 23, 27, 30, 33 and 37 °C in the presence of 1 mM ATP and 75 nM protein kinase A (PKA); the pipette solution contained 10 mM Cl⁻ and the bath solution contained 147 mM Cl⁻; voltage was -50 mV. As a control, we studied wt-CFTR at 23 - 37 °C.

The single-channel current amplitudes of wt- and F508del-CFTR increased linearly as the temperature was raised from 23 to 37 °C (wt: 23 °C, -0.54 ± 0.03 pA; 37 °C, -0.75 ± 0.04 pA; F508del: 23 °C, -0.50 ± 0.01 pA; 37 °C, -0.74 ± 0.02 pA; $n \geq 4$). However, temperature had different effects on the P_o of wt- and F508del-CFTR. At 23 °C, the P_o of wt-CFTR was 0.24 ± 0.02 , which increased as a hyperbola to 0.46 ± 0.04 at 37 °C ($n \geq 4$; $p < 0.05$; Student's unpaired t-test). By contrast, the P_o of F508del-CFTR increased between 23 and 30 °C (0.06 ± 0.01 and 0.09 ± 0.01 , respectively), but then decreased as temperature was raised to 37 °C (0.06 ± 0.01). An analysis of bursts was performed to achieve a better understanding of the effects of temperature on channel gating. For wt-CFTR, a 2-fold decrease in mean burst duration (MBD) and a 7-fold decrease in interburst interval (IBI)

were observed over the temperature range 23 - 37 °C, whereas for F508del-CFTR, MBD and IBI fell 4-fold and 7-fold, respectively.

We have begun to study the effects of CFTR potentiators at different temperatures. The small-molecule UC_{CF}-853 (P4, 10 μM) (CFFT Compound Collection) was tested on wt-CFTR at 23 - 37 °C. The experimental conditions were the same as those above for wt- and F508del-CFTR except that 0.3 mM ATP was used. There was no difference in the current amplitudes of wt-CFTR with or without P4 at 23 - 37 °C. Using 0.3 mM ATP, the P_o of wt-CFTR increased as a hyperbola from 0.15 ± 0.02 at 23 °C to 0.24 ± 0.02 at 37 °C. In the presence of 10 μM P4, it was 0.24 ± 0.04 at 23 °C, and increased to 0.31 ± 0.02 at 30 °C, but did not increase further as temperature was elevated to 37 °C. We are now testing the effects of P4 on F508del-CFTR at different temperatures.

In conclusion, our data suggest that temperature might have different effects on the conduction and gating properties of CFTR, as shown by the fact that it has similar effects on the current amplitudes of wt- and F508del-CFTR, but the gating behaviours of the two channels have different responses to temperature changes. The maximal activity for wt-CFTR occurs at 37 °C, whereas for F508del-CFTR, it is around 30 °C. Studies of P4 show that as temperature increased from 23 to 37 °C, the P_o of wt-CFTR in the absence and the presence of P4 follows a similar hyperbola. This suggests that the compound affects CFTR channel gating non-competitively to the effects of temperature.

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DEVELOPMENT OF ΔF508 CORRECTORS BY NBD1 CONFORMATIONAL RESCUE

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Both an NBD1 conformational defect and altered domain-domain interactions contribute to ΔF508 CFTR misfolding, leading to severely compromised cell surface functional expression of the mutant CFTR. CFTR NBD1 is composed of an amino terminal, β-strand-rich β-subdomain and a carboxy terminal, α-helix-rich α-subdomain. F508 resides in the α-subdomain whereas the DAD endoplasmic reticulum exit code resides in the hinge linking the two subdomains. Using replica-exchange molecular dynamics simulation (REMD), we show a temperature-dependent NBD1 misfolding as a result of the ΔF508 mutation. Consistent with recent biophysical data, we observed a major change in α-subdomain orientation relative to the β-subdomain, which is characterized by: 1) altered DAD orientation; 2) a dislocated ⁵⁰⁹GVS⁵¹¹ loop, which is accompanied by V510 solvent exposure; and 3) transformation of the small helix ⁵³⁴AEKDNIV⁵⁴⁰ into a loop. By molecular docking of two known NBD1-binding small molecule correctors on native NBD1 and its various destabilizing conformations sampled by REMD, we identified a small molecule binding pocket in NBD1 which regulates NBD1 misfolding. Using fragment-based virtual screen and molecular design, we built a putative ΔF508 corrector molecule. Twenty analogues were synthesized and tested for their ability to rescue ΔF508 CFTR. We found that a number of these candidates improve the processing of ΔF508 CFTR in a HEK293 cell line stably expressing ΔF508 CFTR with an efficacy comparable to or above that of a known corrector CFFT VERT-325. While correctors CFNS-0 and CFNS-1 display similar effects on ΔF508 CFTR processing in HEK293 cells, CFNS-1 dramatically enhances cAMP-induced iodide efflux from a CFBE cell line stably expressing ΔF508 CFTR but CFNS-0 slightly inhibits it. As the small molecule binding pocket we identified on NBD1 is flanked by V510, a key residue for ΔF508 rescue and in mediating NBD1-MSD2 interactions, our data point to the possibility that altering NBD1 conformation through small molecule correctors that bind to the identified pocket might simultaneously improve both NBD1 conformation and NBD1-MSD2 interaction, leading to more efficacious rescue. Presently, CFNS-1 is being tested on primary human bronchial epithelial cells from

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patients with $\Delta F508$ cystic fibrosis. Further development and optimization based on existing correctors might lead to the generation of highly efficacious small molecules that rescue $\Delta F508$ CFTR misfolding.

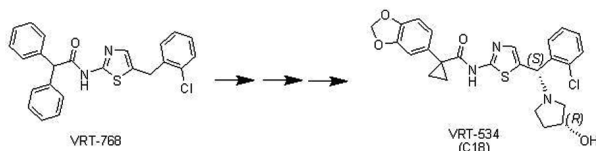
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IDENTIFICATION AND CHARACTERIZATION OF CFTR CORRECTOR VRT-534 (C-18)

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VRT-534 (C-18) is a cystic fibrosis transmembrane conductance regulator (CFTR) corrector made available through the CF Foundation for research purposes. We describe some *in vitro* characteristics of C-18 and the effect of its chiral centers on activity. Extensive medicinal chemistry and SAR analyses on VRT-768, an initial corrector hit, led to several distinct chemical scaffolds, one of which led to VRT-534. The two chiral centers of C-18 allow for four discrete stereoisomers. We studied the corrector activity of the various stereoisomers by monitoring chloride transport in Ussing chamber studies using cultured primary human bronchial epithelial cells derived from patients with cystic fibrosis who are homozygous for the *F508del-CFTR* mutation. The chiral center in the benzylic position was found to display a profound effect on the potency. Thus, C-18 (S,R-configuration) was more potent ($EC_{50}=0.5 \mu M$) than VRT-535 (R,R-configuration) which had an $EC_{50}=2.7 \mu M$. Interestingly, the maximal efficacy of these diastereoisomers was similar. Changing the R-configuration of the hydroxypyrrolidine to S led to 3-fold decrease in potency. To our knowledge, this is the first instance of a clear stereochemical preference for a CFTR corrector; this supports a specific interaction with the putative target. The *in vitro* potency and efficacy of C-18 render it a useful tool for research into CFTR correction.

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IVACAFTOR POTENTIATES MULTIPLE MUTANT CYSTIC FIBROSIS TRANSCONDUCTANCE REGULATOR (CFTR) FORMS

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Clinical studies have shown that ivacaftor, an oral CFTR potentiator, increased CFTR channel activity and improved lung function in patients with CF who have the *G551D-CFTR* gating mutation. The aim of this *in vitro* study was to measure modulation of mutant CFTR by ivacaftor in which the defect was not channel gating. The pharmacological action of ivacaftor on numerous mutant CFTR forms produced by the non-gating missense mutations was evaluated in electrophysiological studies using a panel of recombinant Fischer rat thyroid cells. Ivacaftor potentiated multiple mutant CFTR forms that were tested. These included examples with mild defects in CFTR processing that delivered some functional CFTR to the cell surface. In addition, ivacaftor also potentiated mutant CFTR forms delivered to the cell surface in normal amounts but had mild defects in channel conductance or mild defects in both channel conductance and gating (i.e., R117H-CFTR). These CFTR forms with a mild defect are typically associated with residual CFTR function *in vitro*. A smaller response was observed in some mutant CFTR forms that had severe defects in channel processing and/or channel conductance. This last group is typically associated with minimal residual CFTR function *in vitro*. This *in vitro* study indicated that ivacaftor potentiates chloride transport of multiple mutant CFTR forms with defects beyond channel gating.

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MODULATION OF HSP70 EXPRESSION BY ELP2, A 4-PHENYLBUTYRATE INDUCIBLE ELONGATOR COMPONENT, AND STAT3 DOES NOT REQUIRE STAT3 PHOSPHORYLATION

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In our efforts to understand how sodium 4-phenylbutyrate (4PBA) corrects trafficking of $\Delta F508$ -CFTR in cystic fibrosis (CF) epithelia, we recently demonstrated that a transient increase in Hsp70 expression in CF epithelial cells treated with 4PBA occurs through a process mediated by STAT3 (signal transducer and activator of transcription-3) and Elp2 (Elongator protein 2, Suaud, et al, JBC, 2011). This transient increase in Hsp70 expression may promote correction of $\Delta F508$ -CFTR trafficking. Elp2 (also known as STAT3-interacting protein 1 or StIP-1) is a scaffold protein that is required for activation of STAT3, and is also a component of the Elongator complex that appears to regulate a number of cellular processes including RNA polymerase II activity.

Here, we further probe the mechanism by which 4PBA, through STAT3 and Elp2, stimulated Hsp70 expression. In our previous work, we demonstrated that 4PBA caused transient phosphorylation of STAT3 and translocation of STAT3 into the nucleus. Since Elp2 does not itself contain a nuclear localization signal (NLS), we hypothesized that STAT3 activation/phosphorylation and STAT3's nuclear localization signal (NLS) is required to "bring" Elp2/Elongator into the nucleus. We tested this hypothesis using luciferase reporter assays where luciferase expression was controlled by the human Hsp70 promoter, and with overexpression STAT3 and mutant STAT3 constructs, including a constitutively active STAT3 (STAT3C), STAT3-Y705F that inhibits tyrosine phosphorylation and STAT3-S727A that blocks serine phosphorylation. In control experiments in transfected IB3-1 CF bronchiolar epithelial cells similar to our published data (Suaud, et al, JBC, 2011), 4PBA increased Hsp70 promoter driven luciferase expression 1.5-2 fold, and siRNA mediated depletion of Elp2 decreased this luciferase expression by 50% in both cases. Overexpression of WT STAT3 or STAT3C also increased luciferase expression, but did not reverse the effect of Elp2 depletion on Hsp70 promoter function, further suggesting that Elp2 has a major role in regulating Hsp70 expression. Interestingly, transfection of STAT3-S727A also increased luciferase expression to a similar extent as WT STAT3, and transfection of STAT3-Y705F increased luciferase reporter expression to a 2-fold greater extent than WT or STAT3-S727A. Again, these effects were diminished by siRNA-mediated depletion of Elp2. In control experiments, all of these mutant STAT3's maintained the ability to interact with Elp2 in co-immunoprecipitation experiments. These data suggest that the phosphorylation state of STAT3 is not relevant to the STAT3/Elp2 regulation of Hsp70 expression in response to 4PBA. These data also suggest that testing if the NLS motif of STAT3 is a critical component of the regulation of Hsp70 expression by Elp2/STAT3 in response to 4PBA is warranted.

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SYNERGISTIC CORRECTION OF $\Delta F508$ -CFTR WHEN AN INHIBITOR OF PROTEIN UBIQUITINATION IS COMBINED WITH A SMALL MOLECULE CORRECTOR

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Most wild type CFTR and nearly all $\Delta F508$ -CFTR is trapped in the endoplasmic reticulum (ER) and targeted for ubiquitin and proteasome-dependent degradation because the proteins fail to fold properly. However, conditions that facilitate CFTR and $\Delta F508$ -CFTR folding, or that delay

degradation can improve protein maturation and trafficking to the plasma membrane. Small molecule inhibitors of the proteasome fail to facilitate ΔF508-CFTR correction, suggesting that the protein has passed a “point of no return”. In contrast, small molecule inhibitors of the ubiquitination machinery have not been explored as a means to facilitate ΔF508-CFTR biogenesis. This approach is warranted, as genetic inhibition of the ubiquitination machinery, both alone and in combination with folding correctors, augments ΔF508-CFTR folding and trafficking. We therefore developed an assay to examine the *in vitro* ubiquitination of CFTR and ΔF508-CFTR so that modulators of protein ubiquitination could be screened. This assay employs ER-derived membranes from yeast or mammalian cells that express the channel, which when combined with lysate, ATP, and radiolabeled ubiquitin, recapitulates the polyubiquitination of CFTR and ΔF508-CFTR. Using this system, we tested the effects of two compounds that are known to impact protein ubiquitination, PYR-41, which inhibits the E1 ubiquitin activating enzyme, and Ubistatin B, which inhibits polyubiquitin chain formation. We first discovered that Ubistatin B inhibited CFTR and ΔF508-CFTR ubiquitination. Based on these findings we then tested a series of analogs in order to develop a structure-activity relationship for the inhibition of channel ubiquitination. Based on this analysis, medicinal chemical efforts on the most effective inhibitor of ubiquitination are in progress in order to improve cell permeability. We next discovered that PYR-41 inhibited CFTR and ΔF508-CFTR ubiquitination, although with less potency. Nevertheless, when subcritical amounts of PYR-41 were combined with the CFTR corrector C18 (CF-106951), we observed a statistically significant and synergistic increase in ΔF508-CFTR channel activity when short circuit currents from CFBE/ΔF508-CFTR monolayers were examined in Ussing chambers. Together, we believe that these efforts provide an important proof-of-principle for a therapeutic approach to combine CFTR correctors with refined inhibitors of protein ubiquitination.

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NITRIC OXIDE DONORS DOWNREGULATE CORRECTED F508DEL CFTR AND CL⁻ TRANSPORT IN HUMAN AIRWAY MONOLAYERS
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Background: Nitric oxide (NO) production and inducible nitric oxide synthetase (iNOS) is linked to CFTR expression and localization to the airway epithelial plasma membrane. Also, the fraction of exhaled NO (eNO) is reduced in patients with CF relative to non-CF. NO is typically believed to signal through activation of PKG, but NO is a highly complex molecule that can undergo metabolism to a number of oxidizing and nitrating molecules such as peroxynitrite. It is unclear why the fraction of eNO is reduced in CF, but hypotheses include reduced iNOS localization to the CF airway epithelial cell membrane with reduced NO production, and/or trapping of locally produced NO in the thick mucus blanket characteristic of the CF airway. As NO has been shown to regulate wtCFTR activity in serous glandular airway epithelial cells, we asked whether NO influences F508del CFTR activity following correction.

Methods: CFBE41o- airway cells stably transduced with F508del CFTR cDNA (lentiviral transduction, with CMV promoter) were screened for active correctors of F508del processing using compounds identified by HTS and available through the CFFT Compound Distribution program (Rosalind Franklin University). After identification of chemical corrective conditions in Ussing chambers and by immunoblot (mouse anti-CFTR 596 antibody), monolayers were acutely treated with NO donors (DetoNONOate and SNAP) or cell permeant 8-bromo-cGMP, and then stimulated with CFTR activators (forskolin – 10 μM to raise cAMP and genistein – 50 μM to potentiate F508del CFTR), followed by blockade with CFTRinh172 (10 μM). In complementary experiments, cAMP and cGMP levels of F508del CFTR expressing CFBE41o- cells were measured by ELISA (Cayman Chemicals). Paired T tests were used to statistically assess continuous variables.

Results: Treatment with C18 (an analogue of VX-809, 10 μM) combined with either C3 (10 μM) or C4 (10 μM) reproducibly corrected F508del CFTR, increasing CFTR currents 6-8 fold above baseline (untreated) control monolayers, and led to the detection of C Band CFTR by immunoblot. Treatment with increasing doses of DetoNONOate concentrations (1, 10, 100 μM) five minutes prior to CFTR stimulation with forskolin and genistein inhibited the corrected F508del CFTR currents in a

dose-dependent fashion, reducing CFTR currents to that of control corrected monolayers at the highest DetoNONOate concentration (p<0.02). Both 100 μM DetoNONOate and SNAP abolished corrected F508del CFTR currents (p<0.01), while 500 μM 8-bromo-cGMP had no measurable stimulatory or inhibitory effects. Neither SNAP nor DetoNONOate influenced cAMP levels alone or in response to forskolin (10 μM), and both NO donors had minimal effects on cellular cGMP levels.

Conclusions: NO donors potently reduced the activity of corrected F508del CFTR in polarized human airway cells, and this effect was independent of cGMP signaling. The results indicate that high airway NO levels put corrected F508del CFTR at risk, and identify a condition that may limit the effectiveness of F508del CFTR correctors. Our data suggest that this effect is independent of cGMP or cAMP signaling, and may represent direct effects of NO or NO metabolites on the F508del CFTR protein.

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INTERFERENCE WITH UBIQUITINATION OF LYSINES AT SITES IN THE N TAIL, R, ICL4 AND NBD2 DOMAINS OF CFTR DIFFERENTIALLY AFFECT PROTEASOMAL DEGRADATION AND CELL SURFACE RECYCLING
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Rationale: Post-translational modification of lysine residues in CFTR is predicted to regulate CFTR trafficking from the endoplasmic reticulum (ER) and recycling from the plasma membrane (PM). We hypothesized that disruption of key lysine residues by mutation to arginine to prevent ubiquitination will accelerate destruction of wtCFTR.

Methods: There are 87 lysines that may be accessible to the cytoplasm in CFTR. Site-directed mutagenesis of lysine (K) to arginine (R) was performed in wt and F508del CFTR cDNA vectors to prevent ubiquitination and acetylation. N tail lysines were also mutated to glutamine (Q), which resembles an acetylated lysine residue (Table 1). After transfection in IB3-1, CFTR levels, ubiquitinated CFTR (CFTR-ub) levels and PM CFTR were assayed.

Results: When wtCFTR or the N-lysineless (K0) mutant CFTR were transfected in IB3-1 cells for 24 hr, followed by a cycloheximide chase for 2 hr, the K0 mutant disappeared from the surface biotinylated PM fraction much faster than wtCFTR suggesting that N-tail post translational modification might be one important factor in maintaining cell surface CFTR.

Individual N tail mutants K14R and K68R showed similar loss of wtCFTR as the K0 which reveals K14 and K68 residues are required for CFTR maturation. Mutations of the N tail lysines did not rescue F508del band C. The K710 and K716 residues in the R domain stabilize CFTR expression, and immunoprecipitation of CFTR followed by immunoblotting specific for K48 or K68 ubiquitin poly-linkage shows that K710 and K716 residues tend to be poly-ubiquitinated with K48 and K68 linkages. None of the R domain mutants in F508del CFTR reduced band B or restored band C. K1041R, K1080R, or K1218R mutants showed less expression of CFTR compared to wild type CFTR which reveals that K1041 and K1080 in intracellular loop 4 (ICL4) and K1218 in nucleotide binding domain 2 (NBD2) stabilize the expression of CFTR. Proteasome inhibition rescues K1041R expression to a wild type CFTR level but not for K1080R or K1218R mutants. Lysosomal inhibition restores band C of CFTR in K1218R. Cell surface biotinylated PM fraction and confocal microscopic analysis reveal that band C in the K1218R mutant localizes near the cell surface plasma membrane, but the K1041R mutant does not. None of the ICL4 and NBD2 domain mutants in F508del CFTR reduced band B or restored band C.

Conclusions: Post translational modifications in lysine residues of CFTR can regulate maturation and recycling of CFTR.

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Table 1. Lysine mutations in wt and F508del CFTR

DOMAIN	MUTANTS
N tail	K8R, K14R, K26R, K52R, K64/65R, K68R, K14Q, K64/65Q, K68Q K0 N-tail lysineless (K8/14/26/52/64/65/68R)
R domain	K710R, K716R, K710/716R
ICL4	K1041R, K1080R
NBD2	K1218R

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ALLOSTERIC INTERACTIONS BETWEEN THE TPR AND U-BOX (E3 LIGASE) DOMAINS OF CHIP REGULATE HSC70-MEDIATED CFTR RECOGNITION, UBIQUITINATION AND DEGRADATION

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During CFTR synthesis, heat shock proteins of 70 kDa (Hsc70 and Hsc70), together with their cochaperones, play a key role in deciding whether newly synthesized CFTR successfully traffics to the plasma membrane or is targeted for degradation via the ubiquitin-proteasome pathway. One of the best understood cochaperones in this process is CHIP, a U-box-containing ubiquitin E3 ligase that binds the C-terminus of Hsc70 via its N-terminal tetratricopeptide repeat (TPR) motif. Once bound, CHIP recruits the E2 enzyme UbcH5a into the Hsc70-CFTR complex and thereby initiates CFTR ubiquitination. Previously we showed that the length of time that Hsc70 remains bound to CFTR during a given binding cycle is a major determinant of Hsc70-stimulated degradation, i.e. longer binding favors ubiquitination (Matsumura et al, Mol. Biol. Cell, 2011). These findings raise the possibility that by preventing Hsc70-CHIP interactions it might be possible to stabilize CFTR at the ER membrane and thereby increase the pool of band-B CFTR available for potential export through the secretory pathway. Unfortunately, the mechanism by which Hsc70 binding to substrate coordinates CHIP-client recognition is not well understood.

To determine where in the catalytic cycle of Hsc70 CHIP acts, we used a dominant negative CHIP mutant (P269A), which lacks a conserved proline in the U-box domain required for E3 ligase activity. CFTR was synthesized in a cell-free system that reconstitutes ubiquitination, membrane extraction and ER associated degradation (ERAD), but lacks transcriptional activity and therefore allows manipulation of ERAD components without activating cellular compensatory mechanisms. In this system, addition of recombinant CHIP had minimal effect on CFTR degradation, whereas P269A CHIP inhibited CFTR ubiquitination and degradation in a dose dependent manner. As expected, both the TPR and mutant U-box domains were required for this inhibitory effect. The concentration of mutant CHIP required for 50% inhibition of CFTR degradation was comparable to endogenous Hsc70 (2.5 μ M versus ~2 μ M, respectively), and addition of wild type CHIP, Hsc70, and UbcH5a largely restored CFTR degradation. Thus, P269A CHIP does not simply compete with wild-type CHIP for Hsc70, but rather acts at the level of Hsc70 binding. Interestingly, P269A CHIP did not detectably change CFTR association with Hsc70 or the duration of the Hsc70-CFTR binding cycle. However, the U-box mutation substantially increased CHIP binding to the ADP-bound Hsc70-client complex. These results suggest that reciprocal allosteric interactions between the TPR and U-box domains of CHIP and the substrate-binding and C-terminal domains of Hsc70 regulate the affinity and/or duration of the Hsc70-CHIP complex, which in turn defines protein triage. (Supported by CFFT, NIH, and the Manpei Suzuki Diabetes Foundation.)

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FLUORESCENT ANALYSIS OF SUB-FMOLE QUANTITIES OF IN VITRO-TRANSLATED CFTR NBD1 ON AN IMMOBILIZED SUBSTRATE

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The primary biochemical defect in most CF patients is caused by misfolding and subsequent degradation of Δ F508 CFTR. Phe508 is located in the first nucleotide binding domain (NBD1) of CFTR, where its absence decreases NBD1 folding efficiency (in cells), and thermodynamic stability (in vitro). An important goal in CF therapeutics, therefore, is to understand how NBD1 acquires and maintains its folded state in the cellular environment and to devise pharmacological strategies to improve folding efficiency. To address this problem we developed a FRET-based method to define the NBD1 folding pathway as the nascent chain is synthesized in a cell free extract (Khushoo et al, Mol. Cell. 2011). Our approach is based on the principle that two complementary fluorophores incorporated at specific sites in a nascent protein are brought into close proximity as the domain folds into its native state. Such a change can be detected by an increased efficiency of energy transfer between fluorophores as nascent chain length is increased.

Using this system we showed that NBD1 folding is initiated by compaction of an N-terminal ATP-binding subdomain (residues 389-495), followed by alpha-subdomain folding (residues 495-565) and lastly, formation of the central α/β core containing a six stranded largely parallel β -sheet (residues 567--650). The ability to monitor NBD1 folding directly on the ribosome is appealing because these complexes are easily generated in vitro, require little purification, and allow one to access folding intermediates that exist only transiently during CFTR synthesis. Thus they provide a potential means to pharmacologically manipulate the cotranslational folding pathway. While cell free translation systems enable quantitative incorporation of fluorescent probes with extraordinary specificity and selectivity, the concentration of protein obtained from stalled ribosomes is exceedingly low (~1nM). This poses significant challenges to identify small molecules that might promote NBD1 stability or folding efficiency. Miniaturization of this system to monitor folding on a solid support would therefore be a major advantage in extending this system for screening purposes. Towards this goal, we have investigated strategies to affinity capture in vitro-synthesized fluorescently labeled NBD1. Preliminary results demonstrate that as little as 10-100 attomole (10^{-18} mole) of protein immobilized on agarose beads can be readily detected by fluorescence microscopy. This signal is amenable to quantitative photobleaching, thus providing a potential means to measure FRET and hence monitor NBD1 folding on a large scale from a single translation reaction. Studies are underway to further optimize nascent chain capture, validate FRET measurements, and examine nascent chain folding properties in this solid state system. (This work was supported by CFFT and NIH.)

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ANOCTAMIN 6 IS AN ESSENTIAL COMPONENT OF THE OUTWARDLY RECTIFYING CHLORIDE CHANNEL (ORCC) AND IS UPREGULATED BY CFTR EXPRESSION

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Outwardly rectifying chloride (Cl^-) channels (ORCC, ICOR) of intermediate single-channel conductance of around 50 pS are ubiquitously expressed, but have remained a mystery since their description more than 25 years ago. These channels have been shown to be activated on membrane excision and depolarization of the membrane voltage and by cAMP in the presence of CFTR.

We have recently shown that anoctamin 6 (Ano6), a member of the recently identified family of putative Cl^- channels, is the crucial component of ORCC single channel and whole-cell currents in airway epithelial cells and Jurkat T lymphocytes [1]. CD95 cell death (Fas) receptor ligand (FasL) and staurosporine (STS), known to activate ORCC and to induce apoptosis, also activated Ano6-mediated whole-cell and single-channel currents in Jurkat cells. STS-activated ORCC was suppressed by siRNA-Ano6. Moreover, determination of surviving cells (FACS analysis) indicated that STS-induced apoptosis of Jurkat cells was attenuated by Ano6 siRNA and ORCC/Ano6 was activated by ceramide.

Here we further examined the effect of CFTR on Ano6/ORCC using A549 airway epithelial cells stably expressing a tetracycline-inducible CFTR construct [2]. Induction of CFTR expression led to augmented ORCC/Ano6 activity in A549 cells. Ano6 was shown to be a component of cAMP-activated whole-cell currents in CFTR expressing cells because cAMP-activated whole-cell conductance was reduced after knockdown of Ano6. Furthermore, after CFTR activation by IBMX and forskolin, the ORCC blocker AO1 inhibited a larger portion of the current.

The present results may help to understand the functional coupling between CFTR, ORCC, and cell shrinkage-mediated apoptosis. Moreover, since CFTR has been shown to regulate intracellular ceramide levels and to affect apoptosis by controlling glutathione transport, our data provide further mechanistic insight into this regulatory network.

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MECHANISTIC STUDIES OF CFTR CORRECTORS

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Cystic fibrosis (CF) is a monogenic, recessive disease, caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Loss of function of this Cl⁻-channel leads to multi-organ disease, the most important patho-physiological consequence being impaired mucus clearance from the lung. The most common mutation, found in 90% of CF patients, is a deletion of phenylalanine at position 508, termed ΔF508-CFTR. This mutation causes the protein to misfold, and hence be retained in the endoplasmic reticulum and consequently degraded. Importantly, the mutant protein is appropriately translated in the correct cells. Thus, a possible strategy to treat the disease is to discover small molecules which correct the mistrafficking of the mutant protein and restore its normal cellular function. A number of small molecules have been identified which partially restore ΔF508-CFTR Cl⁻-channel function, some of these are represented in the CFFT panel. Recent work in our lab (Mendoza *et al.*, Cell 2012), indicates that at least two defects are caused by the ΔF508 mutation, leading to misfolding and consequent degradation of the mutant protein: 1) decreased stability of the nucleotide binding domain 1 (NBD1), the domain containing the site of the deletion, and 2) poor assembly of the native multi-domain structure. To determine if CFFT panel small molecules act on different steps in CFTR folding, we utilized second-site mutations, which have been demonstrated to counteract each of the defects, stability or assembly and assays of isolated domain folding. First, we assessed the isolated NBD1: To evaluate domain stability, *in vitro* thermal melts of the purified protein were performed. Second, in combination with step specific second site suppressor mutations we assessed full-length trafficking using biochemical methods. Third, we performed functional assays on these combinations to evaluate the Cl⁻-channel function. These data suggest that some of the CFFT panel compounds target CFTR directly and act preferentially on one of the folding steps, but not the other, consistent with their limited efficacy. Understanding of the mechanism of action of these small molecules provides a rational path to effective combinations and to genotype specific treatment of other CFTR folding mutations.

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AUGMENTATION OF CFTR MATURATION BY S-NITROSOGLUTATHIONE REDUCTASE IN EPITHELIAL CELLS

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S-Nitrosothiols (SNOs) are endogenous nitrosonium (NO⁺) donors with several cell signaling effects and potential relevance to human lung disease. Biochemical evidence suggests that SNOs act on post-translational protein modifications through mechanisms involving S-nitrosylation reactions. Airway epithelial S-nitrosylation signaling disorders have been observed in a wide range of diseases, including cystic fibrosis (CF). SNOs are normally present in the human airway, but concentrations tend to be low in CF patients. This is likely attributable to up-regulated S-nitroso-glutathione reductase (GSNOR) activity. GSNOR is an enzyme that up-regulates nitric oxide and S-nitrosothiol homeostasis through catabolism of GSNO in tissues and is widely expressed in many tissues, including the lungs. Loss of endogenous GSNOR activity increases cellular NO levels and bioactivities, including CFTR maturation in airway epithelial cells. Previously, we have shown that different SNOs increase the expression, maturation and function of CFTR in polarized and non-polarized human bronchial airway epithelial (HBAE) cells (Mol. Pharmacol. 70: 1435-1442, 2006). Our studies have indicated that the effect of SNO on CFTR maturation may be mediated through the degradation of Hsp70/Hsp90 organizing protein (Hop), a co-chaperone of the major molecular chaperones Hsp70 and Hsp90. We have recently shown that GSNO treatment decreases Hop expression and Hop-

CFTR interaction in the endoplasmic reticulum of HBAE cells, and that this effect is necessary for the maturation and cell-surface expression of CFTR. We have also determined that SNOs affect Hop expression by S-nitrosylation of cysteine residue C403. Mutation of this residue to C403S prevents GSNO-induced Hop degradation and S-nitrosylation (Proc Natl Acad Sci USA 107: 11393-11398, 2010). We showed that GSNOR activity is significantly elevated in the mutant HBAE cells expressing ΔF508 CFTR when compared to the wild type HBAE cells. Furthermore, we demonstrated the cellular co-localization of Hop and GSNOR in CFBE41o- cells by immunoprecipitation and confocal microscopy. From this experiment, it appears that GSNOR and Hop interact directly. More recently, we were able to demonstrate that GSNOR knockdown with GSNOR siRNA duplexes increases expression of ΔF508 CFTR and also decreases Hop expression. At present, we seek to determine whether increasing levels of endogenous GSNOR lead to lower CFTR expression. We also tested the effect of GSNOR inhibitor on CFTR maturation. We transfected HBAE cells with 1 and 2 μM FLAG-tagged GSNOR using Lipofectamine 2000. After 48h of transfection, cells were rinsed with PBS and lysed directly on plates using lysis buffer containing protease inhibitors. IB analysis was performed using an anti-CFTR monoclonal antibody. Interestingly, cells transfected with FLAG-tagged GSNOR caused a dose-dependent decrease in maturation of CFTR and GSNOR inhibitor reversed the effect. This data suggests that GSNOR inhibitors could potentially become an attractive therapeutic target for CF. This research is supported by CF Foundation and the NIH.

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HSP27 LEADS TO SUMO-2/3 MODIFICATION OF F508DEL CFTR AND ITS DEGRADATION BY A STUBL

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As a substrate for endoplasmic reticulum associated degradation (ERAD), the mechanism of misfolding and disposal of the cystic fibrosis transmembrane conductance regulator (CFTR) remains incompletely understood. Steps in ERAD usually include the recognition, ubiquitin modification and proteasomal proteolysis of misfolded proteins. Due to its complex folding and domain assembly requirements, much of WT CFTR and ~100% of the common folding mutant, F508del CFTR, are degraded in most systems.

Small heat shock proteins (sHsps) bind to destabilized proteins during cell stress and disease, but their physiological functions are less clear. Model protein studies show that sHsps bind to partially denatured proteins and they have been linked to the ubiquitin proteasome system [1]. We previously found that Hsp27, which is highly expressed in airway epithelial cells, could selectively interact with F508del CFTR and target the mutant for proteasomal degradation. Similar to Hsp27, the SUMO conjugating E2 enzyme (Ubc9) selectively promoted F508del CFTR proteolysis and it interacted with Hsp27 [2]. These findings link sHsp-dependent degradation of mutant CFTR to the small ubiquitin-related modifier, SUMO.

To better understand the properties of this pathway in F508del CFTR degradation, we first determined the SUMO isoform involved in CFTR modification *in vivo* by immunoprecipitation of F508del CFTR and blotting with antibodies to SUMO-1 or SUMO-2/3 (nearly identical isoforms that cannot be distinguished immunologically). Co-expression of Hsp27 preferentially promoted the modification of F508del CFTR with SUMO-2/3, which produced high molecular mass forms suggesting poly- or multi-sumoylation. *In vitro* studies with purified NBD1 showed greater SUMO modification of F508del NBD1 vs. WT NBD1, which was also selective for SUMO-2/3 and enhanced by incubation with Hsp27 protein. In addition to SUMO modification, Hsp27 also promoted ubiquitylation of F508del CFTR via a SUMO-Targeted Ubiquitin Ligase (STUbL) such as RNF4, which binds poly-sumoylated clients. Co-expression of RNF4 promoted the selective degradation of F508del CFTR vs. WT CFTR *in vivo*, whereas dominant-negative RNF4 did not. Furthermore, disabling the SUMO pathway by knock-down of the SUMO E1 increased the expression of F508del CFTR and blocked the action of RNF4 to reduce mutant CFTR expression.

Our results suggest that Hsp27-mediated F508del CFTR modification by SUMO-2/3 targets the mutant CFTR to RNF4 and the ubiquitin-proteasome system. [Supported by the NIH (DK68196 & DK72506) and the Cystic Fibrosis Foundation.]

Poster Session Abstracts

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POLYUBIQUITIN CHAINS DIFFERENTIALLY REGULATE $\Delta F508CFTR$ BIOGENESIS

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The conjugation of ubiquitin (Ub) chains to specific protein substrates is necessary for Ub-mediated proteasomal degradation. Ub contains seven lysine residues (K6, K11, K27, K29, K33, K48, and K63). All seven lysine residues of Ub can be conjugated to another to form polyUb chains on substrates with different topologies, which provide sorting signals for different pathways. To gain further mechanistic insights of Ub-mediated $\Delta F508CFTR$ degradation, we examined all seven lysine residues of Ub in $\Delta F508CFTR$ biogenesis by making Ub containing lysine only mutants (the rest by lysine-to-arginine mutation) in position 6 (UbK6), 11 (UbK11), 27 (UbK27), 29 (UbK29), 33 (UbK33) 48 (UbK48), 63 (UbK63) and found that two lysine-linked polyUb chains (UbK11 and UbK48) facilitate $\Delta F508CFTR$ degradation and three lysine-linked polyUb chains (UbK63, UbK33 and UbK6) protect $\Delta F508CFTR$ from degradation. UbK11, instead of K48, is more potent for $\Delta F508CFTR$ degradation. Overexpression of UbK11 resulted in a profound reduction of $\Delta F508CFTR$ expression (~80%, $p < 0.01$). Surprisingly, UbK33 and UbK63 protect $\Delta F508CFTR$ from degradation. Overexpression of UbK33 and UbK63 increased $\Delta F508CFTR$ expression by 4-5 fold. The effect of UbK33 and UbK63 on the kinetics of $\Delta F508CFTR$ biogenesis was examined in cycloheximide chase experiments. Under control conditions, $\Delta F508CFTR$ had a half life of approximately 60min. However, when UbK33 or UbK63 was expressed, the Ub mutants prolonged $\Delta F508CFTR$ half life to ~7hrs. To further dissect the roles of degradation and protection polyUb chains in $\Delta F508CFTR$ biogenesis, we made a Ub degradation vector containing all degradative Ub mutants and a Ub stabilization vector including all protective Ub mutants. Overexpression of the degradation vector virtually eliminated $\Delta F508CFTR$ expression, whereas overexpression of the stabilizing mutant yielded a significant protection of $\Delta F508CFTR$ from degradation. Relative to the degradation mutant, overexpression of the stabilizing Ub mutant led to ~90-fold increase in $\Delta F508CFTR$ steady-state expression, indicating that the site of Ub modification has opposite functional outcomes in $\Delta F508CFTR$ biogenesis. UbK33 conjugated $\Delta F508CFTR$ was accumulated as detergent-insoluble aggregates and elicited a mild unfolded protein response. Overexpression of the Ub mutants including UbK33 and UbK63 was not observed to result in any impairment of proteasomal degradation function; however, $\Delta F508CFTR$ polychains formed by UbK33 and UbK63 are failed to be recognized by Ub receptors on the proteasome, a prerequisite step for proteasomal degradation. The physiological role of the polyUb chain formation was tested by using UbK48 or UbK63 specific antibodies to perform co-immunoprecipitation and revealed that $\Delta F508CFTR$ can be modified by these Ub linkages in CFBE41o- ΔF cells. Taken together, our data highlight novel mechanisms showing that $\Delta F508CFTR$ utilizes multiple Ub conjugation pathways for its biogenesis. The finding that UbK33 mediated $\Delta F508CFTR$ aggregation formation may provide new insights to understand pathogenesis of cystic fibrosis. [Supported by CFF grant SUN08G0, and NIH grant HL096800.]

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PHOSPHORYLATION OF CFTR SER-737 RESIDUE BY LMTK2 REGULATES CFTR ENDOCYTOSIS

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CFTR expressed at the apical plasma membrane of fluid-transporting epithelia is internalized by clathrin-mediated endocytosis. Previously, we showed that kinases may have a role in this process – as both spleen tyrosine kinase (SYK) and the 4th member of with-no-lysine kinases WNK4 regulate CFTR levels at the membrane [1, 2]. Nonetheless, the protein interactions that control CFTR endocytosis in epithelial cells have only been partially explored. Lemur tyrosine kinase 2 (LMTK2) is a transmembrane protein with serine/threonine kinase activity that has been shown in vitro to phosphorylate Ser-737 in the regulatory region of CFTR [3]. LMTK2 controls intracellular trafficking and binds directly to myosin VI [4], a motor protein that facilitates CFTR endocytosis.

Here, we aimed to determine whether LMTK2 regulates CFTR endocytosis in polarized human airway epithelial cells (CFBE41o- and Calu-3).

Our data showed that endogenous LMTK2 co-immunoprecipitated with CFTR. Silencing LMTK2 increased the plasma membrane abundance of CFTR by attenuating CFTR endocytosis.

An LMTK2 fragment containing the transmembrane and kinase domain deficient in kinase activity (TM+KD-K168M) inhibited the plasma membrane abundance of CFTR and attenuated CFTR endocytosis compared to control.

Taken together, our results demonstrate that LMTK2 facilitates CFTR endocytosis in human airway epithelial cells by a mechanism requiring phosphorylation of Ser-737 in CFTR. Previously published data demonstrate that phosphorylation of Ser-737 inhibits CFTR mediated Cl^- currents [5].

Our results reveal that such Ser-737-dependent inhibition of CFTR-mediated Cl^- currents is due, at least partially, to LMTK2-dependent CFTR endocytosis.

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EXTRACELLULAR LOOP 1 IS INVOLVED IN MAINTAINING OUTER VESTIBULE PORE ARCHITECTURE OF CFTR

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The CFTR protein includes 6 extracellular loops (ECL1-6). Many amino acids in ECL1 have been identified as sites of CF disease mutation, including A107G, S108F, Y109C/N, D110H/Y/N, P111A/L, E116K/Q, and R117C/G/H/P/L. It was reported that R117H-CFTR exhibited reduced channel amplitude while D110H-, E116K-, and R117C/L/P-CFTR exhibited altered channel behavior. We asked whether these amino acids are directly involved in ion conduction and permeation in CFTR or contribute to stabilizing the outer vestibule architecture, using cRNA-injected oocytes combined with electrophysiological techniques to probe the possible functions of these amino acids. We found that: (1) Mutants A107G-, S108F-, Y109C-, D110R-, E116R-, and R117A-CFTR exhibited multiple open states with

significantly shortened burst durations compared with WT-CFTR, while charge-retaining mutants R117K-, D110E-, and E116D-CFTR showed primarily the full open state with normal open burst durations; (2) R117A-, D110R-, and E116R-CFTR unlike WT-CFTR failed to be locked into the open state by AMP-PNP; (3) Y109C-, D110C-, E116C-, and R117C-CFTR were not functionally modified by extracellular MTSES- or MTSET+; (4) D110C-, E116C-, and R117C-CFTR exhibited weakened block by GlyH-101 compared to WT-CFTR; and finally (5) GlyH-101 strongly blocked T338A- and R352A-CFTR and completely lost its effect on R334C- and R334A-CFTR. R334, T338, and R352 are amino acids in TM6 which is the most important TM that determines ion permeation in CFTR. The data so far suggest that: (1) R117, D110, and E116 are not involved in ion conduction and permeation of CFTR directly; (2) amino acids of ECL1 contribute to stabilizing the CFTR channel pore but do not directly contribute to block by GlyH-101; and (3) amino acids in ECL1 probably interact with other residues to help maintain CFTR's outer vestibule architecture. (Support: NIH-R01-DK056481)

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MEMBRANE-INSERTED CFTR CATALYZES ADENYLATE KINASE ACTIVITY

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CFTR is an apical membrane Cl⁻ channel and a member of the ATP-binding cassette (ABC) family of proteins. It is well established that ABC proteins are ATPases, i.e., they can hydrolyse ATP to ADP (adenosine 5'-diphosphate) and P_i (inorganic phosphate) (ATP+H₂O→ADP+P_i). Recent studies with CFTR and two other ABC proteins, the DNA repair enzyme Rad50 and a structural maintenance of chromosome (smc) protein, challenge the model that the function of all ABC proteins depends solely on their associated ATPase activity. CFTR opening and closing depends on ATPase activity, if ATP is the only nucleotide present. However, patch-clamp studies using excised membrane patches containing CFTR showed that in the presence of physiological concentrations of AMP (adenosine 5'-monophosphate), adenylate kinase activity (ATP+AMP↔2 ADP) is coupled to CFTR channel function (Cell 115, 837-50). Rad50 (Mol. Cell 25, 647-61) and smc (J. Mol. Biol. 401, 265-73) were also shown to catalyze both ATPase and adenylate kinase reactions. However, in the case of membrane-bound CFTR, biochemical data demonstrating adenylate kinase activity to complement the electrophysiological data are missing. Here we tested the hypothesis that full-length CFTR displays adenylate kinase activity when it is in the membrane. We predicted that if membranes containing CFTR were incubated with radioactive [³²P]GTP and non-radioactive N₃-AMP, CFTR adenylate kinase activity would catalyze transfer of the radioactive γ-phosphate of [³²P]GTP onto N₃-AMP forming radioactive [β³²P]N₃-ADP. Subsequent exposure to UV light would crosslink [β³²P]N₃-ADP to the protein via the azido (N₃)-group. Thus, CFTR would become radioactively labeled. We used [³²P]GTP rather than [³²P]ATP because CFTR accepts GTP as well as ATP, whereas the protein kinases that phosphorylate CFTR do not. Experimental testing demonstrated radioactive labeling of CFTR. Significantly more labeling was achieved when we used 2-N₃-AMP versus 8-N₃-AMP. In addition, labeling depended on the presence of non-radioactive N₃-AMP and on UV irradiation indicating that it depended on the transfer of the radioactive γ-phosphate of [³²P]GTP onto N₃-AMP and subsequent UV-dependent crosslinking of [β³²P]N₃-ADP to CFTR. Consistent with adenylate kinase activity we found that excess ATP and AMP as well as the adenylate kinase inhibitor Ap₅A reduced labeling. No CFTR labeling occurred when the interaction of [³²P]GTP with ATP-binding site 2 was blocked by the S1248F mutation. These results show that membrane-bound CFTR has adenylate kinase activity and suggest that the interaction of nucleotide triphosphate with ATP-binding site 2 is required for this activity.

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PURIFICATION AND BIOPHYSICAL ANALYSIS OF THE CYSTIC FIBROSIS TRANSCONDUCTANCE REGULATOR PROTEIN EXPRESSED IN *SACCHAROMYCES CEREVISIAE*

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Treatment regimens for cystic fibrosis typically address the symptoms of the disease but cannot correct its cause, namely dysfunction of the cystic fibrosis transconductance regulator (CFTR) protein. To develop specific drugs targeting CFTR we must extend our understanding of the protein; this requires plentiful and pure samples for biochemical, biophysical and structural studies. We aim to purify sufficient CFTR for crystallographic studies, and to obtain a high-resolution structure of the protein.

To produce the protein we have developed an *S. cerevisiae* heterologous overexpression system for CFTR. The protein carries N- and C-terminus poly-histidine (His) tags to facilitate its purification by immobilized metal affinity chromatography (IMAC). A C-terminus green fluorescent protein (GFP) tag enables rapid detection of the CFTR during expression and purification by fluorescence microscopy and spectroscopy. Analytical gel permeation chromatography (GPC), thermal unfolding and light scattering techniques will provide information on the stability and conformational state of the purified CFTR, and may also lay the foundations for future drug screens.

Crystallization of membrane proteins is a notoriously unpredictable art, requiring large quantities of pure, monodisperse protein. Subtle differences in the primary sequence of the protein and the purification method may affect crystal growth. To maximize our chances of producing crystallizable protein, we have screened the expression of orthologs of CFTR from diverse sources, including the human wild-type and ΔF508 variants. We have attempted to optimize the expression of full-length CFTR using additives to the growth media.

Orthologs of CFTR that were abundantly expressed and correctly trafficked to the plasma membrane have been identified using fluorescence microscopy and SDS-PAGE analysis.

We have scaled up expression of four highly-expressed orthologs of CFTR (mouse, chicken, salmon, platypus) in an 18 L bioreactor. A protocol for the purification of mouse CFTR using the detergent lysophosphatidylglycerol-14 (LPG-14) has been established yielding ~100 μg purified protein per litre of yeast cell culture (μg/L) at 90% purity [1]. A second protocol using dodecylmaltoside (β-DDM) has also been developed. Microsomes containing CFTR were solubilized using β-DDM, and CFTR was purified from the total solubilized material by IMAC and GPC. Each ortholog purified in this way yielded ~25 μg/L CFTR at 80% purity. The detection of ATP hydrolysis by purified samples of CFTR provided evidence that purification of the protein in DDM preserved its native fold.

We continue to refine this protocol in order to identify conditions that enable the maximum recovery of pure, functional and monodisperse protein. Ultimately we intend to increase the production of the protein in order to undertake 2D and 3D crystallization and to generate a high-resolution structure of CFTR.

Supported by the Cystic Fibrosis Foundation.

Reference: [1] O'Ryan, L. et al (2012), J. Vis. Exp. (61), <http://www.jove.com/video/3860/>

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CFTR-NHERF2-LPA₂-CONTAINING MACROMOLECULAR COMPLEX FUNCTIONS AS INHIBITORY MACHINERY FOR CFTR (WILD TYPE AND MUTANTS) CHANNEL FUNCTION AT THE PLASMA MEMBRANE

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Background: CFTR has been found to form a macromolecular complex with type 2 lysophosphatidic acid receptor (LPA₂) and Na⁺/H⁺ exchanger regulatory factor-2 (NHERF2) at the plasma membrane of epithelial cells lining airway and gut.

Objectives: To test the hypothesis that CFTR-NHERF2-LPA₂-containing macromolecular complex functions as inhibitory machinery for CFTR (wild type and mutants) channel function at the cell surface, and to explore the possibility of targeting this macromolecular complex for therapeutic interventions of CFTR-associated diseases.

Methods: Two approaches were adopted to test the hypothesis. (i) Strategy No.1: to identify specific LPA₂-NHERF2 inhibitors and test their effects on CFTR channel function. If our hypothesis holds true, such inhibitors would potentiate CFTR channel function. (ii) Strategy No.2: to develop specific LPA₂ agonists and test their effects on CFTR channel function. If our hypothesis holds true, such agonists would attenuate CFTR channel function.

Results: (i) The specific inhibitors for NHERF2-LPA₂ (e.g., CO-068) can disrupt LPA₂ from the complex and abolish the inhibitory effect of LPA₂-dependent events on adenylyl cyclase (AC), thus increasing compartmentalized cAMP levels in proximity to CFTR and, consequently, augmenting wild type (wt) CFTR channel function. Moreover, the inhibitor can further augment the channel function of temperature- and CFTR corrector-rescued ΔF508-CFTR in CFBEo- cells. We are now using structure-based drug design and virtual screening approach to identify more specific and potent inhibitors for LPA₂-NHERF2 interaction. (ii) The specific agonist for LPA₂ (e.g., C143) can activate LPA₂ in the complex and potentiate the inhibitory effect of LPA₂-dependent events on AC, thus decreasing compartmentalized cAMP levels in proximity to CFTR and inhibiting wt-CFTR channel function as evidenced in CFTR-mediated *I*_{sc} measurements and CFTR-dependent intestinal fluid secretion in a mouse acute diarrhea model. We are also using structure-based drug design and virtual screening approach to identify more specific and potent LPA₂ agonists.

Conclusion and Perspective: It seems that (i) an inhibitory machinery (a macromolecular complex containing CFTR, NHERF2, LPA₂ and other intermediate molecules) exists at the cell surfaces which inhibits the channel function of wt-CFTR and also mutants (e.g., ΔF508-CFTR); (ii) This macromolecular complex can be targeted for fine tuning CFTR channel function, thus having the potential for therapeutic interventions of CFTR-associated diseases. Specifically, inhibitors for LPA₂-NHERF2 interaction can further potentiate ΔF508-CFTR channel function rescued by CFTR correctors, thus can be explored for therapy of CF in combination with a CFTR corrector (such as VX-809). Specific LPA₂ agonists can inhibit wt-CFTR channel function, thus can be explored for therapy of enterotoxin-induced secretory diarrheas such as cholera.

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SMALL-MOLECULE CFTR INHIBITOR WITH LOW NANOMOLAR POTENCY

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CFTR is an important drug target for development of small-molecule inhibitors. Activation of CFTR in intestinal enterocytes occurs in secretory diarrheas caused by bacterial enterotoxins, such as cholera and traveler's diarrhea. CFTR is also required for active fluid secretion into cysts in autosomal dominant polycystic kidney disease. CFTR inhibitors are thus pre-

dicted to have therapeutic values in secretory diarrheas and polycystic kidney disease, and, in cystic fibrosis research, for elucidation of CFTR cellular activity and functions. We previously identified several classes of small-molecule CFTR inhibitors, including thiazolidinones (eg. CFTRinh-172), glycine hydrazides (eg. GlyH-101) and pyrimido-pyrrolo-quinoxalinediones (eg. PPQ-102), and showed their efficacy in models of polycystic kidney disease and cholera toxin-induced secretory diarrhea. We recently reported an analogue of PPQ-102 (IC₅₀ ~ 90 nM), the benzopyrimido-pyrrolo-oxazinedione BPO-27 (Snyder et al. J. Med. Chem. 54:5468-5477, 2011), which inhibited CFTR with IC₅₀ ~ 8 nM, and had greatly improved in vitro metabolic stability and aqueous solubility compared to PPQ-102. BPO-27, which was synthesized and tested as a racemic mixture (R:S 50:50), prevented and reversed renal cyst formation in an embryonic kidney culture model of polycystic kidney disease. Here, we separated the enantiomers of BPO-27, which contains a single chiral center, and determined their absolute configuration, activity, and metabolic stability. Following purification by chiral supercritical fluid chromatography, enantiomer R inhibited CFTR chloride conductance with IC₅₀ ~ 4 nM, while the S enantiomer was inactive. Crystals of the ethyl ester derivative of BPO-27 for x-ray structure analysis were obtained by vapor diffusion crystallization using toluene and hexane. X-ray analysis revealed absolute enantiomer configuration, and provided high-resolution coordinates for CFTR docking computations. In vitro metabolic stability in hepatic microsomes showed both enantiomers as stable, with <5 % metabolism in 4 h. Following bolus intraperitoneal administration in mice, serum BPO-27 decayed with t_{1/2} ~ 2 h, giving sustained therapeutic concentrations in kidney. BPO-27 is a potential development candidate for secretory diarrheas and polycystic kidney disease.

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FROM MULTIPLE CELL LINE HIGH THROUGHPUT SCREENING (HTS) TO THE RESCUE OF ΔF508-CFTR IN PRIMARY HBE CELLS

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Deletion of phenylalanine 508 on the CFTR gene (ΔF508-CFTR) is the most prevalent disease-causing CF mutation. ΔF508-CFTR causes impaired protein trafficking and maturation, and improper functional gating as a chloride channel in epithelial cell membrane. Such defects may be attenuated by small molecule modulators, such as CFTR correctors for protein trafficking and CFTR potentiators for gating. At Flatley Discovery Lab, we are conducting an extensive high-throughput screening (HTS) campaign with a YFP-based iodide influx assay, seeking small molecule CFTR correctors from a large collection of compound libraries. One of the most challenging obstacles in this effort is the translation of cell-based HTS results to detectable ion current increases in ΔF508-CFTR hBE primary cells in the Ussing chamber assay, the gold standard ex-vivo assay for ΔF508-CFTR correction. The low throughput of the Ussing assay requires reliable methods for triaging and selecting compounds for testing.

The YFP flux assay was initially developed with Fisher Rat Thyroid (FRT) cells (Pedemonte et al, 2005; Sui et al, 2009). Recently, a modification to replace FRT with mammalian cell lines has been reported (Pedemonte et al, 2010). However, it is unclear which cell line produces the highest rate of translatable hits that display corrector activity in primary hBE cells. We deployed a mammalian cell line (CFBE41o-) in our primary HTS, and used a low activity threshold for selecting hits that were then screened in three cell lines (FRT, CFBE, and A549) in confirmatory HTS and multiple dose SAR screening formats. Our hit selection decisions were based on the collective results from all three cell lines. Upon comparing the three cell lines, we found that CFBE cell data had a slightly better correlation to hBE assay data than the others, but no cell line appeared definitively superior to the others. However, the best ΔF508-CFTR correctors showed high activity in all three cell lines, confirming the value of the three cell line approach in our campaign.

To accommodate the increased throughput required to validate HTS hits in primary cells, we have implemented a conductance-based assay for CFTR correction in hBE cells to select and prioritize compounds for detailed assessment in the Ussing chamber assay. We found that metrics for correction correlate across multiple compound classes between the conductance and Ussing assays. Our data indicate that this conductance assay may serve as a medium throughput bridge from the HTS flux assay to the Ussing

chamber. Implementation of the multi-cell line approach in our HTS platform, coupled with the medium throughput conductance assay and the high quality “gate-keeping” Ussing chamber assay, formed a logical and productive workflow. This approach significantly improved both our throughput and the rate of detecting hBE-active correctors in our drug screening campaign.

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A ROBUST AND EFFICIENT VISIBLE FLUORESCENCE ASSAY FOR HUMAN NBD1 FOLDING AND STABILITY DESIGNED FOR MECHANISTIC STUDIES AND HIGH-THROUGHPUT SCREENING FOR CORRECTOR COMPOUNDS

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The F508del mutation – deletion of a single phenylalanine residue at position 508 in the cystic fibrosis transmembrane conductance regulator (CFTR) – is the most frequent mutation carried by cystic fibrosis (CF) patients. This destabilizing mutation triggers rapid degradation of CFTR by cellular quality control systems, preventing its export from the endoplasmic reticulum (ER) to its site of function in the epithelial cell membrane. One important objective of ongoing CF drug-discovery efforts is to find small compounds that can stabilize F508del-CFTR and thereby rescue it from the degradation in the ER. While some lead compounds with such activity have been discovered via high-throughput screens, none of the compounds identified so far fully correct the defective processing of F508del-CFTR in human tissue culture cells or patients.

Recently, this group and others have demonstrated that the F508del mutation facilitates the unfolding of nucleotide-binding domain 1 (NBD1) of CFTR, which triggers aggressive aggregation that is likely to be a key contributor to pathogenesis. These findings form the foundation of a new approach to assaying the conformational stability of NBD1 in isolation or in situ in full-length CFTR, where the F508del mutation is also believed to perturb functional interdomain interactions. Specifically, we have used computational protein engineering methods to construct a stable variant of human NBD1 (hNBD1) covalently labeled with a visible fluorophore at a single site in the domain. Unfolding of the labeled domain leads to strong fluorescence self-quenching due to subsequent aggregation. This assay can be used to evaluate the conformational stability of hNBD1 either in isolation in aqueous solution or when incorporated into detergent-solubilized CFTR. We have evaluated its use for high-throughput screening for small molecules that stabilize isolated F508del-hNBD1. Calibration screens show a Z-factor of ~0.8 either in the presence or absence of a compatible non-ionic detergent commonly used to purify membrane proteins. This fluorescent screen provides a promising approach to identifying novel compounds correcting the molecular pathologies caused by the F508del mutation in CFTR. It is also applicable to optimizing procedures for expression, purification, and stabilization of full-length CFTR based on its coupling of a simple visible fluorescence signal to the conformational state of NBD1.

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THE CONSERVED Q-LOOP GLUTAMINE 1291 INTERACTS WITH AP₅A

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Previous work showed that in the presence of AMP (adenosine 5'-monophosphate) CFTR channel activity was coupled to adenylate kinase activity (ATP+AMP↔2 ADP). The adenylate kinase inhibitor Ap₅A (P₁,P₅-di(adenosine-5') pentaphosphate) inhibited current. Inhibition was attenuated by increasing the ATP concentration or by adding AMP (1). Ap₅A contains two adenosine groups (1 and 2) connected by five phosphate groups

allowing it to bind simultaneously to an ATP and an AMP binding-site of an adenylate kinase. While it is well established where ATP interacts with CFTR and other ATP-binding cassette (ABC) proteins, the AMP binding-site of CFTR has remained elusive. Two other ABC proteins, the DNA repair enzyme Rad50 (2) and a structural maintenance of chromosome (SMC) protein, are also known to interact with AMP and to exhibit adenylate kinase activity. The crystal structure of the nucleotide-binding domain (NBD) of the *Pyrococcus furiosus* SMC protein in complex with Ap₅A (3) showed the two adenosine moieties of Ap₅A attached to two binding sites separated by ~15Å. A Mg²⁺ ion, adenosine 1, plus α-, β-, and γ-phosphates of Ap₅A bind the canonical Mg²⁺-ATP-binding site on lobe I of the SMC NBD. Adenosine 2 stacks onto the side chain of a conserved glutamine of the Q-loop at the interface of lobe I and lobe II. Based on this structure we hypothesized that the homologous glutamine in CFTR, Q1291, would interact with Ap₅A. To test this hypothesis we studied the effect of single amino acid substitutions of Q1291 on Ap₅A inhibition of CFTR currents and CFTR photolabeling with [β₃³²P]8-N₃-Ap₄A, a photoactivatable analogue of Ap₄A (P₁,P₄-di(adenosine-5') tetraphosphate), which also inhibits CFTR current in a similar manner as Ap₅A. We found that substitution with phenylalanine (F), an amino acid with a larger and bulkier side chain, interfered with Ap₅A inhibition without affecting the potency of ATP to stimulate current or changing the single channel opening and closing rates in the presence of ATP. When we eliminated the Q1291 side chain by substitution to glycine we observed again current inhibition by Ap₅A and photolabeling with [β₃³²P]8-N₃-Ap₄A. However, the apparent K_i of Ap₅A to inhibit current increased. In summary, our data suggest that Q1291 interacts with Ap₅A but it does not directly interact with ATP. Thus, Q1291 may be part of the AMP-binding site. Identifying key amino acids that are involved in the interaction of Ap₅A and AMP with CFTR may aid development of new treatments for CFTR-related diseases, like cystic fibrosis or cholera-toxin induced secretory diarrhea.

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SECOND GENERATION CORRECTOR SCREENING BASED ON CORRECTION OF DISTINCT ΔF508-CFTR FOLDING DEFECTS

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A second-generation corrector screen was developed and implemented based on the emerging paradigm that ΔF508-CFTR misfolding is the consequence of two or more distinct structural defects, such as NBD1 misfolding and impaired domain-domain interactions. The principle of this approach is to screen for compounds in the presence of a known corrector (with reasonably established mechanism as a pharmacological chaperone) or second site mutations (that stabilize NBD1 or the NBD1-MSD2 interface), with the goal of identifying compounds whose additive or synergistic effects can restore ΔF508-CFTR surface targeting and function close to that of wild-type CFTR. CFBE41o- cells (a CF human bronchial epithelium-derived cell line homozygous for the ΔF508 mutation) were transduced with lentiviral particles encoding human ΔF508-CFTR containing horseradish peroxidase (HRP) in its fourth extracellular loop, controlled by a tetracycline-activated transactivator. ΔF508-CFTR-HRP was ER-retained under control conditions, but corrected by low-temperature and pharmacological/chemical chaperones including VX-809. The screening assay utilized a robust luminescence read-out of extracellular HRP activity as a measure of ΔF508-CFTR cell surface expression. Incubation with VX-809 for 24 h at 37°C gave a ~5-fold increase in luminescence signal with maximum correction at 3 μM VX-809, which was comparable to that of low temperature rescue (27°C for 24 h). Initial screening was done using collections of ~4000 approved/investigational drugs and ~15000 synthetic small molecules. More than ten active compounds were identified that gave >50% increase in lumi-

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nescence signal over that of VX-809. Several potentially interesting drugs/compounds emerged, including the anti-inflammatory suprofen, the anti-hypertensive drug enalapril, the PDE inhibitor zaprinast, and the anti-inflammatory meclofenamate. The additivity of effects suggests distinct corrector mechanisms that may involve individual targets, which will be elucidated using biochemical and genetic assays. Second generation corrector screening based on synergy, may yield novel corrector combinations with high efficacy.

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BIOPHYSICAL CHARACTERIZATION OF NBD1 CONFORMATIONAL EQUILIBRIA AND DYNAMICS

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The thermodynamic stability of the first nucleotide binding domain (NBD1) of CFTR is closely linked with the overall stability of CFTR. Mutations which destabilize NBD1, such as deletion of F508, also destabilize the full-length molecule and lead to processing and gating defects. Thus, the mechanism by which F508 destabilizes NBD1 is directly related to the pathology. Structural studies show that the F508del mutation has only a minor effect on the structure of the ground state of NBD1. Using NMR relaxation experiments we have previously shown that fast timescale (ns-ps) dynamics of the ground state of NBD1 are very similar in the WT, F508del and I539T variants, corroborating the structural studies. The similarity in the pattern of fast dynamics for the three variants indicates that dynamical changes to the ground state of NBD1 do not provide an explanation for deleterious effects of the F508 deletion. However, our NMR dispersion studies show that deletion of F508 increases intermediate timescale (ms- μ s) sampling of an excited state, which might be responsible for inefficient processing of the channel. Similarly, published thermal unfolding transition midpoints for NBD1 are significantly above 37°C, while low temperature rescue of CFTR requires temperatures below 37°C, apparently improving even below 26°C, supporting the idea that defective processing involves an excited state, as recently proposed. Temperature dependent changes in sampling of an excited state may be more directly relevant to understanding misfolding of CFTR than the overall stability of the domain. The nature of the excited state(s) is currently unknown, though hypothesized to involve the α -subdomain. We will show results of residue specific hydrogen-deuterium exchange experiments aimed at identifying regions of NBD1 that sample amide exchange competent excited state conformations. Together these studies provide insight into the NBD1's energy landscape and yield critical clues into the nature of excited state(s) relevant to channel processing and gating defects. This work was supported by the CF Foundation Therapeutics.

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THE EFFECT OF CAL-COMPLEXED E3 UBIQUITIN LIGASE ON CFTR CORRECTOR

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Previously, we have demonstrated the role of PDZ-domain containing protein CAL and its associated proteins in the regulation of the abundance of complex-glycosylated, mature CFTR. We have shown that CAL and the Qbc-type N-ethylmaleimide-sensitive factor attachment protein receptor (Qbc-SNARE) protein syntaxin 6 (STX6) form a complex and both proteins bind to CFTR. Manipulation of either CAL or STX6 abundance and function by overexpression and small interfering RNA (siRNA) mediates silencing in multiple cell lines, defining their role as negative regulators of stability of mature CFTR. Recently, we identified the membrane associated ring CH (MARCH) E3 Ubiquitin ligase II (MARCH II) as part of the CAL-complex. Overexpression of MARCH II but not a catalytic dead mutant led to increased ubiquitination of CFTR. Ubiquitination plays a critical role in targeting misfolded CFTR to degradation in both ER and peripheral quality control systems. Overexpression of MARCH II led to dose-dependent degradation of mature CFTR, while silencing of endogenous MARCH II

increased the abundance of mature CFTR. Immunoprecipitation of MARCH II brought down CAL, STX6 and CFTR. Furthermore, degradation of CFTR by MARCH II requires the presence of CAL as well as the PDZ motif of CFTR. MARCH II has no effect on ER-localized delta F508 CFTR. Treatment of a HeLa cell line stably expressing delta F508-CFTR with CFTR corrector C18 for 24 hours led to the appearance of mature CFTR. Overexpression of MARCH II led to degradation of C18-rescued CFTR. Importantly, silencing of endogenous MARCH II increased the abundance of rescued CFTR. Taken together, these data suggest that as a part of CAL-complex MARCH II plays an important role in CAL-mediated degradation of mature CFTR, and manipulation of MARCH II levels can influence the effect of CFTR correctors in rescuing delta F508 CFTR. Supported by the Cystic Fibrosis Foundation and the National Institutes of Health.

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MICROFLUIDICS PLATFORMS FOR DISCOVERY AND CHARACTERIZATION OF CHLORIDE CHANNEL-MODULATING DRUGS

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We previously developed and applied fluorescence, cell-based high-throughput screening to identify inhibitors and activators of wildtype CFTR, potentiators and correctors of Δ F508-CFTR, and inhibitors and activators of CaCCs including TMEM16A. Motivated by the relatively high efficiency and low cost of microfluidics (lab-on-a-chip) technology, here we developed fluorescence, cell-based microfluidics platforms for discovery and characterization of chloride channel modulators, focusing on inhibitors and activators of CFTR and TMEM16A. As done for plater-based screening, the microfluidics assay utilized FRT cells stably expressing CFTR or TMEM16A together with the iodide-sensing, genetically encoded fluorescent protein YFP-H148Q/I152L/F46L. A multiplexed microfluidics platform was developed for single-shot determination of full concentration-activity relations in which a 5 x 5 mm square area of adherent cultured cells was exposed for 5-10 min to a pseudo-logarithmic gradient of test compound generated by iterative, two-component channel mixing. Cell fluorescence was imaged following perfusion with an iodide-containing solution to give iodide influx rate at each point in the image field, thus quantifying modulator effects over a wide range of concentrations in a single measurement. IC₅₀ determined for CFTR and TMEM16A modulators by single-shot microfluidics were in close agreement with conventional plater-based measurements. For automated compound screening, a microdroplet-based approach was developed in which cells together with test compound were incubated in a stable aqueous droplet surrounded by oil, and then fused with an iodide-containing droplet to rapidly establish an iodide gradient. Iodide influx was determined from the time course of droplet fluorescence following fusion as measured using an intensifier/CCD camera with submillisecond resolution. The droplet microfluidics platform was validated using known CFTR and TMEM16A modulators. Our results establish microfluidics-based technology for chloride channel drug discovery, which, compared to conventional methods, could reduce costs and increase efficiency.

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COMBINATION OF SMALL-MOLECULE CORRECTORS WITH ATTENUATION OF CELLULAR LIPID LEVELS OR INHIBITION OF INTERNALIZATION AUGMENTS RESCUE OF APICAL Δ F508 CFTR IN AIRWAY EPITHELIAL CULTURES

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Cystic fibrosis (CF) is caused by mutations in the CFTR gene, which codes for a highly regulated apical chloride channel of airway epithelial cells. The most common mutation in CF is a deletion of phenylalanine at position 508 (Δ F508), which results in a misfolded protein that does not reach the apical membrane of airway epithelial cells. Rescue of surface expression of Δ F508 CFTR by treatment with small-molecule correctors is an important therapeutic approach for improving the quality of life of CF patients. Recent studies revealed that Δ F508 CFTR rescue efficiency is dependent on the cell model. We study the efficacy of small-molecule cor-

rector compounds in well-differentiated primary human airway epithelial (HAE) cultures grown at air-liquid interface as a physiological system that recapitulates the morphology of the human airway epithelium. Viral expression of extracellularly tagged $\Delta F508$ CFTR was used to visualize turnover of apical $\Delta F508$ CFTR by confocal immunofluorescence microscopy and to quantify $\Delta F508$ CFTR that has reached the apical membrane by On-Cell Western (Cholon et al. 2010 *Am J Physiol Lung Cell Mol Physiol.*, 298:L304-14). Western blotting of whole-cell lysates and short circuit current measurements in Ussing chambers were used to characterize rescue of endogenous corrector-treated $\Delta F508$ CFTR in HAE cultures. We found that small-molecule corrector compounds, such as VX-809, can restore some apical $\Delta F508$ CFTR function and that various combinations of corrector compounds can act synergistically. However, trafficking studies of corrector-rescued $\Delta F508$ CFTR show that the rescued mutant protein is less stable at the cell surface and has a significantly shorter half-life than wild-type CFTR. Once internalized, wild-type CFTR is efficiently recycled back to the cell surface while rescued $\Delta F508$ CFTR is targeted for lysosomal degradation. Application of the specific inhibitors of dynamin- or clathrin-dependent internalization, Dyno-4a and Pitstop 2, respectively, further increased the efficiency of corrector rescue. Attenuation of cellular lipid levels, such as cholesterol extraction by methyl- β -cyclodextrin, or inhibition of glycosphingolipid synthesis by miglustat, also enhanced the corrector rescue of $\Delta F508$ CFTR by augmenting the amount of mutant protein at the surface. Because cells expressing $\Delta F508$ CFTR were shown to accumulate cholesterol, and high cholesterol levels appear to be of disadvantage for rescued $\Delta F508$ CFTR stability, lowering cholesterol levels may be an effective approach for increasing the amount of $\Delta F508$ CFTR at the surface of airway epithelial cultures. Successful small-molecule rescue of $\Delta F508$ CFTR in HAE cultures requires restoration of protein stability and re-establishment of proper trafficking to increase steady-state abundance and ion channel function. Because normal lung function requires sufficient amounts of functional CFTR at the cell surface, data obtained from these studies are directly relevant for improving treatments for CF patients.

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TGF- β 1 IMPAIRS CFTR-MEDIATED ANION SECRETION ACROSS CULTURED PORCINE VAS DEFERENS EPITHELIAL MONOLAYER VIA THE P38 MAPK PATHWAY

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Transforming growth factor β 1 (TGF- β 1), a cytokine that modifies cystic fibrosis lung disease severity, is present at high concentrations in human semen. Since cystic fibrosis is commonly associated with male infertility, experiments were conducted to examine the impact of TGF- β 1 on cultured PVD9902 epithelial cells that were derived from porcine vas deferens. In modified Ussing-style flux chambers, PVD9902 monolayers exposed to TGF- β 1 (5 ng/ml, 24 hr) exhibited a reduced forskolin-stimulated elevation in short circuit current (Isc). TGF- β 1 exposure also decreased Isc induced by a cell permeable cAMP analog. The effect of DASU-02, a selective CFTR inhibitor, on forskolin-stimulated Isc was reduced by TGF- β 1. Together these results suggest that TGF- β 1 impairs cAMP-mediated anion secretion across vas deferens epithelia by affecting a target downstream from cAMP generation. RT-PCR revealed that TGF- β 1 reduced the abundance of mRNA coding for the cystic fibrosis transmembrane conductance regulator (CFTR). Biotinylation and Western blot analysis revealed that TGF- β 1 reduced both total CFTR and apical cell surface CFTR expression. Attenuation of the forskolin response by TGF- β 1 was fully abrogated in the presence of TGF- β 1 receptor inhibitor SB431542. Further, SKF86002, an inhibitor of p38 MAPK, reduced the effect of TGF- β 1 on forskolin-stimulated anion secretion as well as CFTR mRNA abundance, while anisomycin, an activator of p38 MAPK, mimicked the effect of TGF- β 1 on the forskolin response. These data derived from cultured porcine vas deferens epithelial cells, indicate that TGF- β 1 binds to its cognate receptor to activate the p38 MAPK pathway and down-regulate CFTR expression, ultimately attenuating anion secretion, which is expected to comprise male fertility. Similar effects on CFTR expression may occur in other epithelial tissues. [NIH HD058398 and RR017686.]

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PDZ-BINDING MOTIF MUTATION IN SLC26A9 ENHANCES ITS PLASMA MEMBRANE ABUNDANCE AND FUNCTION

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CFTR is known to interact with several members of the solute carrier 26 (SLC26A) family of anion channels. Previously, we demonstrated that SLC26A9, which is expressed in lung and gastrointestinal epithelial cells, exhibits chloride channel-like properties. Co-expression of SLC26A9 and wtCFTR in HEK293 cells produced both constitutive currents and enhanced currents stimulated by cAMP/PKA, suggestive of a coordinated regulation between these channels. A physical interaction was also shown using co-immunoprecipitation assays (*J Gen Physiol.* 2009 Apr;133 (4):421-38). Both CFTR and SLC26A9 contain Class 1 PDZ motifs, which have been proposed to facilitate their interaction by tethering these proteins in close proximity via interactions with PDZ domain proteins. CFTR is known to be regulated by two PDZ domain proteins: CFTR-associated ligand (CAL) and Na⁺/H⁺ Exchanger Regulatory Factor (NHERF). The half-life and activity of CFTR in the plasma membrane was observed to be reduced by CAL mediated lysosomal degradation, and enhanced by its interaction with NHERF at the plasma membrane (*J Biol Chem* 2002 Feb 1; 277(5):3520-9). In the present study, we examined the role of PDZ interactions on SLC26A9 expression and function, as well as its interaction with CFTR. In order to examine the role of the PDZ binding motif in SLC26A9, the essential threonine at residue 789 was replaced with alanine by site directed mutagenesis. Using a biotinylation assay, HEK cells expressing SLC26A9 T789A alone showed a significant increase in the expression of the channel in the plasma membrane, compared with wt SLC26A9 expressing HEK cells. Co-expression of SLC26A9 T789A with wtCFTR showed a similar increase in plasma membrane expression of SLC26A9 T789A compared to cells co-expressing wtCFTR with SLC26A9; however, the expression of CFTR at the plasma membrane was not significantly altered. The enhanced plasma membrane abundance of SLC26A9 T789A co-expressed with wtCFTR correlated with a significant increase in the constitutive current, using whole cell patch clamp recordings (-27 ± 3 pA/pF, n=6, SLC26A9 T789A, vs. -17 ± 3 , n = 8, SLC26A9, p<0.05), while the response to cAMP/PKA stimulation was unaffected (-189 ± 61 pA/pF, CFTR+SLC26A9 T789A, vs. -236 ± 32 pA/pF, CFTR+SLC26A9), consistent with the biotinylation results. Finally, co-immunoprecipitation assays revealed an interaction between SLC26A9 and CAL. We propose that the enhanced cell surface abundance and function of SLC26A9 T789A is due to the disruption of its interaction with the PDZ domain protein CAL, which otherwise leads to its lysosomal degradation. In addition, maintenance of the enhanced response to cAMP/PKA stimulation suggests that, with its sufficiently high level of plasma membrane expression, SLC26A9 T789A maintains its functional interaction with wtCFTR in the absence of PDZ domain protein interactions, as has been previously shown for other SLC26A family members (*Nature Cell Bio* 2004 6(4):343).

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DIRECT BINDING OF CFTR MODULATORS TO HUMAN CFTR NBD1

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Understanding the mode of action of CFTR modulator compounds is key for optimization of CF therapeutics as well as obtaining insights into the molecular mechanisms of CFTR function and malfunction. We have utilized NMR and differential scanning calorimetry to probe the potential direct binding of a number of CFTR modulator compounds to the isolated human CFTR NBD1 (ARIARE), with both the otherwise wild-type and the F508del mutation. We have previously demonstrated direct binding of CFFT-001, a dual acting corrector-potentiator, to NBD1. Chemical shift changes observed in NMR spectra provide evidence of CFFT-001 binding to the surface of β -strands S3, S9 and S10 of NBD1 and displacing the transiently engaged C-terminal helices H8 and H9, leading to loss of their helicity. Binding to CFFT-001 also leads to a negative T_m shift or decrease in melting temperature for NBD1, consistent with binding to the NBD1 core and disruption of the C-terminal helices. Recent sequence analysis indicated that the C-terminal strands of NBD1, S9 and S10, are conserved in a CFTR-specific manner. Taken together with our previous work on phosphoregulation of NBD1 interactions with the R region, these results suggest that the S3, S9, S10 surface may be responsible for critical regulatory interactions with helices H8 and H9 and the R region and support this locus as a binding site for a CFTR-specific modulator compound. We have used this experimental data to now generate a docking model for CFFT-001, requiring enhanced sampling REMD simulations to open up the binding site. These simulations are consistent with binding to an excited state of NBD1. A model was proposed for CFFT-001 binding in which reduction of H8 and H9 interactions facilitated removal of the R region from the NBD1 surface including the heterodimer interface, enhancing NBD1:NBD2 interactions and thus the efficiency of gating and folding. Analysis of chemical shifts in NMR spectra upon binding of CFFT-001 now reveal long-range allosteric effects, potentially also contributing to the effect of the CFTR modulator. Results of ongoing studies to probe the similarities and differences in effects of direct binding of other CFTR modulators, including C18, will be reported. This work was supported by the CF Foundation Therapeutics.

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CO-EXPRESSION OF CFTR AND SLC26A9 INCREASES THE SINGLE CHANNEL CONDUCTANCE OF CFTR

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Several members of the SLC26A family of anion transporters have been found to associate with CFTR and participate in a coordinated regulation of both CFTR function and SLC26A activity. Since the different SLC26A family members show significant diversity in function, substrate, and tissue specificity, their coordinated regulation by CFTR may confer the unique anion permeability characteristics of the different organs that are affected by CF. We previously characterized the activity of SLC26A9, and its coordinated regulation by wt CFTR (J Gen Physiol 133:421 2009), using the whole cell patch clamp method in HEK293 cells and human bronchial epithelia. In these studies, SLC26A9 interacted with wt CFTR to enhance both constitutive and cAMP/PKA-stimulated chloride secretion. SLC26A9 expressed alone or with wtCFTR resulted in a constitutive current of -22 ± 6 pA/pF (n=9). During cAMP/PKA activation, cells expressing SLC26A9 plus wtCFTR exhibited a peak current of -311 ± 39 pA/pF (n = 11), compared to

-214 ± 38 pA/pF (n=21) for cells expressing wt CFTR alone. In the present study, we utilized excised, inside-out patch clamp methods to determine whether SLC26A9-CFTR interactions modulate the single channel properties of CFTR. In transiently transfected CHO cells expressing wt CFTR alone, single channel deflections with a conductance of 9 ± 1.02 pS (n=25) were observed, consistent with previous studies of wtCFTR. In cells co-expressing SLC26A9 with wtCFTR, the observed single channel conductance was primarily 12 ± 1.1 pS (n=23) following activation of CFTR by PKA + ATP (2mM). No evidence for two populations of channel events was observed, suggesting that this conductance increase was due to CFTR itself. In order to confirm this, a construct consisting of the STAS domain and PDZ motif of SLC26A9 was expressed, and whole cell patch clamp confirmed that this STAS domain construct did not result in a constitutive current. When this STAS domain construct was co-expressed with wt CFTR, the observed single channel conductance upon PKA + ATP activation increased to 11.5 ± 1.1 pS (n=10). These findings suggest that the STAS domain of SLC26A9, in association with CFTR, increased its single channel conductance, as did expression of the full-length SLC26A9. Interestingly, the higher conductance state of CFTR observed in the presence of SLC26A9 showed reduced sensitivity to CF1172 inhibition, from $90 \pm 5\%$ (n=4) to $72 \pm 7\%$ (n=11; p<0.01). A similar reduction in CF1172 inhibition has been observed in whole cell patch experiments. Under the conditions utilized in these experiments, single channel deflections uniquely associated with SLC26A9 were not observed. At this time, it is not clear whether the single channel conductance of SLC26A9 is significantly less than CFTR, or whether additional co-factors required for SLC26A9 function are lost with patch excision. We conclude that the interaction of the STAS domain of SLC26A9 with wt CFTR increases the single channel conductance of CFTR. This increased conductance may account for the increase in the cAMP/PKA-stimulated chloride secretion observed in whole cell experiments. [Supported by CFF R883-CR02 and BERTRA12G0, and NIH P30 DK072506.]

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14-3-3 PROTEINS AS NOVEL REGULATORS OF CFTR BIOGENESIS

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Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. The major impact of F508del, the most common CFTR variant, is the inability of the protein to fold properly and thus being intracellularly retained in the endoplasmic reticulum (ER). Indeed, the ER quality control (ERQC) recognizes incorrect folding of F508del-CFTR and sends it to early degradation, thus preventing it from trafficking to the cellular membrane. CFTR biogenesis involves protein interactions that assist in CFTR folding, but can also initiate its degradation when the folded state is not achieved. Prior studies have implicated 14-3-3 proteins in the forward trafficking or cell surface stability of ion channels and receptors through interactions with phosphorylated motifs or by shielding ER retention motifs (RXR) as in prior K channel studies. CFTR contains RXR and kinase phosphorylation sites that serve as candidates for 14-3-3 protein interactions. We previously have shown high expression of 14-3-3 β , γ and ϵ isoforms in airway cells, and further, that physical and functional interactions between 14-3-3 and CFTR (both bands B and C) involve the N-terminus and R domain as shown in pairwise binding studies, the latter increased by R region phosphorylation¹. Additionally our data indicate that 14-3-3 binding and CFTR expression are enhanced by PKA stimulation. We therefore are currently examining the 14-3-3/CFTR interactions in phospho-sites and RXR motif mutants. Preliminary results show that the impact of 14-3-3 β overexpression is lost when it is co-expressed with phospho-site mutants of CFTR. Conversely RXR mutants remain sensitive to the action of 14-3-3 β to increase CFTR biogenesis. These data suggest that the major effect of 14-3-3 proteins to increase CFTR expression stems from their interaction with phospho-sites in the R region rather than RXR sites. We are at this time looking at individual interaction sites to determine what role 14-3-3 proteins play in CFTR biogenesis and potentially in its stability at the plasma membrane. Understanding the role of 14-3-3 proteins in the processing of WT and F508del-CFTR should identify novel targets for therapeutic modulation of CFTR biogenesis.

¹Liang X., et al (2012) *Mol Biol Cell*. 23(6): 996-1009.

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LONG-RANGE ALLOSTERIC COUPLING WITHIN CFTR NBD1 REVEALED BY ICL4 PEPTIDE BINDINGDawson, J.E.; Forman-Kay, J., *Hospital for Sick Children, Toronto, ON, Canada*

Cystic fibrosis is caused by mutations in the chloride channel CFTR, leading to loss of function and changes in the ion and fluid flow across epithelial surfaces. Like other members of the ABC transporter family, CFTR contains two membrane spanning domains (MSDs) and two cytoplasmic nucleotide binding domains (NBDs). The formation of an NBD1/NBD2 heterodimer drives channel opening. The coupling helices at the base of the intracellular domains (ICLs) couple the NBDs to the MSDs of the channel. How are changes on the heterodimer interface transmitted across NBD1 to ICLs? The sensitivity of NMR spectroscopy reveals how the ICL4 binding site of NBD1 is allosterically linked to its heterodimer interface. Titration of an ICL4 "coupling helix" peptide led to chemical shift changes at the predicted binding site near the alpha-subdomain of NBD1, as well as in residues of the C-terminal NBD1 helices 8 and 9 (H8/H9), consistent with their destabilization and release from the NBD1 core via an allosteric mechanism. In addition, correlated chemical shifts were observed for residues near the RI-deletion site. We hypothesize that perturbations in one of these correlated regions should cause a reciprocal change in another region. F508del, a CF-causing mutation in the alpha-subdomain, reduces the effects of ICL4 binding on H8/H9. In contrast, F508del-suppressor mutations, F494N and V510D, increase these effects. Helix 8 mutation, Q637R (also a F508del-suppressor), increases the binding effects in the ICL4 binding site. Q637R also alters the dynamics in this region, suggesting that the internal motions of NBD1 are involved in transmitting changes across this plastic domain. The destabilization and release of H8/H9 from the heterodimer interface is strikingly similar to that of the regulatory extension (RE) and R region, which follow helix 9 and become more disordered and less bound to NBD1 upon phosphorylation. The RE and R region regulate NBD dimerization and, ultimately, channel opening and closing. The allosteric effects provide insight into how changes in the dimerization interface may be communicated to the rest of CFTR. This work was supported by the CF Foundation.

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SYNTAXIN 4 MEDIATES THE REGULATED TRAFFICKING OF CFTR AT THE APICAL MEMBRANE OF CALU-3 AIRWAY CELLSPeters, K.W.; Bertrand, C.A.; Liang, X.; Frizzell, R.A. *Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*

The cystic fibrosis transmembrane conductance regulator (CFTR) must reside in the apical plasma membrane to perform its primary function as a chloride channel that mediates cAMP-dependent salt and water secretion in epithelial cells. The significance of CFTR's involvement in fluid secretion is evidenced by the most common mutation, F508del, which produces CF disease because the misfolded protein is unable to leave the endoplasmic reticulum. Airway disease is the major cause of mortality and morbidity in CF, so we chose to investigate apical membrane CFTR trafficking in Calu-3 cells, an airway serous cell model that expresses endogenous CFTR. Data from several laboratories have suggested that not all CFTR goes directly into the membrane from the protein secretory pathway but is retained in a sub-apical vesicle population that can be mobilized to the membrane upon stimulation. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are known to be responsible for donor/acceptor membrane trafficking and compartment specificity through interactions of vesicle- and target-SNAREs. We examined the expression of the t-SNAREs, syntaxins 1-4, in Calu-3 cells and identified the expression of syntaxins 2 and 4. In primary human bronchial epithelial (HBE) cultures, we detected syntaxins 3 and 4. To our surprise, in Calu-3 and HBE cells, we did not detect syntaxin 1A, either by PCR analysis of RNA or by immunoblot for protein analysis, although this syntaxin has been implicated as a negative regulator of CFTR trafficking and activity. Syntaxin 4 and CFTR interacted as evidenced by co-immunoprecipitation experiments and both co-localized in the apical membrane by indirect immunofluorescence. When RNA from both proteins was co-injected into *Xenopus* oocytes, CFTR Cl⁻ currents were doubled in contrast to oocytes expressing CFTR alone. Biotinylation

experiments showed that cells subjected to cAMP stimulation by forskolin and 3-isobutyl-1-methylxanthine (IBMX) exhibited greater quantities of CFTR in the plasma membrane. Using adenovirus-mediated transduction of Calu-3 cells together with functional assays measuring 6-methoxy-N-(3-sulfoethyl) quinolinium (SPQ) fluorescence, we demonstrated that the knockdown of syntaxin 4 expression reduced forskolin-stimulated anion efflux compared with cells expressing EGFP alone. These data suggest that syntaxin 4 mediates the insertion of CFTR into the apical membranes of Calu-3 cells to control its apical density and the magnitude of stimulated anion efflux. [Supported by NIH DK068196, DK072506 and the CF Foundation.]

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TARGETED MOLECULAR DYNAMICS SIMULATIONS OF THE CFTR GATING CYCLERahman, K.¹; Cui, G.²; McCarty, N.A.²; Harvey, S.C.¹ *1. Petit Institute of Bioengineering and School of Biology, Georgia Institute of Technology, Atlanta, GA, USA; 2. Emory+Children's Center for CF Research and Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA*

Advances in CF therapeutic research have been hampered by the lack of information about the conformational changes that CFTR undergoes during its gating cycle. Here, we present a new all-atom structural model of the ATP-free conformation of CFTR that is based on homology modeling using the experimentally-determined structure of P-glycoprotein, a closely related mammalian ABC transporter, as a template. We believe that this inward-facing model, which is supported by experimental data, corresponds to the closed state of the CFTR channel, and is notably different from prior homology models of nucleotide-bound CFTR that have been proposed to represent the putative open state conformation. In addition, we performed targeted molecular dynamics (TMD) simulations to explore the conformational transition pathway between these putative end states. Our simulations reveal that the gating transition is initiated by conformational changes in the nucleotide-binding domains (NBDs) of CFTR, consistent with the notion that ATP binding regulates the CFTR gating cycle. Analysis of the TMD trajectory also identifies important translation and rotation motions of helices in the transmembrane domain that alter the pore structure of the CFTR molecule as it transitions between the open and closed states. (Support: NIH-DK056481 to N.A.M.)

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MODELING THE TRANSLOCATION MECHANISM OF THE ABC TRANSPORTER P-GLYCOPROTEIN USING TARGETED MOLECULAR DYNAMICSSpeir, E.¹; Rahman, K.¹; McCarty, N.A.²; Harvey, S.C.¹ *1. School of Biology and Petit Institute of Bioengineering, Georgia Institute of Technology, Atlanta, GA, USA; 2. Emory+Children's Center for CF Research and Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA*

P-Glycoprotein (P-gp) (MDR1, ABCB1) is a transmembrane protein, clinically important due to its role as a multidrug resistance efflux transporter. Like CFTR, it is a full transporter belonging to the ATP-binding cassette (ABC) superfamily of proteins. As with most other ABC transporters, P-gp is hypothesized to operate under an alternating access mechanism, transitioning between inward- and outward-facing states during its transport cycle. Molecular models of the outward-facing conformation of human P-gp have previously been constructed by homology modeling using the nucleotide-bound structure of a related bacterial ABC transporter, Sav1866, as a template. The subsequent release of an inward-facing, nucleotide-free crystal structure of murine P-gp has made it possible to investigate the conformational transitions that accompany the transport cycle of this protein. In this study, we performed targeted molecular dynamics (TMD) simulations between the inward- and outward-facing states to model the transport cycle of human P-gp as it exists in the membrane. Our simulations provide insights into the order and relative importance of conformational changes in various structural domains within the protein, and analysis of changes in the pore structure during the gating cycle provides evidence for the alternating

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access hypothesis of transport in P-gp. Furthermore, pore analysis also indicates a possible pathway through which the translocation cycle of a typical ABC transporter may be modified to lock the pore open at both ends, thereby enabling the channel activity seen uniquely in CFTR. (Support: NIH-DK056481 to N.A.M.)

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CYTOPLASMIC EXTENSIONS OF CFTR MEMBRANE-SPANNING HELICES STRONGLY INFLUENCE SINGLE-CHANNEL PROPERTIES

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Although a channel gate has yet to be defined, a great deal has been learned about the anion permeation path across the CFTR channel, with most focus on residues in trans-membrane helices (TMs) and putative charged vestibules near their external and internal ends (Norimatsu et al, *Biochemistry*, 51(11):2199-212, 2012). Less is known about the role in CFTR function of the long cytoplasmic extensions (TMEs) of the TMs that are characteristic of the exporter family of ABC proteins, but Wang et al. (*Proc Nat Acad Sci* 107(8), 3888-93, 2010) have shown that specific substitutions of residues in two of these TMEs have profound effects on channel activity, increasing P_o and changing ATP dependence. We have studied six mutations in four different TMEs that had somewhat similar effects on gating accompanied by substantially decreased single channel conductance relative to the wild-type. For some of these variants, the unitary conductances were reduced by as much as 40%. Despite their diminished magnitudes, the temperature and ion concentration dependencies of the reduced single channel conductances of these variants were not different from those of wild-type CFTR. Therefore, the channel pore or rate-limiting step in the permeation pathway was not altered by these substitutions. Consistent with this conclusion, preliminary calculations of pore diameter in structural models of the wild-type and mutant CFTRs revealed that the size of the pore did not significantly differ between the wild type and variant structures. However, although the static structure was unaffected, protein dynamics may vary in the mutants as compared to the wild type, which could significantly change channel behavior. Thus, we postulate two possible mechanisms of the strong impact of TME changes on channel conductance: either that the residues changed may normally contribute to a cytoplasmic vestibule where ions are concentrated proximal to the pore or, alternatively, that they may be dynamically coupled to residues within the pore. To distinguish between these possibilities, we are performing further single channel and biochemical measurements, as well as Molecular Dynamics simulations to detect structural fluctuations and dynamic coupling between distant regions of the channel. Supported by the NIH and CFF.

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THE IMPORTANCE OF THE NBD DOMAINS IN THE SELECTION OF DETERGENTS FOR CFTR PURIFICATION

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Generation of sufficient amount of purified, homogeneous full-length CFTR represents a major barrier to the comprehensive studies of its structure and function. The choice of detergents for the initial solubilization and subsequent purification is critical to the success of this goal. The main focus of the published research efforts have been on the interactions between detergents and the transmembrane domains. However, in order to achieve functional purification of integral membrane proteins, maintaining the stability and function of the extramembranous domains is as important as the transmembrane domains. We therefore studied the interactions between detergents and the first nucleotide-binding domain of human CFTR (hNBD1), an extramembranous domain. We selected members from the

three different classes of detergents, non-ionic, zwitterionic and anionic, that were commonly used in membrane protein purification. For example, lysophosphatidylglycerol (LPG) and perfluorooctanoate (PFO) were anionic detergents that were widely used in CFTR extraction and purification. Differential scanning calorimetry (DSC) and circular dichroism (CD) were used to study the effects of the detergents on the thermal stability and secondary structures of isolated hNBD1. The results show that all detergents destabilized hNBD1, causing decreases in both unfolding temperature and enthalpy, albeit to different degrees. Anionic detergents were the stronger denaturants, causing complete denaturation of hNBD1 at modest concentrations; while non-ionic detergents only destabilized it to some extent, without drastic effects on its secondary or tertiary structures. Zwitterionic detergents were harsher than non-ionic detergents, but complete denaturation only occurred at very high concentrations. These effects were similar to the effects of detergents on egg white lysozyme, a well characterized soluble protein. The reversibility of the denaturing effects and the feasibility of using mixed micelle systems will be discussed. In summary, the studies reported here represent the first systematic studies of interactions between detergents and the extramembranous domain of an integral membrane protein. Similar studies can be carried out on other membrane proteins to assist the detergent optimization. The results suggested that the usage of anionic detergents in membrane protein purification is limited due to their denaturing effects on the soluble domains.

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ASSOCIATION OF CFTR WITH ENAC SUBUNITS CARRYING LIDDLE'S SYNDROME MUTATIONS

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The proposed role of CFTR-ENaC association in the pathophysiology of cystic fibrosis (CF) is controversial. In a recent study, we demonstrated a close physical association of WT-CFTR and WT-ENaC. We have also shown that the F508del CFTR fails to associate with ENaC unless the mutant protein is rescued pharmacologically or by low temperature. In the present experiments, we investigated physical association of WT-CFTR with ENaC subunits carrying Liddle's syndrome mutations. We show that all three ENaC subunits truncated at the C-termini could be co-immunoprecipitated with WT-CFTR. The biochemical studies were complemented by fluorescence resonance energy transfer (FRET) measurements. FRET is a distance-dependent approach that monitors protein-protein interactions between fluorescently labeled molecules. HEK cells were co-transfected with vectors encoding CFTR N-terminally tagged with ECFP (ECFP-CFTR) and $\alpha\beta\gamma$ -ENaCs. One subunit in $\alpha\beta\gamma$ -ENaC was truncated at the C-terminus and N-terminally tagged with EYFP (termed EYFP- α - Δ C- $\beta\gamma$ -ENaC, EYFP- β - Δ C- $\alpha\gamma$ -ENaC, or EYFP- γ - Δ C- $\alpha\beta$ -ENaC). Our measurements revealed significantly increased FRET between CFTR and all ENaC combinations compared to controls (ECFP and EYFP co-transfected cells), and suggest that CFTR can influence ENaC activity by means of a direct intermolecular interaction even in the absence of its carboxy terminus.

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RESCUE OF CFTR MUTANTS BY SMALL MOLECULE CORRECTORS

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Correction of the processing of mutant CFTR by small molecules is a major goal in the development of new therapies for cystic fibrosis. Recently the corrector VX-809 shows some promise in recent clinical trials in patients carrying the Δ F508 mutation. The aim was to study how correctors function on wild-type and various mutant forms of CFTR with the goal of increasing our understanding of their mechanism of action. The mutants studied included Δ F508, A455E, Δ 264 and Δ 27-264 CFTR (missing the first

four transmembrane segments of CFTR) and TNR CFTR (missing TMD2 and NBD2).

Correctors C3, C4, C18 and CFFT 108548 and 108756 all increased the steady state expression of the C band of wild-type CFTR. As we expected, all of these correctors also increased the maturation of $\Delta F508$ CFTR from B to C band to varying degrees.

Correctors C3 and C4 both increase the processing of band B to C band of A455E CFTR similar to what we found for $\Delta F508$ CFTR. Interestingly, correctors C3, C4, and CFFT 108548 and 108756 all increased the steady state expression of $\Delta 27$ -264 CFTR whereas C18 had little to no effect suggesting that C18 is more specific for $\Delta F508$ CFTR.

TNR CFTR resides in the ER but is stable and not rapidly degraded. Interestingly TNR protein expression is not affected either by C3 or C4.

Previously, we showed that $\Delta 264$ CFTR enhanced the maturation of $\Delta F508$ from B to C band by transcomplementation. In cells cotransfected with $\Delta F508$ CFTR and $\Delta 264$ CFTR and treated with increasing doses of either C3 or C4, there was a dramatic increase in the mature C band of $\Delta F508$ CFTR compared to the cells cotransfected with $\Delta F508$ and $\Delta 264$ CFTR in the absence of correctors or cells transfected with $\Delta F508$ CFTR alone. This indicates a synergy between C3 and C4 and transcomplementation. In sharp contrast, C18 did not show any enhancement of transcomplementation.

Conclusion: Rescue of $\Delta F508$ CFTR occurs when cells are treated with the small molecule correctors or when cotransfected with $\Delta 264$ or $\Delta 27$ -264CFTR. Taken together our data shows that C3 and C4 most likely rescue mutant CFTR by interfering with several pathways and are strongly synergistic with transcomplementation by $\Delta 264$ or $\Delta 27$ -264 CFTR. C18 on the other hand is more specific for $\Delta F508$ CFTR and may function by correcting $\Delta F508$ CFTR more directly. The absence of synergy with transcomplementation suggests that C18 may be correcting a similar defect. Funded by CFFT and NHLBI.

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POST-TRANSLATIONAL MODIFICATIONS OF CFTR GLYCOFORMS IDENTIFIED BY HIGH RESOLUTION, HIGH MASS ACCURACY MASS SPECTROMETRY

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Post-translational modifications (PTMs) such as phosphorylation, ubiquitination, and sumoylation are important regulators of CFTR trafficking and channel gating. Mass spectrometry is a powerful tool for identifying the precise locations of such PTMs; however, difficulties purifying large quantities of CFTR have limited previous studies of this type. We are pursuing recombinant technologies and purification methods that recover amounts of CFTR suitable for 1) proteolytic digestion and global analysis of CFTR by MS/MS and 2) monitoring specific locations of predicted PTMs by MRM-MS. An initial approach using a qQTrap mass spectrometer led to confirmation of seven previously described sites of CFTR phosphorylation and four novel positions for this PTM. We have also shown that several CFTR residues are methylated, provided the first description of CFTR palmitoylation, and identified a single (putative) site of ubiquitination. Recent improvements in CFTR purification have increased yields more than 10-fold, and together with a new high resolution, high mass accuracy qQTOF mass spectrometer, we have been able to further refine the analysis. One striking result has been identification of over twenty sites of CFTR ubiquitination (a modification known to govern CFTR degradation from both the ER and plasma membrane compartments). Precise locations of this PTM have been established by MS/MS. In addition, the improved methods allow investigation of PTMs specific to immature (band B) versus mature (band C)

CFTR glycoforms as well as wild-type versus F508del CFTR. Our findings also suggest the possibility of previously unappreciated molecular “switches” in which two PTMs in close proximity may regulate CFTR gating. For example, four well characterized phosphorylation sites (S686, S700, S712, and S795) are each separated from positions of ubiquitination by a single amino acid, a finding that may help explain the well described refractoriness of F508del CFTR (which is heavily ubiquitinated) to kinase-dependent activation. In other membrane proteins, switch mechanisms such as these control aspects of ion channel function, protein biogenesis, exit from the endoplasmic reticulum, and insertion in the plasma membrane. Further studies regarding interplay of PTMs are therefore expected to provide valuable information regarding wild-type and F508del CFTR biogenesis and gating.

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S-PALMITOYLATION MEDIATES TRAFFICKING OF CFTR TO THE CELL SURFACE

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S-Palmitoylation is a critical regulator of membrane protein localization, maturational processing and function of ion channels and ABC transporters. The post-translational modification (PTM) is characterized by attachment of a 16-carbon saturated fatty acid to cysteine sulfhydryl groups. We used both multiple reaction ion monitoring (MRM) and tandem (MS/MS) mass spectrometry to establish that full-length CFTR is palmitoylated in human cells and confirmed this observation by metabolic labeling with ³H-palmitic acid. We also established that pharmacologic inhibition of palmitoylation with 2-bromopalmitate (2-BP): 1) results in decreased steady state levels of both mature and immature CFTR, 2) abrogates maturation of CFTR band B → C (pulse-chase analysis), and 3) decreases CFTR activity in the plasma membrane. The F508del CFTR mutant is also covalently labeled by ³H-palmitic acid. Although 2-BP causes no obvious effect on F508del CFTR steady state expression or activity at 37°C, it significantly decreases low temperature correction of the mutant protein. MS/MS and MRM-MS have so far localized palmitoylation to CFTR residues 524 and 1395. Alanine replacement of the cysteine at position 1395 has no effect on wild-type CFTR steady state expression, maturational efficiency, or cell surface activity. In contrast, C524A (within NBD1) led to diminished expression of fully glycosylated band C, blocked maturation of band B → C, and reduced CFTR function in living cells. Detailed studies of other cysteine residues (with predicted palmitoylation consensus motifs and at positions in proximity to plasma membrane) are currently in progress. In addition, palmitoyl acyl transferases (PATs) comprise a family of 24 gene products that contain a DHHC motif and mediate palmitoylation of diverse cellular proteins. Recombinant expression of PATs in epithelial cells led to robust effects on CFTR expression and palmitoylation as judged by Western blot and metabolic labeling. Co-immunoprecipitation established a direct interaction between several PATs and either wild-type or F508del CFTR. Together, these findings indicate that palmitoylation plays an important role during trafficking of CFTR from the endoplasmic reticulum to the cell surface. Continued studies of this pathway may reveal novel therapeutic strategies for overcoming the processing defect attributable to F508del CFTR.

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DIFFERENTIAL PDZ INTERACTIONS ACCOUNT FOR INCREASED CELL SURFACE MOBILITY OF RESCUED Δ F508CFTR COMPARED TO WILDTYPE CFTR

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Deletion of phenylalanine 508 (Δ F508) in the cystic fibrosis transmembrane conductance regulator (CFTR) is the most common mutation causing cystic fibrosis (CF). Δ F508CFTR is retained at the endoplasmic reticulum; however, several approaches promote trafficking of partially functional Δ F508CFTR to the cell surface. Rescued (r) Δ F508CFTR is conformationally and metabolically unstable and subject to ubiquitination, rapid endocytosis and lysosomal degradation. To test the role of scaffold interactions in r Δ F508CFTR stability, quantum-dot single particle tracking was used to probe cell surface CFTR interactions in live cells. In several transfected cell types, immediately after low-temperature rescue, the diffusion of r Δ F508CFTR was identical to that of wild type (wt) CFTR. However, thermal destabilization of r Δ F508CFTR by incubation at 37 °C, which causes ubiquitination and enhanced endocytosis, increased r Δ F508CFTR diffusion. Similar increases in Δ F508CFTR diffusion compared to wtCFTR were found when Δ F508CFTR biosynthetic processing was corrected by small molecules (VX-809, CoPo-22), activation of alternative trafficking pathways (GRASP overexpression) or thapsigargin treatment. Perturbation of PDZ domain-interactions that tether CFTR to the actin cytoskeleton by overexpression of the CFTR carboxy-terminus (fused to mCherry) resulted in comparable increased diffusion of wild type and r Δ F508CFTR. These results suggest that PDZ domain-containing scaffold proteins interact with r Δ F508CFTR at the cell surface, and contribute to channel confinement, albeit to a lesser degree than for the wtCFTR. Modulation of the expression or function of CHIP, a peripheral protein quality control protein, by siRNA knockdown or overexpression of a dominant negative CHIP mutant, reduced r Δ F508CFTR diffusion, implicating the involvement of CHIP in modulating r Δ F508CFTR-scaffold interactions. To quantitatively relate r Δ F508CFTR mobility to endocytosis, a cell line overexpressing the CFTR carboxyl terminus (fused to mCherry) was generated in which wtCFTR mobility was increased to that of r Δ F508CFTR levels. In this cell type, CFTR endocytosis was increased by ~50%. Our data indicate that: (i) wtCFTR and r Δ F508CFTR have different cell surface mobility, which is related to r Δ F508CFTR-PDZ scaffold interactions, (ii) the peripheral quality control protein CHIP is responsible for altered mutant channel mobility, and (iii) at least two distinct mechanisms contribute to enhanced endocytosis of r Δ F508CFTR compared to wtCFTR.

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ORIGIN AND EVOLUTION OF THE CFTR REGULATORY R-DOMAIN

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The cystic fibrosis transmembrane conductance regulator protein (CFTR) is an ion channel, which is a member of the ABC transporter superfamily. CFTR is distinguished from all other related ABC transporters by the presence of an unstructured domain in the middle of the protein's primary sequence known as the regulatory or R-domain. The origin of the CFTR R-domain has been a mystery until this time; it does not encode any known structural elements or folds and does not contain sequence similarity to any known domain. Comparison of the gene sequence of CFTR to its most closely related ABC transporter, ABCC4, reveals a highly conserved exon-intron structure and shows that the R-domain is encoded by an extension of exon 14 in CFTR. The origin of the R-domain can be attributed to the loss of a splice donor site in the CFTR evolutionary lineage after its divergence from ABCC4 via an insertion/deletion event and the subsequent capture of previously intronic sequence as protein coding sequence in CFTR. Evaluation of the phyletic distribution of the R-domain shows that this event

occurred near the base of the vertebrate lineage approximately 550-600 million years ago. The R-domain has evolved under purifying selection since that time, consistent with its functional relevance to the CFTR protein, albeit at lower levels of constraint than the other CFTR domains. The site-specific pattern of the R-domain is also distinct from the patterns seen for the other CFTR domains; there are a few highly conserved sites, primarily serine residues that form consensus sequences for PKA- or PKC-dependent phosphorylation, whereas the remaining sites have evolved under weak selective constraint. Thus, the consensus phosphorylation positions in the R-domain appear to have emerged very early in its evolution and have remained unchanged since that time. Origination of protein coding sequence from non-coding (intronic) sequence is thought to be a rare event in evolution, partly due to the low likelihood of non-coding sequence encoding structural motifs or folds. The R-domain remains unstructured, consistent with its origin from non-coding sequence, but seems to have been able to emerge and remain as protein coding sequence via the contribution of substrates for phosphorylation, which in turn may have played an important role in the emergence of the ion channel function of the protein by facilitating a sustained open state. (Current address for KAB: Univ. Alabama, Birmingham.) (Support: NIH-DK056481.)

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CFTR IS REQUIRED FOR MAXIMAL TRANSEPITHELIAL LIQUID TRANSPORT IN PIG ALVEOLAR EPITHELIA

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A balance between alveolar liquid absorption and secretion is critical for maintaining optimal alveolar subphase liquid height and facilitating gas exchange in the alveolar space. However, the role of cystic fibrosis transmembrane conductance regulator protein (CFTR) in this homeostatic process has remained elusive. Using a newly developed porcine model of cystic fibrosis, in which CFTR is absent, we investigated ion transport properties and alveolar liquid transport in isolated type II alveolar epithelial cells (T2AECs) cultured at the air-liquid interface. CFTR was distributed exclusively to the apical surface of cultured T2AECs. Alveolar epithelia from CFTR^{-/-} pigs failed to increase liquid absorption in response to agents that increase cAMP, whereas cAMP-stimulated liquid absorption in CFTR^{+/+} epithelia was similar to that in CFTR^{+/+} epithelia. Expression of recombinant CFTR restored stimulated liquid absorption in CFTR^{-/-} T2AECs but had no effect on CFTR^{+/+} epithelia. In ex vivo studies of non-perfused lungs, stimulated liquid absorption was defective in CFTR^{-/-} alveolar epithelia but similar between CFTR^{+/+} and CFTR^{+/+} epithelia. When epithelia were studied at the air-liquid interface, elevating cAMP levels increased subphase liquid height in CFTR^{+/+} but not in CFTR^{-/-} T2AECs. Our findings demonstrate that CFTR is required for maximal liquid absorption under cAMP stimulation, but it is not the rate-limiting factor. Furthermore, our data define a role for CFTR in liquid secretion by T2AECs. These insights may help to develop new treatment strategies for pulmonary edema and respiratory distress syndrome, diseases in which lung liquid transport is disrupted.

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CFTR ACTIVATION BY THE SOLVENT ETHANOL: IMPLICATIONS FOR CF DRUG TESTING AND DELIVERY

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CFTR activating molecules identified by high throughput drug screening may exhibit limited solubility in water and are therefore dissolved in solvents such as DMSO or ethanol for experimental testing. During evaluation of novel compounds obtained in this fashion, we serendipitously discovered that agents solubilized in low concentrations of ethanol produce strong activation of wild type CFTR-mediated Cl⁻ transport in well characterized primary cell culture models of murine nasal septal (MNSE) and human sinonasal respiratory epithelium (HSNE). Dysfunctional mucociliary clearance (MCC) is a hallmark of many airway diseases, including cystic fibrosis (CF), chronic obstructive pulmonary disease, and chronic rhinosinusitis (CRS). An approach to enhance transepithelial Cl⁻ secretion with low concentrations of ethanol, a solvent already present in numerous aerosolized pharmaceuticals, is being pursued by our laboratory. The objectives of the current study were to investigate ethanol for effects on transepithelial Cl⁻ secretion and characterize the mechanism that underlies this form of CFTR modulation. Primary MNSE [wild type (wt) and transgenic CFTR^{-/-}] and HSNE cultures were subjected to transepithelial ion transport measurements using pharmacologic manipulation in Ussing chambers. CFTR R-domain phosphorylation and cAMP levels were examined as a test of PKA-dependent activity. Time-dependent and dose-dependent toxicity were also measured using LDH based protocols. A strong increase in CFTR-mediated Cl⁻ transport (change in short-circuit current, ΔISC) was demonstrated with activation stable at 0.5% ethanol [MNSE; 18.2 ± 2.5 vs. 0.0 (control); p<0.001 and HSNE; 4.6 ± 0.4 vs. 0.0 (control); p<0.001]. Total stimulation (ethanol+forskolin 100nM) was elevated in MNSE [32.7 ± 2.2 vs. 26.6 ± 3.8 (control); p=0.12] but significantly additive to forskolin in HSNE [20.7 ± 1.2 vs. 13.0 ± 0.5 (control); p<0.01]. Effects of ethanol were completely abrogated by INH-172 and CFTR dependence confirmed by lack of stimulation in transgenic CFTR^{-/-} MNSE. There was no increase in cellular cAMP attributable to ethanol at the concentrations studied. R-domain phosphorylation, as judged by a biochemical gel shift assay of recombinant R-domain, was also unchanged. Incubations up to 24 hours were performed using all concentrations tested and no cellular toxicity noted. The observation that ethanol stimulates transepithelial Cl⁻ secretion via CFTR indicates possible use as a topical aerosol delivered alone or in combination with other CFTR activators for treatment of dysfunctional MCC. The same intervention might be used to activate residual CFTR activity (e.g. in the setting of F508del-CFTR pharmacologic correction) and ameliorate underlying mucus obstruction in CF-associated sinusitis. Finally, it should be noted that CFTR activation by potentiators dissolved in ethanol may overestimate potency, and our results emphasize the need for stringent vehicle controls during experimental testing of CFTR modulators in vitro and in vivo.

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YFP AS A SENSOR FOR CHLORIDE CHANNEL ACTIVITY OF CFTR IN *PICHA PASTORIS*

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Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The protein acts as a selective channel for chloride ions. CFTR activity is regulated by cAMP dependent protein kinase A and requires ATP binding and hydrolysis to open and close the ion channel. Mutation affects chloride secretion of the epithelial cell lining of the lungs, pancreas and sweat glands. Still little is known about how mutations affect folding and function at the protein level. We have expressed CFTR in the yeast *Pichia pastoris* for large scale production of wild-type and mutant proteins. To assess their function we have explored the use of yellow fluorescent protein (YFP) as a molecular probe for chloride channel

activity. Halide binding to chloride sensitive YFP mutant H148Q/I153L causes protonation of the chromophore and thus quenching of the YFP fluorescence, which may serve to monitor CFTR's channel activity.

Our approach was to use purified YFP protein as a chloride sensor in membrane vesicles or purified, reconstituted CFTR proteoliposomes. For this vesicles expressing wild-type human CFTR a codon-optimized CFTR* variant are loaded with YFP protein and fluorescence changes monitored after exchange into iodide containing buffers. Quench occurs due to CFTR channel opening causing an influx of iodide that leads to quenching of the YFP fluorescence. Control experiments using an ATP hydrolysis deficient CFTR* mutant or a channel gating mutant confirmed the fluorescence quench is due to CFTR specific activity. This assay is invaluable for assessing purified CFTR proteins and may be developed into a high throughput screening tool for drugs that correct cystic fibrosis.

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REGULATED TRAFFICKING ROUTES OF CFTR FROM THE CELL SURFACE

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The regulated trafficking of membrane proteins to and from the cell surface allows cells to rapidly respond to physiological stimuli. Activation of CFTR by protein kinase A (PKA) phosphorylation increases its open probability and facilitates ion transport. Our aim was to investigate whether elevated PKA activity not only increased CFTR gating but also promoted trafficking from an intracellular pool to the cell surface in order to augment cellular conductance. In order to monitor the dynamic and rapid movements of CFTR, we used a fluorogen activating protein fused to the N-terminus via an extra transmembrane linker to selectively label CFTR at the plasma membrane (PM). In a prior study we used this approach to quantify the actions of known F508del CFTR correctors both alone and in combination to detect additivity and synergy. Here, we tracked the movement of CFTR from the cell surface to intracellular compartments and recycling back to the cell surface with live cell fluorescence microscopy. We observed internalization and accumulation from the PM into a coalesced perinuclear compartment at the microtubule organizing center (MTOC) which we confirmed by tubulin staining. This intracellular CFTR compartment colocalized closely with the endosomal recycling complex markers, Rab11 and EHD-1 and reached steady-state distribution around 25 minutes. F508del-CFTR which was rescued either by corrector treatment or low temperature incubation also internalized from the PM to a Rab11/EHD1 positive compartment. Little or no colocalization was observed for CFTR WT and the lysosomal marker, Lamp1, consistent with efficient recycling and slow turnover. Alternatively, thermal destabilization of temperature rescued F508del-CFTR resulted in increased targeting to lysosomes. Acute increases in PKA activity by forskolin stimulation depleted this intracellular pool of CFTR by 55%. Stimulation with forskolin was found to inhibit CFTR endocytosis and dramatically reduce intracellular accumulation. Furthermore, PKA activation induced the translocation of CFTR from an intracellular recycling compartment and promoted insertion into the plasma membrane which we observed using TIRF microscopy. These findings indicate that the itinerary of internalized surface CFTR leads into a regulated recycling pathway that contributes to augmented PM channel density in response to secretory stimuli. [Supported by NIH U54 RR022241, P30 DK072506, DK68196 and a grant from the Cystic Fibrosis Foundation, FRIZZE05XX0.]

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PROGRESS TOWARD ELUCIDATING THE 3D STRUCTURE OF FULL-LENGTH CFTR

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Elucidating the 3D structure of CFTR will enhance our understanding of mechanism of CFTR function, provide insight into the effects of mutations associated with clinical disease, and underpin structure-based discovery of small molecules for treating cystic fibrosis. Herein, we describe innovative

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strategies for and recent research results on the expression and solubilization of full-length CFTR. Full-length human CFTR was expressed in 293HEK cells exploiting a molecular strategy (SUMO*-CFTR.901FLAG-GFP*) that enabled controlled, high yield batch production of CFTR in mammalian cells while integrating an armamentarium of early assessment precrystallization assays. Our results demonstrated a robust doxycycline inducible expression system for 10 liter (30-50 billion cells) batch culture production of recombinant CFTR in 293HEK and CHO cells. By 24-36 hrs after induction, CFTR expression levels reached 3-5 pg/cell. Analysis by mass spectrometry demonstrated > 10% of the total recombinant CFTR was associated with the plasma membrane. Single channel analysis of microsomal membranes, indicated that CFTR was biologically active. Importantly, affinity purified CFTR exhibited ATPase activity. Using high-throughput self-interaction chromatography (HSC), data will be presented for elucidating solution conditions that improve the solubility of full-length CFTR protein and the NBD1 and NBD2 CFTR domains. Combining HSC with fluorescence size-exclusion chromatography, we will also present a two-step chromatographic protocol for rapid purification of milligram quantities of full-length, homogeneous human CFTR expressed via the mammalian expression system described above. Several solution conditions for improved solubility and physical stability of CFTR and NBD1/NBD2 domains will be presented. The solutions contain different combinations of detergents and/or lipids combined with solubilizing additives, assessed at different pH and ionic strength values. Characterization of purified protein includes solubility and homogeneity analysis using size-exclusion chromatography, Coomassie gels, Western blots, electron microscopy and dynamic laser light scattering.

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AIRWAYS PHYSIOLOGY & PATHOPHYSIOLOGY

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REDUCED CAV-1 EXPRESSION IN CF MACROPHAGES CHALLENGED WITH LPS LEADS TO A DERANGED HO-1 CELLULAR COMPARTMENTALIZATION, PROMOTING HYPER-INFLAMMATION

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We have reported that the TLR4 signaling regulation is compromised in CF macrophages (MΦs) and that the unregulated TLR4 signaling contributes to the robust production of pro-inflammatory cytokines in response to lipopolysaccharide (LPS) isolated from *P. aeruginosa* (PA) (Bruscia et al. JI, 2011). The heme oxygenase (HO-1)/carbon monoxide (CO) is a key cytoprotective cellular pathway that, once induced, modulates the redox status of the cells, the inflammatory response, and cell survival. The HO-1 enzyme also acts as a negative regulator of the TLR4 signaling in MΦs. HO-1/CO-dependent TLR4 negative regulation requires the expression of the scaffold protein caveolin 1 (Cav-1), which has binding motifs for both the HO-1 and the TLR4 TIR domain. The HO-1/Cav-1 complex binds TLR4 and favors the detachment of the MyD88 adaptor from the TLR4, thus terminating the signaling (Wang et al. JI, 2009).

Here, we demonstrate by western blot on cellular protein fractions and by immunofluorescence studies that in CF MΦs, HO-1 does not compartmentalize to the cell surface in response to LPS and most of the protein accumulates intracellularly. The total amount of the HO-1 protein in CF MΦs is slightly reduced compared to WT cells, perhaps due to its enhanced degradation triggered by the abnormal cellular localization. Because the HO-1 function depends on the presence of Cav-1, we assessed whether the expression of Cav-1 in CF MΦs is altered. CF MΦs have decreased Cav-1

expression after 4h and 6h following LPS stimulation ($p < 0.001$) and half as much protein after 15 hours of LPS challenge, as compared to WT cells. Studies on Cav-1 KO MΦs demonstrate that Cav-1 expression is required for orchestrating HO-1 cell surface distribution in MΦs. Thus, during LPS challenge the HO-1/Cav-1 axis is impaired in CF MΦs.

Over-expressing HO-1 with adenoviral vector or by delivering CO with the CO releasing molecule (CORM2) enhances not only HO-1 expression but also Cav-1 protein expression by two-fold in CF MΦs, suggesting that HO-1, via CO production, is an inducer of Cav-1 expression. In addition, these manipulations reestablish HO-1 and Cav-1 protein co-localization with TLR4 on the CF MΦs cell surface.

Consistent with the restoration of HO-1/Cav-1 TLR4 negative regulation, genetic or pharmacological (CORM2) enhancing of this pathway decreased the production of pro-inflammatory cytokines in CF MΦs treated with LPS.

In conclusion, our results demonstrate that the counter regulatory HO-1/CO pathway, which is critical in balancing and limiting the inflammatory response, is defective in CF MΦs through a Cav-1-dependent mechanism, exacerbating the CF MΦ's response to LPS. This pathway could be a potential target for therapeutic intervention for CF lung disease.

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ACTIVATION OF THE PGE2 RECEPTOR EP4 PROTECTS AGAINST OXIDANT-INDUCED APOPTOSIS IN AIRWAY EPITHELIAL CELLS

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Heme oxygenase (HO)-1 is expressed in airway epithelial cells, and its expression induced in pulmonary diseases characterized by oxidant stress, including CF. HO-1 over-expression protects against *P. aeruginosa* infection, suggesting induction of HO-1 in CF is cytoprotective in nature and that increasing its expression could potentially be therapeutic against bacterial infection and inflammation. Our laboratory is interested in the role of prostanoid receptors in mediating oxidant stress in airway epithelia. Recently, it was reported that PGE2 can protect against oxidant-induced apoptosis via upregulation of HO-1 mediated by the EP2 receptor. Therefore, using model human airway epithelial cells we have investigated whether activation of EP2 and EP4 receptors induce HO-1 expression and, if so, can this protect the cells against oxidant-induced damage. 16HBEo- cells were treated with 400 μ M H₂O₂ for 6 hours, a protocol we initially determined induces apoptosis, but not significant levels of death by necrosis (as determined by lactate dehydrogenase assay). Apoptosis was measured via a caspase-3 colorimetric assay. As a positive control, we initially induced HO-1 using the known inducer cobalt protoporphyrin IX (10 μ M), which protected against H₂O₂-induced apoptosis, and increased HO-1 expression as determined via quantitative PCR and immunoblotting. Pharmacological activation of the EP2 and EP4 receptors using butaprost and PGE1-OH (both at 10 μ M) respectively revealed that EP4 receptor activation protected against H₂O₂-induced apoptosis, while EP2 receptor activation had no effect. PGE2 similarly protected against apoptosis. Interestingly however, whilst protecting against apoptosis, EP2 and EP4 receptor activation had no significant effect on HO-1 expression, either at the gene or protein level. Finally, we investigated whether HO-1 induction or prostanoid receptor activation were protective against H₂O₂-induced lipid peroxidation. Treatment with cobalt protoporphyrin IX, butaprost, PGE1-OH or PGE2 were not effective at reducing H₂O₂-induced lipid peroxidation. We conclude that whilst EP4 receptor activation is protective against oxidant-stress induced apoptosis in human airway epithelial cells, this mechanism is independent of HO-1 induction, and likely reflects stimulation of PGE2 production. This suggests the EP4 receptor can be protective against oxidative-induced cell death and may represent a novel target for airway diseases characterized by oxidative stress, such as CF.

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DEFICITS IN CFTR ACTIVITY CAN BE ACQUIRED AND TRANSMITTED BY A SYSTEMIC PATHWAY

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Inherited mutations in CFTR are the proximate cause of CF, however less is known regarding the role of acquired defects in CFTR function and their clinical significance. Cigarette smoking is known to induce CFTR dysfunction in airway models *in vitro* and in human subjects. As such, whole cigarette smoke (WCS) is one of several endogenous and exogenous stimuli reported to alter CFTR activity as are hypoxia, unfolded protein response, alcohol, cadmium, and neutrophil elastase. To further characterize the extent and severity of acquired CFTR abnormality in smokers with and without COPD, we evaluated CFTR function in pulmonary and non-pulmonary organs of mice and humans.

Prior studies by our group and others have indicated that healthy smokers exhibit reduced chloride transport by NPD (Δ Cl⁻ free isoproterenol; 40% of controls, $P \leq 0.0005$) and reduced lower airway PD, a measure of CFTR activity in the lung (50% of Controls, $P \leq 0.05$). Our latest studies analyze CFTR function remote from the respiratory system and demonstrate elevated sweat chloride in healthy smokers (-30.8 mEq, $P \leq 0.01$) and COPD smokers (-30.7 mEq, $P < 0.05$) compared to controls (-17.5 mEq, $n > 20$ /group). This is indicative of ~40% reduction in CFTR activity, a value similar to that observed in CFTR related disorders and the first report of acquired CFTR dysfunction at a site not directly exposed to cigarette smoke. Similarly, compared to non-smokers, CFTR-dependent intestinal currents were also reduced in smokers (45% of Controls, $P < 0.05$, $n = 15, 6$). The systemic nature of the CFTR decrement was further supported by reduced CFTR activity (68%, $P < 0.01$, $n = 14$) in non-CF HBEs exposed to plasma from smokers for 24 hrs compared to plasma from normal controls, suggesting a circulating factor could confer CFTR dysfunction. To confirm these findings *in vivo*, non-CF mice were exposed twice daily to WCS for 5 and 17 weeks. WCS-exposed mice had decreased CFTR activity by NPD (28.9% of controls, $P < 0.005$, $n = 10$), trachea Isc (53%, $P < 0.05$, $n = 9$) and Isc of the distal ileum (84.3%, $P < 0.05$, $n = 10$; and 45%, $P < 0.001$, $n = 8$, after 5 and 17 weeks, respectively). Acrolein is a reactive component of WCS that also circulates due to endogenous production related to inflammation. Acrolein content in the serum proteins of smokers was ~twice that of healthy non-smokers ($P < 0.05$, $n = 12$) and free serum acrolein was higher by 35% ($P < 0.05$) in COPD smokers. In HBEs, basolateral exposure to acrolein mimicked the inhibitory effects of plasma from cigarette smokers on CFTR function (72% block at IC_{max}, $P < 0.05$), and could be attenuated by N-acetyl cysteine ($P < 0.05$).

Cigarette smoking causes an acquired systemic defect in CFTR function and suggests a potential explanation for the increased incidence of CF-associated extra-pulmonary disorders in smokers including pancreatitis, male infertility, constipation, and osteoporosis. Acrolein is an endogenous and exogenous circulating factor that could transmit systemic CFTR abnormality, and may also be relevant to functional CFTR deficits in CF patients.

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THE EFFECT OF SULFORAPHANE IN BROCCOLI SPROUTS ON NRF2 ACTIVATION, GLUTATHIONE, MARKERS OF OXIDATIVE STRESS, AND NEUTROPHIL MIGRATION

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The link between defective CFTR and inflammation is unknown. CF epithelial cells decrease Nrf2 activity, a transcription factor that attenuates NF κ B-driven inflammation. Nrf2-regulated antioxidant proteins are decreased in CF. We sought to determine whether activation of Nrf2 by consumption of sulforaphane in broccoli sprouts would activate Nrf2 in monocytes and nasal epithelial cells, reduce oxidative metabolites, and reduce PMNs in healthy volunteers (HVs) and subjects with CF. Ten HVs and 5 CF subjects ate 100 gms of broccoli sprouts daily for 5 days. Outcomes included Nrf2 in nasal cells, plasma glutathione (GSH), intracellular GSH and Nrf2 activation in monocytes, urine bromotyrosine, and oral PMNs. Nrf2 was isolated in the cytoplasm of all lymphocytes but in the nucleus from lymphocytes from 1 CF and 7 HVs. HVs had more Nrf2 in lymphocyte cytoplasm than did CF (median pre-treatment values in CF vs. HV: 121,460 vs. 516,095 OD x mm³, $p = 0.22$). Nrf2 was increased post treatment in lymphocyte cytoplasm in 4/5 CF subjects (pre- vs. post-treatment: 121,460 pre-treatment vs. 402,261 OD x mm³) compared to HVs (pre- vs. post-treatment: 516,095 vs. 640,867 OD x mm³). The change in Nrf2 did not differ between CF and HV ($p = 0.86$). Nrf2 decreased in the nucleus of lymphocytes from HV (median values pre- vs. post-treatment: 178,961 vs. 9,559 OD x mm³, $p = 0.08$). Nrf2 was detected in epithelial cell cytoplasm of 4/5 CF but only 4/10 HVs. Mean changes in lymphocyte GSH did not differ between the two groups. There was a trend towards increased lymphocyte GSH in HV ($p = 0.14$). In CF, there was a trend towards decreased plasma GSH ($p = 0.07$). Four of five CF subjects showed declines in plasma GSH. The mean change in plasma GSH was more negative in CF than in HVs ($p = 0.023$). Post-treatment lymphocyte GSH tended to increase, and plasma GSH was unchanged in HVs. The lack of Nrf2 activation would suggest that less GSH is made in CF cells, thus reducing redox protection. Nine of ten HVs had decreases in mouthwash PMN pre- to post-treatment ($p = 0.022$). Four of five CF subjects demonstrated decreases in mouthwash PMN. Mean change in log₁₀(PMN) did not differ between the two groups ($p = 0.48$). The mean change in log₁₀(urine bromotyrosine) did not differ significantly from zero in either CF or HVs, and the mean change over time did not differ between the two groups. CF subjects had a higher baseline urine bromotyrosine than did HVs, thus suggesting a high baseline oxidative state ($p = 0.0013$). There were no SAEs during this study. Results suggest that subjects with CF have a high baseline oxidative state and that there is an abnormality in Nrf2 activation. Results also suggest that sulforaphane may attenuate inflammation in CF, possibly through the activation of Nrf2. Supported by the CF Foundation.

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ALTERNATING OR CONCURRENT ABSORPTION AND SECRETION OF SURFACE FLUID IN SMALL AIRWAYS

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Native small airways must remain wet enough to be pliable and support ciliary clearance, but dry enough to remain open for alveolar gas flow. Thus the airway epithelium must both absorb and secrete electrolytes to maintain a constant volume of fluid to bathe the airway surface. Despite their frequent

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involvement in lung diseases, the miniscule size of native peripheral airways has limited studies of fluid and electrolyte movements in these structures. For this, we assembled a novel capillary-Ussing chamber (area 1 mm^2) to measure electrolyte transport in small airways ($\approx 1 \text{ mm } \Phi$) from pig lung. Transepithelial potentials (V_t) were recorded in open circuit conditions and during brief constant current pulses across the epithelial surface of dissected airways to determine transepithelial conductance (G_t) and equivalent short circuit current (I_{sc}^{eq}) in the presence and absence of selected inhibitors and agonists. In bilateral Ringer's solution, spontaneous V_t , G_t and I_{sc}^{eq} were: $-2.1 \pm 0.2 \text{ mV}$, $21.2 \pm 0.9 \text{ mS/cm}^2$, and $44.4 \pm 3.9 \text{ } \mu\text{A/cm}^2$ ($n = 37$; mean $\pm$ SEM), respectively. Both the Na^+ conductance inhibitor amiloride and the Cl^- conductance inhibitor niflumic acid depolarized V_t , decreased G_t and I_{sc}^{eq} , but unexpectedly, CFTR Cl^- conductance inhibitor GlyH-101 hyperpolarized V_t and decreased G_t while slightly increasing I_{sc}^{eq} . Activating CFTR with cAMP agonists significantly depolarized V_t and increased G_t without a significant effect on I_{sc}^{eq} . On the other hand, putatively activating Ca^{++} activated Cl^- channels (CaCC) with the purinergic agonist UTP, acutely hyperpolarized V_t and increased G_t and I_{sc}^{eq} significantly above spontaneous levels. When the impermeant anion gluconate completely replaced luminal Cl^- , V_t hyperpolarized markedly by nearly -40 mV . A lumenally directed 2:1 NaCl gradient, hyperpolarized V_t indicating that transepithelial Cl^- : Na^+ selectivity is at least 3:1. These data are consistent with a native airway epithelium that is highly anion-selective and is composed of separate, constitutively and concurrently, active electrolyte secreting and absorbing epithelia. Since small airways are formed of long longitudinal, accordion-like rows of pleats and folds, we propose that the pleats may consist mainly of dedicated secretory cells while the folds consist of absorptive cells.

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ERP29 REGULATES ENAC FUNCTIONAL EXPRESSION BY PROMOTING CHANNEL CLEAVAGE

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The epithelial Na^+ channel (ENaC) is a key regulator of airway surface liquid volume. In the cystic fibrosis (CF) airway, relative hyperactivity of ENaC is hypothesized to cause depletion of the periciliary liquid, which impairs mucociliary clearance and portends bacterial colonization of the airway. In our group's studies of the correction of ΔF508 -CFTR trafficking by 4-phenylbutyrate (4PBA), we demonstrated that ERp29, a novel 29 kDa molecular chaperone of the lumen of the endoplasmic reticulum (ER), had increased abundance in CF epithelial cells after treatment with 4PBA, and that ERp29 promoted trafficking of both WT and ΔF508 CFTR (Suaud, et al, JBC, 2011). ERp29 is homologous to the thioredoxins, which are enzymes that catalyze the formation and rearrangement of disulfide bridges. However, ERp29 has only a single cysteine residue (C157) and lacks the C-X-X-C motif that is characteristic of the thioredoxins. We tested the hypotheses that ERp29 would regulate ENaC trafficking and functional expression, and that its single cysteine, C157, would have a critical role in ERp29's regulation of ENaC. To test this hypothesis in epithelial cells, we selected ENaC-overexpressing MDCK cell lines with doxycycline (dox)-inducible expression of either WT ERp29 or a mutant ERp29 where C157 was changed to serine (C157S ERp29). In Ussing chambers, dox-induced overexpression of WT ERp29 caused an increase in amiloride-sensitive I_{sc} , or ENaC functional expression. In contrast, overexpression of C157S ERp29 caused a decrease in amiloride-sensitive I_{sc} . These opposite effects correlated with the influence of WT or C157S ERp29 on the whole cell expression of the cleaved form of ENaC's γ -subunit. Overexpression of WT ERp29 increased its whole cell expression, while overexpression of C157S ERp29 decreased it. We also tested the influence of C157S ERp29 on the fraction of cleaved, higher P_o versus uncleaved, lower P_o ENaC at the apical surface by examining the influence of trypsin on amiloride-sensitive I_{sc} in Ussing chambers. Application of $10 \text{ } \mu\text{g/mL}$ trypsin to the apical surface of MDCK cells overexpressing C157S ERp29 caused a significantly greater fractional increase of amiloride-sensitive I_{sc} compared to the control cells, suggesting that C157S ERp29 inhibited ENaC cleavage. Interestingly, the effect of siRNA-mediated depletion of ERp29 expression on amiloride-sensitive I_{sc} was similar to that of C157S ERp29. When MDCK cells were

treated with ERp29 siRNA, the fraction of total amiloride-sensitive I_{sc} that was induced by trypsin was significantly greater than control cells treated with non-targeting siRNA. In contrast, overexpression of WT ERp29 decreased the fraction of total amiloride-sensitive I_{sc} induced by trypsin. Finally, overexpression of either WT or C157S ERp29 increased the recovery of β -ENaC after immunoprecipitation of ERp29. These data support the hypothesis that ERp29 interacts with ENaC and can regulate its functional expression by regulating ENaC subunit cleavage. These data also suggest that C157 is critical for ERp29's regulation of ENaC, but is not a determinant of ERp29's interaction with ENaC.

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THE EFFECT OF TGF- β 1 ON ION TRANSPORT: ROLE OF THE LARGE CONDUCTANCE, Ca^{2+} ACTIVATED AND VOLTAGE-DEPENDENT K^+ CHANNELS (BK CHANNELS)

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Increased TGF- β 1 levels have been found in inflammatory diseases and in CF. Bronchoalveolar lavage fluid levels of TGF- β 1 are elevated in cystic fibrosis in comparison to non-CF controls, and are associated with neutrophilic inflammation, diminished lung function and increased rates of hospitalization. TGF- β 1 has been identified as a modifier gene in cystic fibrosis.

Large conductance, Ca^{2+} activated and voltage-dependent K^+ channels (BK channels) control a variety of physiological processes in different tissues. In normal human bronchial epithelial cells grown at the air-liquid interface (NHE), apical BK channels open in response to ATP and are critical for the maintenance of adequate airway surface liquid volume.

We studied the effect of a 20h treatment of TGF- β 1 (10 ng/mL) on NHE transport, and in particular, in BK channels expression and activity, by using electrophysiology, qPCR, Western blot, flow cytometry, immunocytochemistry and ciliary beat frequency (CBF). Filters from cells from 3 or more donors were used in all the experiments. All data is expressed as mean $\pm$ SEM and Student's T test was used to detect statistical differences between control and TGF- β 1 treated samples.

Ussing chamber experiments in basolaterally permeabilized cells indicated that TGF- β 1 reduces the ability of ATP to cause BK-mediated potassium secretion from $15.84 \pm 3.224 \text{ } \mu\text{A/cm}^2$ ($n=9$) in the control to $8.168 \pm 1.406 \text{ } \mu\text{A/cm}^2$ ($n=9$, $p=0.0445$) in the TGF- β 1 treated cells. mRNA levels of the pore forming subunit KCNMA1 (Slo-1) were increased in TGF- β 1 treated cells. Flow cytometry indicated similar percent of cells expressing the KCNMA1 (Slo-1) pore forming subunit in treated and nontreated cells (75.1 ± 10.19 and 69.9 ± 12.61 , $n=5$, $p=0.7567$). Significant differences in protein quantity could not be detected by Western blot or immunocytochemistry.

TGF- β 1 induced decrease in BK apical activity is accompanied by the reduction of CBF ($4.3 \pm 0.24 \text{ Hz}$ in treated vs. $6.6 \pm 0.59 \text{ Hz}$ in control cells, $n=8$, $p=0.0038$). Lower CBF in TGF- β 1 group is restored to control values by apical addition of buffer, suggesting that decrease of CBF is caused by reduction of water secretion.

Parallel studies with bronchial human airway epithelial cells obtained from CF patients who underwent lung transplant, led to similar results, TGF- β 1 (10 ng/mL , 20h) decreases ATP-induced apical BK current from $16.1 \pm 1.26 \text{ } \mu\text{A/cm}^2$ to $8.5 \pm 2.12 \text{ } \mu\text{A/cm}^2$ ($n=7$, $p=0.0095$).

Therefore, TGF- β 1 causes a decrease of BK channel activity, which influences water secretion, promoting a drier phenotype. This could help explain the relationship between increased TGF- β 1 and decreased lung function in cystic fibrosis.

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SYNERGY BETWEEN NANOMOLAR CARBACHOL AND MICROMOLAR FORSKOLIN INDUCES GLAND SECRETION WITHOUT AIRWAY NARROWING IN CF HUMANS AND FERRETS

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Objective: Defective mucus secretion from CF human airways glands can be partially overcome via Ca^{2+} agonists, but these cause bronchoconstriction that limits their use. The combination of low dose forskolin or VIP with carbachol synergistically induces fluid secretion from airway glands via a CFTR-dependent mechanism (Choi, JCI, 2007;117:3118). However, higher concentrations of forskolin (3 μM) combined with 100 nM carbachol, stimulated airway submucosal fluid secretion in CFTR-/- piglets, indicating a CFTR-independent mechanism (Joo, JCI, 2010;120:3161). We hypothesized that this combination might produce a therapeutic amount of gland secretion without causing bronchoconstriction.

Methods: To test the hypothesis, we concurrently measured gland secretion and airway muscle tension in humans and in ferrets, which express human-like CFTR in their tracheal glands and display human-like lung disease in the absence of CFTR (Sun, JCI, 2010;120:3149). Individual airway gland secretion rates were measured optically in situ and airway muscle contractions were measured with a force transducer. Isolated airway segments from 20 WT ferrets, 3 CFTR-/- ferrets, 9 non-CF and 7 CF humans were studied.

Results: In WT ferrets, 100 nM carbachol plus 3 μM forskolin synergistically stimulated gland secretion at a rate ~4.4-fold greater than predicted from their additive effects (n=8, paired t-test, p=0.0016). Synergistic secretion was also seen between 1 μM phenylephrine + 3 μM forskolin (6.4-fold greater than their predicted additive effects, n=6, p=0.01), and between 100 nM substance P + 1 μM VIP (2.7-fold greater than their predicted additive effects (n=6, p=0.01). Changes in contractile force (delta milli-Newtons) showed dramatic differences with the combined treatments compared to carbachol alone; 3 μM forskolin relaxed the muscle and abolished contraction to concurrent stimulation with 100 nM carbachol (n=6, paired t-test, p=0.038). Gland secretion in CF human bronchi was also synergistically stimulated with 100 nM carbachol + 10 μM forskolin, which produced a secretion rate almost 4.6-fold greater than predicted from their additive effects: (in $\mu\text{l}/\text{min}/\text{g}$): 100 nM carbachol: 18.4; 10 μM forskolin: 45.0; combined: 291.3 (n=7, p<0.01). In non-CF human trachea, 100 nM carbachol + 10 μM forskolin, produced a secretion rate ~1.7-fold greater than predicted from their additive effects: (in $\mu\text{l}/\text{min}/\text{g}$): 100 nM carbachol: 24.3; 10 μM forskolin: 150.1; combined: 299.7 (n=9, p=0.01). In non-CF (n=6) and CF (n=1) human airway muscle, 10 μM forskolin also abolished contractions to concurrent stimulation with 100 nM carbachol as in WT ferrets. CFTR-/- ferrets also showed synergic fluid secretion to 3 μM forskolin + 100 nM carbachol (2.8 fold, p=0.012).

Conclusions: These results suggest that conventional bronchodilators combined with low dose cholinergic agonists might induce glandular fluid secretion and bronchodilation in CF airways, which could have therapeutic potential for CF lung disease. Supported by the Cystic Fibrosis Foundation.

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THE ROLE OF CELL SURFACE-ATTACHED MUCINS IN AIRWAY HEALTH

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The clearance of airway mucus represents a primary innate defense mechanism that protects the airways from infectious and toxic agents inhaled during normal breathing. The airway surface layer (ASL) is a critical component of the mucus clearance system and consists of two principal components: 1) the motile mucus layer that traps inhaled particles and 2) the

periciliary layer (PCL) which provides a favorable environment for ciliary beating and cell surface lubrication. This system has been traditionally viewed as "gel-like" mucus layer propelled by cilia beating in a low viscosity "watery" periciliary (or sol) layer. However, this view of the PCL does not adequately explain why mucus does not freely penetrate into the ~200 nm space between cilia. In fact, using a two-dye labeling technique to quantify the permeability of the PCL (i.e. mesh size), we found that polymer molecules and beads with molecular sizes as small as 40 nm in diameter were completely excluded from the PCL of human bronchial epithelial (HBE) cell cultures, whereas only molecules < 5 nm diameter could gain access to the cell surface. Coupled with data from electron microscopy studies which reveal an electron-dense meshwork surrounding the cilia, we hypothesized that cell surface-tethered glycoproteins form an extracellular brush with a sufficiently high concentration to establish a mesh that prevents both mucus and inhaled particles from penetrating the PCL and reaching the cell surface. In an effort to identify the molecules responsible for the PCL mesh, immunohistochemistry studies revealed the presence of very large membrane-spanning mucins (MUC1, MUC4, and MUC16) attached to cilia, microvilli, and the cell surface of HBE cultures. To understand whether these mucins are involved in establishing the PCL brush, we generated a transgenic mouse lacking Muc1, Muc4, and Muc16. Using excised tissue and cultured cells from wild-type mouse airways, we observed that, like the human airways, molecules of 40 nm diameter were completely excluded from the mouse PCL. In contrast, we found that the PCL mesh permeability was significantly altered in airways derived from the triple MUC knock-out mouse. Here, molecules of 40 nm diameter were capable of penetrating the PCL down to a depth of 2.3 μm from the cell surface, compared to 4.1 μm in tissues from wild-type animals (i.e. complete exclusion). (Note, the cilia of the mouse respiratory tract are ~4 μm in length, compared to ~7 μm in human). Taken together, our results suggest that tethered mucins play a vital role in protecting the airways by establishing a permeability barrier to prevent small infectious agents (e.g. Influenza A, ~100 nm, and RSV, ~120 nm) from gaining access to the cell surface. This work was supported by the Cystic Fibrosis Foundation.

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SUPERIOR EFFECT OF DRY-POWDER ICALM OVER AEROSOLIZED HYPERTONIC SALINE IN MAINTENANCE OF AIRWAY HYDRATION AND CILIA BEATING

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The importance of regulating the hydration of the airway surface layer (ASL) is readily apparent in patients with cystic fibrosis (CF), where dysregulation of ion transport leads to unregulated fluid absorptions, mucus plugging, failure of mucociliary clearance (MCC), and eventually, chronic lung disease. Hyperosmotic solutions, such as hypertonic saline (HS), have been used clinically to treat patients with CF, by acting osmotically to draw water out of cells and rehydrate the thick, sticky, mucus. We have previously shown that aerosolized HS applied to cultured human bronchial epithelial (HBE) cultures, produces only a transient increase in the ASL height (an index of airway hydration), as a result of epithelial sodium channel (ENaC)-mediated fluid reabsorption. Furthermore, we have previously shown that HS produces a transient, but significant, slowing of cilia beating frequency (CBF), and thus MCC, when delivered as an aerosol to the airway surface. The goal of this study was to compare the effects of both liquid and dry powder formulations of HS (NaCl) to a novel hyperosmotic calcium-based formulation (iCALM) on ASL hydration and CBF. In these studies, we utilized novel nebulization and dry powder delivery systems, capable of mimicking the *in vivo* delivery rates of hyperosmotic solutions and powders to the lower airways. Consistent with the application of an osmotic agent to the luminal surface of the airways, we found that liquid and powder NaCl and iCALM formulations produced a robust increase in the total ASL height. However, the rates of fluid reabsorption following treatment with the powder reagents (both NaCl and iCALM) were significantly slower than the liquid formulations. As a result, the total hydration power, an integrated measure of change in ASL height overtime, was significantly higher in the powder treated conditions. Second, we found that powder formulations of NaCl and iCALM had a significantly milder affect on CBF, as compared to the liquid formulations. Interestingly, iCALM (both liquid and powder) had a significantly

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faster recover of CBF following delivery, compared to NaCl. While the mechanism for the hyperosmotic-mediated slowing of CBF is unknown, it is hypothesized to involve cell shrinkage during exposure to hyperosmolar agents. Consistent with this hypothesis, we found that iCALM (liquid and powder) and NaCl (powder) produced significantly less cell shrinkage, compared to the traditional liquid NaCl (HS) formulation. Taken together, these studies demonstrate that dry powder iCALM, and to a lesser effect NaCl, have superior effects in maintaining airway hydration while minimizing the effect on cilia beating. The net effect of both is a prolonged facilitation of MCC rates. This material is based upon work supported by, or in part by, the US Army Research Laboratory and the US Army Research Office under grant number W911NF-10-1-0382.

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AIRWAY MUCIN INTERACTOME COMPOSITION IN RESPONSE TO DEFECTIVE MUCUS CLEARANCE AND GENETIC DELETION OF SECRETED MUCINS

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The role of airway mucus in lung defense is often associated with trapping of particles and pathogens, which are cleared by mucociliary clearance. Recent studies on human purified gel-forming mucins, i.e., MUC5AC and MUC5B, revealed the existence of macromolecular complexes, called mucin interactome, which involve immune/regulatory proteins and might contribute to maintaining lung immune homeostasis. Defective mucus clearance contributes to the pathology of diverse muco-obstructive lung diseases, including cystic fibrosis (CF), but the impact of airway mucus obstruction on the mucin interactome is currently unknown. Experimentally, defective mucus clearance has been generated by airway-targeted overexpression of the epithelial Na⁺ channel β subunit (encoded by the *Scnn1b* gene) in murine lungs. *Scnn1b*-transgenic (*Scnn1b*-Tg) mice exhibit CF-like lung pathology including airway mucus obstruction, inflammation, and susceptibility to spontaneous bacterial infections. We began to characterize the mucin interactome in *Scnn1b*-Tg mice and WT littermates using bronchoalveolar lavages (BAL). Pellet and supernatant fractions were obtained by low speed centrifugation, and the pellet was washed with PBS to remove loosely associated components. Guanidine hydrochloride (6M) was added to break non-covalent interactions and disperse the mucus. Refractive index measurement combined with gel filtration was used to determine the mass of total biomolecules (G25 Sephadex) and large complexes (CL2B column, V_0 volume). As expected, the total biomass was higher in *Scnn1b*-Tg BAL than in WT. Most of the biomass recovered from *Scnn1b*-Tg BAL was in the pellet fraction and consisted primarily of large aggregates. The ratio of large aggregates (CL2B) to total biomolecules (G25) was not different between WT and *Scnn1b*-Tg pellets, indicating coordinate regulation of all mucus components. The CL2B V_0 fraction was digested with trypsin and subjected to LC-MS/MS mass spectrometry. Surprisingly, a series of low Mr proteins, such as chitinases, Lplunc1, histones, SP-A, SP-B, SP-D, were detected in the high Mr complexes in addition to Muc5b, indicating interaction of these proteins with mucins. Unfractionated BALs in 6M urea were also subjected to CL2B gel filtration and mass spectrometry. Using a novel label free quantitation technique, ~50 proteins were detectable only in *Scnn1b*-Tg samples (vomeromodulin, calgranulins, peroxiredoxin, cathelicidin, thioredoxin, CD177, lactoferrin, FIZZ, HMGB1, myeloperoxidase), ~15 proteins increased 2-19 fold (Lplunc1, superoxide dismutase, histones, glutathione S-transferase, YM1, SP-D), and ~10 proteins decreased 4-60 fold (α 1-antitrypsin, SP-B, surfactant convertase, contrapsin, CC10, WAP, SP-A) as compared to WT. The effect of selective removal of either gel-forming mucin, i.e., Muc5ac or Muc5b, on the mucin interactome composition is currently being investigated using BALs from mucin-deficient *Scnn1b*-Tg mice and WT littermates. Besides providing insights about the specific function of Muc5ac and Muc5b in airway mucus, this analysis will suggest safety thresholds for therapeutic strategies focused on reducing mucin secretion as a tool to alleviate mucus obstruction.

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AIRWAY LENTIVIRAL CFTR GENE TRANSFER EXTENDS THE LIFETIME OF CF MICE

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Effective airway gene transfer that could functionally correct the CFTR gene defect in CF airways has been the primary goal of gene therapy development in the CF mouse animal model. We have reported long term (> 12 month) partial CFTR correction in timed-group CF mouse studies¹. In recent repeated-measure cohort studies we further examined the success and effect of partial CFTR gene correction in CF mice.

Methods: Male and female CF mice (*cftr*^{tm1unc}) were examined under three experimental nasal-airway gene transfer conditions: Control treatments were: a) pre-treatment with airway surfactant lysophosphatidylcholine (LPC, 0.1%) prior to a lentiviral (LV) vector containing no gene (LV-MT, n=6); (b) PBS pre-treatment prior to delivery of the therapeutic gene (LV-CFTR, n=6). The treatment group received LPC prior to LV-CFTR (n=12). Viral titre was 0.6×10^{10} tu/mL and 2.5×10^{10} tu/mL for LV-MT and LV-CFTR mice respectively. CFTR function was assessed via nasal potential difference (PD) at 1 week, 1 and 3 months, then at 3 monthly intervals until 21 months after LV delivery. Survival data was expressed as Kaplan-Meier plots and the presence of survival differences between the three groups determined by Mantel-Cox log rank test. Outcomes were also compared to historical survival data from our reporter gene studies in normal (C57) mice.

Results: CF mice treated with LPC/LV-CFTR showed significant persistent functional CFTR gene transfer in the nasal airway for up to 15 months (APD_{CL} range 12-54% towards heterozygote normal). The median survival (20.1 mo) was significantly extended compared to either PBS/LV-CFTR (14.4 mo) and LPC/LV-MT (8.8 mo) groups. Survival in normal C57 mice similarly treated with the reporter gene luciferase (Luc) LPC/LV-Luc, PBS/LV-Luc, or with LPC/LV-MT was not different between groups, and all showed a median survival greater than 23 mo. Unexpected deaths within the CF mouse study (as assessed by a veterinary research pathologist) were not different across the three CF mouse groups. No deaths were suggestive of an oncogenic cause. Compared to historical data for the C57 strain the survival of the LPC/LV-CFTR treated CF mice was not significantly different from normal untreated C57 mice.

Discussion: These findings indicate successful *nasal* airway gene transfer in CF mice significantly improves the lifetime of the treated animals, approaching longevity to near that present in normal C57 mice. This is the first report to our knowledge of generalised and physiologically significant health improvement in CF animals due to partial correction of airway CFTR dysfunction by gene transfer. Since nasal dosing can reach lung airways² (as noted in our nasal LV-Luc reporter gene studies) we speculate that "spillover" of gene vector into the lung could induce sufficient low-level CFTR gene transfer to improve direct and indirect CFTR-related transport across lung tissue to improve general health and survival.

Acknowledgements: NHMRC and CURE4CF Foundation Ltd.

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MUCINS AND EXTRACELLULAR DNA IN CF MUCUS: A HIGHER ORDER OF STRUCTURAL COMPLEXITY

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One of the stereotypical findings of cystic fibrosis (CF) is the pathological accumulation of mucus in the lungs, associated with chronic bacterial infection and inflammation. It has been shown previously that increased mucin concentration (2.5% to 8% solids) and large amounts of extracellular DNA alter the viscoelastic properties of CF mucus and compromise its transport. To reduce the viscosity of CF secretions, a recombinant human DNase (dornase alpha) was developed and is widely used to improve mucus clearance. Extracellular DNA was thought to result from degenerating neutrophils in response to chronic infection, but recently a new function of neu-

trophil extracellular traps (NETs) has been discovered whereby neutrophils expulse their nuclear content, forming complex scaffolding of DNA, histones and granular proteins, resembling a spider web, designed to trap pathogens.

We have recently collected pediatric CF bronchoalveolar lavage fluids (BALF) from exacerbating and non-exacerbating patients and analyzed them using confocal microscopy, refractometry and rheological techniques. From our direct observation of cytocentrifuge preparations, we have detected increased numbers of neutrophils and neutrophil extracellular traps (NETs) in exacerbating patients, and this correlates with changes in rheology and increased mucin concentrations. NETs were tightly interwoven with mucins and the structure of these large polymers (e.g. mucin-DNA networks) varied considerably from patient to patient. In some cases, these networks presented complex levels of scaffolding and showed colossal molecular weights (~2,000 MDa) and substantial sizes (radius of gyration ~400nm). In non-exacerbating patients who underwent CT scanning as part of their usual care, increased pellet mass, mucin and extracellular DNA concentrations closely correlated with CT scoring for bronchial wall thickening (BWT), likely an important finding that precedes the onset of bronchiectasis. Unlike exacerbating patients, the majority of cells in these samples were macrophages with fewer neutrophils (mean = 16% as compared to 61% with exacerbation); however, substantial amounts of extracellular DNA that co-localized with citrullinated histones was detected, suggesting that macrophages could also play a role in mucus thickening in CF. Finally, despite the use of rhDNase in many of these patients, our BALF analyses continued to show the persistence of mucin-DNA macrocomplexes, which suggests that rhDNase had incomplete effects on NETs, either because of lack of efficiency or inadequate penetration into the thick mucus.

These data suggest that NET formation may increase during periods of pulmonary exacerbation, and this in turn could contribute to the pathogenesis of CF. Further, it is possible that non-neutrophil cell types (i.e. macrophages) may also contribute to the accumulation of extracellular DNA, perhaps being most important in airways with lesser degrees of chronic inflammation. A better understanding of the role that NETs play in the pathophysiology of CF, and the identification of strategies that better address the problem of mucus-DNA complexes and defective mucus clearance may provide new insights into the treatment of CF.

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A SPLUNC1 DERIVED PEPTIDE INHIBITS ENAC IN CF AIRWAY EPITHELIA

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The epithelial Na⁺ channel (ENaC) is responsible for Na⁺ absorption in the kidney, colon and lung. ENaC is made up of three subunits, α , β , and γ . Previous studies have shown that ENaC is hyperactive in CF airways, due to a lack of negative regulation of ENaC by CFTR. This leads to a decrease in airway surface liquid (ASL) volume which slows or abolishes mucus transport. The activation of ENaC is dependent on its proteolytic cleavage by enzymes such as neutrophil elastase (NE). We have previously shown that the short palate lung and nasal epithelial clone (SPLUNC1) is a potent negative regulator of ENaC and ASL height. SPLUNC1 binds to and prevents the cleavage of ENaC. In this study we have identified the ENaC inhibitory domain of SPLUNC1 and characterized its interaction with ENaC. The region of SPLUNC1 responsible for ENaC inhibition was identified by measuring the amiloride sensitive ENaC current, I_{Na} , in *Xenopus* oocytes co-injected with α , β , and γ ENaC in the presence of C-terminal SPLUNC1 truncants. Deletion of up to 85% of SPLUNC1 resulted in a similar inhibition of ENaC as seen with full-length SPLUNC1. This narrowed down the ENaC inhibitory domain of SPLUNC1 to residues 22-39. A peptide corresponding to this region, named G22-A39, was synthesized and 10 μ M G22-A39 caused a ~2.5 fold decrease in I_{Na} , indicating that we had identified the ENaC inhibitory domain of SPLUNC1. SPLUNC1 has been shown to co-immunoprecipitate with all three ENaC subunits, when they are expressed at the same time. Through a series of peptide pull-down assays we confirmed that G22-A39 was also able to interact in a similar fashion with all three subunits. However, when the subunits were individually expressed, G22-A39 specifically interacted with only the β -ENaC subunit in a glycosylation dependent fashion. To determine if G22-A39 was capable of inhibiting ASL hyperabsorption in CF human bronchial epithelial cultures (HBECs), the change in ASL height in CF HBECs was measured after treatment with

G22-A39. Addition of G22-A39 to CF HBECs resulted in an increase in ASL height from $4.2 \pm 0.6 \mu$ m to $7.9 \pm 0.6 \mu$ m, comparable to normal HBECs. G22-A39 remained active in the presence of activated neutrophil supernatant (ANS), which is derived from human neutrophils and contains high NE and cathepsin activity, suggesting that G22-A39 may prevent ASL hyperabsorption under pathologically relevant conditions. By mass spectrometry, we observed that NE cleavage of purified SPLUNC1 resulted in peptides corresponding to the G22-A39 region. This indicates that the ENaC inhibitory domain is released from SPLUNC1 by neutrophil elastase. The ability of G22-A39 to function in a proteolytically active environment makes G22-A39 a strong therapeutic candidate for restoring ASL height and function in CF patients.

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THERAPEUTIC EVALUATION OF A NOVEL RHESUS THETA DEFENSIN IN NEUTROPHILIC AIRWAY INFLAMMATION

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Background: Cystic fibrosis (CF) is characterized by a chronic cycle of airway obstruction, infection, and inflammation, leading to remodeling, impaired pulmonary function, and eventual respiratory failure. Rhesus macaque θ -defensin (RTD-1) is a unique cyclic antimicrobial octadecapeptide isolated from neutrophil granules of Old World monkeys. RTD-1 exhibits potent immune modulating activity demonstrating 100% survival in a murine model of SARS.

Objective: To evaluate RTD-1 for effectiveness in modulating host immune activity and reducing lung neutrophil burden in a murine model of airway neutrophilia.

Methods: Airway neutrophilia was established in BALB/c mice via insufflation of Pa-LPS. Mice were treated with 0, 0.04, 0.2, 1, 5, or 25 mg/kg RTD-1 (N=6/group). Enumeration of total and differential airway leukocyte, as well as quantification of inflammatory markers (forthcoming) was performed on 24 hour BALF. Pharmacokinetics of SC RTD-1 in plasma was performed by RP-HPLC and MALDI-TOF MS. In-vitro anti-inflammatory activity and cytotoxicity were evaluated in Normal Lung (NuLi-1) and Cystic Fibrosis (CuFi-1) hBECs. IL-1 β was used to induce airway inflammation with or without 5 μ g/mL RTD-1. TNF- α and CXCL8 were analyzed by ELISA and RTD-1 airway cytotoxicity by MTT. In addition, direct proteolytic inhibition was performed using an rh-active MMP peptide substrate activity assay. Lastly, 24 hour RTD-1 stability in rh-active MMP-9 or pooled CF sputum (2 individuals) was determined by RP-HPLC and MALDI-TOF MS. Statistical analysis was conducted using one-way ANOVA with log transformed data (Mean $\pm$ SD). Non-linear regression was used to determine the dose response relationships.

Results: RTD-1 dramatically reduced airway neutrophilia in a dose dependent manner with maximum inhibition at 25 mg/kg (5.6E6 $\pm$ 4.0E5; p<0.001). The dose that resulted in 50% of maximum inhibition (ID₅₀) was found to be 0.5 mg/kg (95 CI: 0.10 to 2.5 mg/kg) (r^2 = 0.51) and corresponded to a 2.8E6 reduction in airway PMNs. The disposition of RTD-1 was characterized by a peak plasma concentration of 965 μ g/mL at 15 min and a half-life of 18 hours. Treatment with 5 μ g/mL of RTD-1 resulted in a significant in-vitro diminution of CXCL8 (55% NuLi; 53% CuFi) and TNF- α (40% NuLi; 40% CuFi) release (n=1). This concentration was 100% tolerated and well below the toxicity concentrations with 50% cell survival (TC₅₀) = 31 μ g/mL (95 CI: 25 to 38 μ g/mL) (r^2 = 0.96). Additionally, RTD-1 inhibited rh-MMP-9 substrate proteolysis with the concentration 50% of maximum (IC₅₀) = 0.785 μ g/mL (95 CI: 0.23 to 2.77 μ g/mL) (r^2 = 0.80). Lastly, RTD-1 in the presence of CF sputum or rh-active MMP9 was >98% stable after a 24 hour incubation.

Conclusion: RTD-1 exhibits potent anti-inflammatory activity as evidenced by a reduction in inflammatory cytokines in-vitro and a diminution of neutrophils into the airspace of mice following stimulation of LPS. Importantly, RTD-1 exhibits favorable pharmacokinetics with a long terminal half-life in plasma and peak levels 30 times below the in-vitro defined TC₅₀.

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PHARMACOKINETICS AND EX-VIVO ANTI-INFLAMMATORY ACTIVITY OF AZITHROMYCIN

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Background: Clinical outcomes following long-term (≥ 6 months) azithromycin (AZM) demonstrate improved pulmonary function (PF) and reduced pulmonary exacerbation frequency in CF patients. PF improvement as noted in the pivotal trial was highly variable ranging from a decline of $>15\%$ to an improvement of $\geq 15\%$. Anti-inflammatory activity has frequently been reported as the mechanism of action. One possible explanation for the variability in PF response is differences in AZM exposures. The objective of this study was to characterize the pharmacokinetics (PK) of AZM in patients with CF and determine the relationship between AZM concentration and anti-inflammatory activity ex-vivo.

Methods: A cross-sectional study was conducted in 46 adult CF patients receiving AZM 250mg daily or 500mg TIW and 9 controls not receiving AZM. Blood was drawn immediately before and 3 h after a dose and AZM plasma concentrations quantified by HPLC MS/MS. Population PK was performed applying a 1-compartment model with first order absorption to plasma concentration data. Ex-vivo anti-inflammatory activity was determined by incubating peripheral blood samples (at AZM trough times) for 24 hours with or without LPS. Multiplex ELISA was used to quantify cytokines. Inflammatory response was defined as the inflammatory marker concentration after LPS stimulation minus the concentration at baseline. Linear correlation analysis was performed to determine the relationship between AZM trough levels and inflammatory response. In addition, patients were grouped into quartiles based on AZM trough levels and ANOVA was performed to determine differences in anti-inflammatory responses between groups.

Results: The CF study cohort had a mean age 30.1 (8.4) years, BMI 24.4 (3.6), and FEV₁ 54.5% (22.2). Significant negative correlations were identified between log AZM trough concentrations and log IL-1 β response ($r=-0.4662$, $P=0.0014$), log IFN- γ response ($r=-0.4105$, $P=0.0051$), log IL-12p70 response ($r=-0.3324$, $P=0.0024$), and TNF α response ($r=-0.3136$, $P=0.0359$). No significant correlations were noted with AZM concentrations and IL-4, IL-8, IL-10, GM-CSF and MCP1 responses. IL-1 β concentrations were significantly higher in patients in the lowest quartile of AZM concentrations when compared with the highest quartile (mean: 4.145 ± 0.51 vs 3.474 ± 0.69 , $P<0.05$). The inflammatory markers IFN- γ , IL-12p70 and TNF α showed no significant difference between quartiles. There was no significant difference in inflammatory marker concentrations or FEV₁ levels between patients between AZM regimens. PK analysis demonstrated a plasma clearance of 58.3 L/h, volume of distribution of 1750 L and half-life of 20.7 h, consistent with published reports. Mean peak and trough concentrations measured 0.325 $\mu\text{g/mL}$ and 0.099 $\mu\text{g/mL}$ respectively demonstrating significant variability.

Conclusions: AZM exhibits anti-inflammatory activity at plasma concentrations achieved in-vivo. Significant variability in plasma AZM concentrations contributed to the differences in anti-inflammatory response indicating some patients may require dosage adjustment to optimize anti-inflammatory activity.

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TYPE 4 CYCLIC NUCLEOTIDE PHOSPHODIESTERASES ARE CRITICAL REGULATORS OF CFTR ACTIVITY IN PRIMARY HUMAN AIRWAY EPITHELIAL CELLS

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Cyclic nucleotide phosphodiesterases (PDEs), the enzymes that hydrolyze and inactivate cAMP, represent a superfamily of isoenzymes that

are divided into 11 PDE families on the basis of their kinetic and pharmacologic properties. We have generated preliminary data that identify Type 4 PDEs (PDE4s) as the main regulators of the cAMP-dependent stimulation of CFTR in primary human bronchial epithelial cells (HBEs), a model highly relevant to human airway disease. PDE4 inhibition increases basal CFTR-dependent short-circuit currents (ISC) to about 30% of the maximal levels induced by forskolin. Given that long-acting β -adrenergic (β -AR) agonists are widely used in COPD and CF therapy, we tested whether PDE4 inhibition modulates β -AR-dependent stimulation of CFTR. PDE4 inhibition potentiated isoproterenol (ISO)-stimulated ISC and delayed the return of ISC to basal levels upon termination of β -AR signaling by treatment with the β -AR antagonist propranolol. PDE3 has been reported to regulate CFTR in the colon adenocarcinoma cell line T84. However, PDE3 inactivation has little effect on basal CFTR activity in HBEs and does not further stimulate ISC induced by PDE4 inhibition or β -AR stimulation. In HBEs from $\Delta F508$ -CF patients, PDE4 inhibition or FSK treatment induced small ISC increases in some but not all cultures. To enhance CFTR function, we tested $\Delta F508$ -cultures after partial rescue of CFTR expression through incubation at low temperature (27°C for 48 h) or treatment with "small molecule correctors". Temperature-corrected $\Delta F508$ -HBEs displayed a similar pattern of responses compared to non-CF cultures; but the amplitude of ISC changes was smaller. PDE4 inhibition increased basal ISC, potentiated ISO-stimulated ISC, and delayed the return of ISC to basal levels upon termination of β -AR stimulation. An initial experiment using cultures treated with the "small-molecule corrector" VRT325 (20 μM ; 37°C) mirrored the effects of PDE4 inhibition in temperature-corrected HBEs. PDE4 inhibition increased basal ISC in $\Delta F508$ -HBEs to only 30% of the levels obtained with FSK. However, PDE4 inhibition can potentiate the effect of cAMP stimuli, such as β -AR agonists, to fully activate CFTR. More importantly, PDE4 inhibition represents a viable therapeutic approach given the clinical use of the PDE4 inhibitor Roflumilast, whereas FSK treatment does not. Since restoring 5% of CFTR function may ameliorate the severity of CF, the effects of PDE4 inhibition on ISC in $\Delta F508$ -HBEs have therapeutic relevance. The efficacy of PDE4 inhibition to increase CFTR-dependent ISC increases proportionally with increased CFTR expression (ΔISC in non-corrected CF-HBEs $< \Delta\text{ISC}$ in corrected CF-HBEs $< \Delta\text{ISC}$ in non-CF HBEs). Thus, a treatment regimen combining a PDE4 inhibitor and a CFTR-corrector is a more promising therapeutic approach for CF than either drug by itself. Supported by the Cystic Fibrosis Foundation and the National Institutes of Health.

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PULMONARY INFLAMMATORY RESPONSES TO BACTERIAL CHALLENGE IN NEWBORN CF AND NON-CF PIGS

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There is broad agreement that inflammation is an early and major contributor to lung disease in individuals with cystic fibrosis (CF). The CF pig model provides a unique opportunity to study the timing of the onset of infection and inflammation in the CF lung. We previously showed that newborn CF pigs exhibit impaired innate immunity in the absence of pulmonary inflammation. This observation suggests that a host defense defect precedes the onset of inflammation in the CF lung; however, it does not address the possibility that CF lung disease may be further complicated by an impaired ability to respond appropriately to inflammatory stimuli. Here we test the hypothesis that acute inflammatory responses are perturbed in the absence of functional CFTR. We first investigated the responses of newborn CF and non-CF pigs to an inflammatory stimulus, heat-killed *Staphylococcus aureus* (SA). SA was aerosolized into the lungs of newborn CF pigs and

non-CF littermate controls. At 4 hours post-instillation, animals were euthanized and pulmonary inflammation was assessed. We found that, for both CF and non-CF pigs, a SA dose of approximately 10^9 CFU equivalents/animal was sufficient to elicit measurable changes in BAL inflammatory cell counts and IL-8 secretion. Overall, total inflammatory cells, PMN counts, and IL-8 production were similar in BAL fluid collected from CF and non-CF lungs after SA exposure, as were levels of the anti-inflammatory mediator lipoxin A_4 (LXA $_4$). Histopathological analysis confirmed that PMN infiltration in CF lung and trachea tissue was similar to non-CF littermate controls. Furthermore, we failed to detect significant differences in tissue myeloperoxidase activity in CF and non-CF pigs in response to SA challenge. In parallel, we studied well-differentiated primary airway epithelial cultures derived from CF and non-CF pigs. In agreement with our *in vivo* results, CF and non-CF epithelia treated with inflammatory stimuli (including TNF, heat-killed SA and gentamicin-killed *Pseudomonas aeruginosa*) produced similar levels of IL-8 and exhibited similar levels of NF- κ B activation at 1 and 20 hours post stimulation. Transcriptome array analysis of CF and non-CF pulmonary tissue and primary cultures is ongoing. In sum, we failed to find evidence that acute inflammatory responses are altered in newborn CF pigs. Our study does not rule out CF-associated problems with the *in vivo* resolution phase of the inflammatory response or genotype specific differences in the response to chronic inflammatory stimuli. These combined *in vitro* and *in vivo* approaches provide new insights into the complex relationship between loss of CFTR function and pulmonary inflammation. Supported by P01 HL-51670, P01 HL-091842, CFF, HHMI, and the Roy J. Carver Charitable Trust.

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ROCK SIGNALING IS AN IMPORTANT MEDIATOR OF AIRWAY GOBLET CELL MUCIN SECRETION

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We recently demonstrated that in addition to apical P2Y $_2$ -receptor agonists (e.g., ATP), serine proteases acting via basolateral protease activated receptor (PAR)1 and PAR2, and neutrophil elastase via unknown basolateral receptor/s are potent mucin secretagogues in airway epithelium. Here, we present data indicating that these different agonists require activation of Rho kinase (ROCK) to promote mucin secretion in both normal and CF airway goblet cells. Thus, mucin secretion induced by either PAR1 or PAR2 agonists, ATP, or elastase was markedly reduced in non-CF and CF primary human bronchial epithelial (HBE) cells pre-treated with the ROCK inhibitors H1152 or Y27632. Moreover, mucin secretion in response to PAR agonists or ATP was diminished in HBE cell cultures stably-expressing shRNAs that specifically decreased expression of ROCK1 or ROCK2. Similarly, in airway goblet-like Calu-3 cells, PAR agonist- and elastase-stimulated mucin secretion was also blocked by ROCK inhibitors H1152 and Y27632, as well as ROCK1 and ROCK2 shRNA.

In sum, our studies revealed that ROCK signaling is required for agonist-mediated mucin secretion in airway epithelial cells. Blocking of this step reduces mucin secretion in *in vitro* models of airway epithelia (i.e., HBE and Calu-3 cells). *In vivo* studies utilizing mouse models are being developed to validate ROCK as a putative therapeutic target to ameliorate mucin hypersecretion in CF lung disease.

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REAL-TIME TIRF MICROSCOPY IMAGING OF EXOCYTOTIC MUCIN RELEASE AND SWELLING IN CALU-3 CELLS

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Abnormally thick and viscous mucus and the resulting obstruction of small airways is the main cause of morbidity and mortality in cystic fibrosis (CF) patients. How this pathology is linked to mutations in the CFTR and abnormal Cl^-/HCO_3^- transport in CF epithelia remains unclear. Mucin formation, granular storage, and secretion into airway lumen are highly-regulated processes critical for normal lung physiology, yet our understanding of the underlying mechanisms is rudimentary. In this study, to gain mechanistic insights into the mucin secretion process we developed a real-time imaging assay based on total internal reflection fluorescence (TIRF) microscopy and applied it to acridine orange (AO)-stained Calu-3 cells. Confocal and fluorescence life time microscopy revealed that AO accumulates in high concentrations in intracellular granules producing a distinct red-shift of the fluorescence maximum (from 525-nm to ~650-nm) and a significant increase in the fluorescence life time (from 4.1-ns to ~12-ns) compared to that in the cytoplasm. TIRF microscopy allows the visualization of the exocytosis of such AO-stained granules and the study of the kinetics of post-exocytotic swelling of their fluorescently-labeled content with high sensitivity and a high temporal resolution. Mechanical stimulation of Calu-3 cells resulted in multiple secretion events that were seen in TIRF images as rapidly-expanding bright-fluorescence patches. The rate of their size expansion had a time constant τ between ~300-ms to ~1.5-s. After full expansion the mucus patch showed gradual decay of its fluorescence intensity with τ of 10- to 25-s. Under our experimental configuration secreted mucus remains in a confined (~0.1 μ m thick) layer between the apical cell membrane and the glass substrate, therefore the decay of fluorescence is mainly due to lateral diffusion of the fluorescent dye AO out of the mucus patch. Analysis of the fluorescence decay indicated that AO diffusion coefficient D (0.75×10^{-6} cm ^{2} /s) was ~10-fold smaller compared to that in bulk aqueous solution, consistent with significantly slower AO diffusion in a mucus gel. Estimates of the final volume of the released granule contents were in the range of 20- to 150- μ m ^{3} , which represent several-hundred-fold volume expansion for a typical granule of 0.4- μ m to 1- μ m diameter. In summary, the TIRF imaging assay allows the study of exocytosis of single mucin granules and the monitoring in real-time of post-exocytotic mucin swelling with high sensitivity and temporal resolution. This approach should help future investigations of mucin secretion in normal and CF epithelial cells and to test the role of CFTR in this process. (Supported by Cystic Fibrosis Canada and NCI R21 CA149897.)

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BICARBONATE HAS A ROLE IN MUCUS RELEASE IN ISOLATED PORCINE DISTAL AIRWAYS

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Introduction: Accumulation of mucus in lung distal airways has detrimental and fatal consequences in cystic fibrosis (CF). Bicarbonate has recently been demonstrated to play a crucial role in the release of mucus in intestinal and reproductive tissues. However, the participation of bicarbonate in mucus clearance in distal airways has not been demonstrated.

Hypothesis: Bicarbonate participates in the release and clearance of mucus in distal airways.

Objectives: To determine the role of bicarbonate in the release of mucus in porcine lung distal airways, and to identify proteins that may participate in the bicarbonate transport.

Methods: Isolated porcine distal airways of approximately 0.5-1 mm were incubated in the presence or absence of PGE2 and bicarbonate to study mucus release and its distribution. Morphological analysis of the distribution of mucus was performed in frozen sections from distal airways stained with

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periodic acid-Schiff (PAS). Effect of HCO_3^- on mucus release was analyzed by using inhibitors of bicarbonate transport 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS), 4,4'-dinitro stilbene-2,2'-disulfonate (DNDS), acetazolamide, and GlyH-101. RT-PCR technology was used to identify specific proteins that putatively participate in HCO_3^- transport.

Results: Histochemical analysis of PAS staining showed that mucus distribution and its accumulation in the isolated porcine distal airways was mainly observed around clusters of cells near the base of pleats of the small airways, as well as occasionally scattered cells along the epithelium. Isolated distal airways incubated in the presence of Ringer's buffer containing bicarbonate together with PGE2 apparently showed a qualitative increase in the distribution and release of mucus over the epithelial surface. Distal airways incubated only in the presence of either PGE2 or bicarbonate alone did not show visible change in mucus release. Distal airways incubated in the presence of inhibitors DIDS, DNDS with acetazolamide, and GlyH-101 showed inhibition of mucus release and its accumulation over the lining airway epithelium. RT-PCR analysis demonstrated the presence of the $\text{Na}^+/\text{HCO}_3^-$ NBCe1B cotransporter as well as the expression of CFTR and Slc26a6 in the porcine distal airway.

Conclusions: Our results provide further evidence that bicarbonate plays an integral part in mucus release in general and more specifically herein, in porcine distal airways. This process may be mediated by both basolateral NBC1 and luminal transporters CFTR and Slc26a6.

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100★

BICARBONATE PERMEATION OF PORCINE BRONCHIAL EPITHELIUM

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Anion secretion across the surface and glandular epithelia of airways is mediated by both CFTR and TMEM16A anion channels which function as the efflux pathway for Cl^- and HCO_3^- across the apical membrane of airway epithelial cells. Loop diuretics inhibit Cl^- secretion across airway epithelia through selective blockade of NKCC cotransporters in the basolateral membrane of secretory cells. A previous study with cannulated ex vivo porcine bronchi demonstrated that the loop diuretic bumetanide inhibited acetylcholine-stimulated mucous liquid secretion by 70% (Trout et al. *Am J Physiol* 275:L1095, 1998). Although it was assumed that all of the bumetanide-insensitive liquid secretion in this study was driven by the active secretion of HCO_3^- , the concentration of HCO_3^- in the mucous liquid was only 63.2 ± 5.0 meq/L after 2 hr, much lower than the expected anion total of approximately 150 meq/L. This previous finding has two potential explanations. First, the bumetanide-pretreated airways secrete 150 meq HCO_3^- /L with the majority of this anion diffusing out of the airway lumen across the epithelial barrier and being replaced by Cl^- influx during the 2 hr experiments. Second, a bumetanide-insensitive Cl^- secretion pathway is also present in porcine airway epithelia that sustains Cl^- secretion in the presence of this loop diuretic. We tested the first possibility by instilling either isosmotic NaHCO_3 (initially 0 meq Cl^- /L) or isosmotic NaCl (initially 0 meq HCO_3^- /L) solutions into the lumen of isolated, cannulated porcine bronchi that were immersed in normal Krebs solution (25 meq HCO_3^- /L; 122 meq Cl^- /L). Acetazolamide (10 μM) was added to the bath to inhibit carbonic anhydrase activity, but no acetylcholine was present. After 2 hr, the HCO_3^- concentration in the nominally 150 meq HCO_3^- /L instillates had fallen to 67.8 ± 11.9 meq HCO_3^- /L ($n=8$), a value that approximated what was previously measured in the study by Trout et al. In the airways that were filled with isosmotic NaCl (initially HCO_3^- free), the HCO_3^- concentration had risen to 8.2 ± 2.0 meq HCO_3^- /L ($n=8$). These data confirm the notion that the porcine bronchial epithelium is permeable to HCO_3^- and thus cannot maintain large HCO_3^- gradients across this barrier over time.

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MUCOCILIARY TRANSPORT IN NEWBORN PIGS

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Cystic fibrosis (CF) is a common genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Dysfunction of CFTR affects multiple organ systems, but most mortality results from lung disease. At birth the CF lung lacks the infection, inflammation, mucus plugging and airway remodeling that characterizes later CF lung disease. Some human studies suggest that mucociliary transport is defective in CF. This has led to the hypothesis that dysfunctional mucociliary transport initiates CF lung disease. However, such studies have not been conducted in newborns and thus mucociliary transport defects might be secondary to disease, rather than directly due to loss of CFTR function. We recently generated a CF pig model that recapitulates many findings seen in humans with CF, including the spontaneous development of lung disease. Within hours of birth, they manifest a host defense defect against bacteria. We hypothesized that newborn CF pigs would exhibit reduced mucociliary transport compared to non-CF pigs. To measure mucociliary transport, we developed a computerized tomography (CT) based assay. Briefly, we delivered radiopaque particles to airways and subsequently acquired serial CT images. Using a Cartesian coordinate system, we determined individual particle locations and quantified individual particle velocities and trajectories. Analysis of particle trajectory revealed that particles preferentially travel to the anterior trachea in a gravity-independent manner. We found that newborn pig non-CF maximum particle velocity was 11 ± 3 mm/min. We are currently analyzing particle velocities in newborn pig CF airways. These studies provide in vivo assessment of 3-dimensional mucociliary transport in newborn pigs. These studies will aid understanding of mucociliary transport and its role in the origin of CF lung disease.

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A RANDOMISED TRIAL OF HYPERTONIC SALINE NEBULISATION DURING HOSPITALISATION FOR PULMONARY EXACERBATION IN ADULTS WITH CYSTIC FIBROSIS

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Objectives: To determine the effects of hypertonic saline nebulisation during hospitalisation for an exacerbation of CF lung disease on length of hospital stay, day of exacerbation resolution, and improvement in lung function, symptom severity, oxygenation, bacterial load, exercise tolerance and quality of life.

Methods: A total of 132 adults, mean (SD) age 28 (9) y, FEV1 48 (20) %predicted, were randomised to thrice-daily inhalation of 4mL nebulised 7% saline or a taste-masked control of 0.12% saline throughout their hospital admission.

Results: No participants had an acute fall in their FEV1 of >15% after the first dose of their allocated inhalation solution. Length of stay was 12 days in the treatment group and 13 days in the control group (mean difference 1 day, 95%CI 0 to 2). Exacerbations (as defined by Fuchs 1994) resolved on day 10 in the treatment group and day 11 in the control group (mean difference 1 day, 95%CI 0 to 2). Participants were significantly more likely to have returned to their baseline FEV1 at discharge in the treatment group (75%) than the control group (57%), number needed to treat 6 (95%CI 3 to 65). On a visual analogue scale of symptom severity (0 to 100 mm) recorded daily throughout the hospital admission, the treatment group had significantly greater improvement than the control group in: sleep disturbance by 15 mm (95%CI 6 to 23), chest congestion by 9 mm (95%CI 4 to 14), and dyspnoea by 6 mm (95%CI 1 to 12). The greater improvement in the treatment group was of borderline statistical significance for fatigue (8 mm, 95%CI 0 to 15) and cough (6 mm, 95%CI 0 to 11). At discharge, the treatment group had less severe: sleep disturbance by 13 mm (95%CI 4 to 23), chest congestion by 10 mm (95%CI 3 to 18), and dyspnoea by 8 mm

(95%CI 1 to 16). Other outcomes did not differ significantly between groups.

Conclusion: The resolution of exacerbation symptoms was greater in rate and magnitude in the treatment group than the control group, and a greater proportion of the treated participants returned to their baseline FEV1. Thus, for a similar length of stay in hospital, nebulised hypertonic saline allows patients to leave hospital with greater resolution of their exacerbation.

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THE PROINFLAMMATORY CYTOKINES INTERLEUKIN-1 BETA, INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-ALPHA STIMULATE CFTR-DEPENDENT SECRETION ON AIRWAY SUBMUCOSAL GLANDS FROM SWINE

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Airway submucosal glands contribute most of the mucus and antimicrobial factors in the upper airways, which are important for the normal lung host defense. There is increasing evidence that submucosal glands function abnormally in the CF airways. Airway submucosal glands from CF patients and animal models of CF fail to trigger secretion when stimulated with some secretagogues (e.g. vasoactive intestinal peptide and substance P). Thus, it has been proposed that CF airways suffer from reduced delivery of antimicrobial factors and mucus that would abate mucociliary clearance and facilitate infection. However, the effect of inhalation of bacteria on submucosal gland mucus secretion is not fully understood. Our objective is to study the response of airway submucosal gland to bacteria inhalation and the role of CFTR. When inhaled pathogens land on the airway, they trigger the release of proinflammatory cytokines including interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α and these are elevated in CF airways. Thus, we tested the effects of IL-1 β , IL-6 and TNF- α on submucosal glands mucus secretions and the role of CFTR. Secretion assay experiment show that all the proinflammatory cytokines tested, IL-1 β (50 ng mL⁻¹), IL-6 (50 ng mL⁻¹) and TNF- α (50 ng mL⁻¹), stimulate submucosal glands mucus secretion through CFTR-dependent mechanism, which can be blocked with CFTRinh 172 (100 μ mol L⁻¹). Also, the results indicate that IL-1 β stimulation triggers a cAMP intracellular second messenger pathway. Inhibition of adenylate cyclase with the blocker SQ22536 (0.5 mmol L⁻¹) reduced mucus secretion by IL-1 β -stimulated submucosal glands while treatment increasing cAMP level with the phosphodiesterase inhibitor IBMX (1 mmol L⁻¹) increased mucus secretion. Using scanning Ion-selective Electrode Technique (SIET), that directly measures the flux and species of ion transported across epithelia, we were able to determine that both IL-1 β and IL-6 act directly on the serous acini of the glands. These cytokines stimulate transepithelial transport of Na⁺ and Cl⁻ but not K⁺ through a cAMP-PKA pathway. The Na⁺ and Cl⁻ transport triggered by IL-1 β and IL-6 was blocked by the CFTR inhibitor, CFTRinh172. The results indicate that IL-1 β , IL-6 and TNF- α , released in response to bacteria, stimulates CFTR-mediated transport that would increase mucus and antimicrobial factors that would help expel the pathogen from the airways. In CF airways, however, this response may be lost, resulting in reduced delivery of antimicrobial factors and development of CF airway disease. This is the first direct measurement of ion transport across a single anatomical domain of the submucosal glands. In the future, the role of the other segments of airway submucosal gland on modulating the ion composition of the secreted fluid will be determined.

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INCREASED MUCUS SECRETION BY TRACHEAL SUBMUCOSAL GLANDS IN RESPONSE TO PSEUDOMONAS AERUGINOSA USING SYNCHROTRON RADIATION

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There is evidence showing that cystic fibrosis lung disease reflects the failure of the innate defense mechanisms of the lung against inhaled organisms such as *Pseudomonas aeruginosa*. Normal airways are protected from inhaled "insults" by a complex immune defense system that includes mucus containing antimicrobial factor that traps and inactivates bacteria favoring clearance from the airways. Recently, it has been suggested that airway submucosal gland dysfunction is a primary defect in CF that may contribute to airway pathobiology. In normal airways inhalation of bacteria would trigger antimicrobial factors and mucus secretion by submucosal glands in the upper airways, but this response would be missing in CF resulting in reduced delivery of antimicrobial factors, thus facilitating infection. However, the response of airway submucosal glands to inhalation of bacteria has never been directly tested. One of the main obstacles to study bacteria-airway interaction in situ is the lack of suitable techniques with appropriate resolution and contrast. There is substantial evidence that synchrotron-based phase contrast imaging (PCI) and diffraction enhanced imaging (DEI) can be used to investigate the air/fluid interface in the lung. The large differences in refractive index between the air and fluids/tissue give a very strong refraction signature that is easily detected using phase related imaging methods.

Our objective is to test the viability of synchrotron-based phase related imaging methods to image the air/mucus interface in isolated tracheas to study the effects of bacteria-laden agarose beads on submucosal glands mucus secretion.

Our results show that synchrotron-related PCI and DEI technique can be used to image the air and mucus interfaces in isolated tracheas. Although it is hard to visualize the mucus/tissue interface, we manage to determine the position of the tissue relative to the mucus layer using agarose beads loaded with the contrast agent barium sulfate. The energy of X-ray used in the experiment was 20 KeV, and the resolution of the detector was 8.75 μ m. Agarose beads, which have a size range from 200 to 600 μ m, were made by mixing inactivated clinical isolates of *P. aeruginosa* (boiled for 10 min) with agarose to 4% W/V in PBS. We added 233 g L⁻¹ of barium sulfate; however, since this compound is not soluble in aqueous solution, it did not contribute to the osmotic pressure of the agarose beads. Our results show that 30 minutes after instillation of the agarose beads, the mucus layer height increased by 34.5 $\pm$ 3.8 and 9.5 $\pm$ 6.1 μ m for bacteria-laden agarose beads and bacteria free beads, respectively. For future experiments, we will use the same technique to measure the response of submucosal glands to inhaled bacteria-laden agarose beads in living swine, both wild type and CFTR-/-.

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CF AIRWAY CELLS DO NOT HYPERABSORB FLUID FOLLOWING NEBULIZED HYPERTONIC SALINE

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Introduction: An underlying dogma of CF holds that CF epithelial cells lack chloride secretion due to defective CFTR and hyperabsorb sodium through an unregulated Na⁺ channel, resulting in dehydration of the airway surface liquid (ASL) and thickened airway mucus. Inhaled hypertonic saline (HS) has shown to be effective at stimulating mucus clearance and improving lung function. The proposed mechanism of action of HS is that an osmotic gradient is created on the airway surface, leading to temporary rehydration of the mucus layer. We hypothesized that regardless of tonicity, when nanoliter volumes of fluid are nebulized onto the airway surface, CF cells will hyperabsorb fluid from the ASL faster than non-CF cells.

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Methods: Well differentiated CF and normal human bronchial epithelium (HBE), cultured on air-liquid interface, were examined under XZ-confocal microscopy to determine ASL height. Cells were treated acutely with nebulized doses of either normal saline (NS) or 7% HS to achieve a steady state ASL height increase of ~ 50 μm . Reabsorption rates were then calculated as cells were allowed to absorb excess ASL fluid.

Results: When subject to treatment with NS, CF cells had a faster rate of ASL fluid absorption compared to normals (mean rate for CF 1.92 $\mu\text{m}/\text{min}$ vs. normals 0.76 $\mu\text{m}/\text{min}$, $p=0.049$). In contrast, we found that in response to nebulized HS, the rate of fluid absorption from CF cells was not significantly different from that of normals (1.12 $\mu\text{m}/\text{min}$ vs. 1.75 $\mu\text{m}/\text{min}$, $p=0.289$). While the reabsorption rate between NS and HS was not statistically significant in the CF group, the trend was clearly for faster absorption in the presence of NS compared to HS. This is in contrast to normal cells, where the trend was towards faster absorption in the presence of HS. Outcomes were the same whether analyzing resorption rates or integrated area under the curve of total hydration time.

Conclusions: Our data presented here reinforces the concept of sodium hyperabsorption of CF cells compared to normal cells when both are exposed to isotonic fluid conditions. However, our data do not support a statistically significant difference in reabsorption rates between CF and normal cells when the excess fluid is administered as HS. Interestingly, we found that normal cells tended to absorb faster in the presence of HS-induced airway hydration, whereas CF cells exhibited slower reabsorption. The mechanism by which these differences in reabsorption rates occur is currently not known.

Sustained ASL rehydration in response to HS powder deposited on CF HBE has been previously documented; however our earlier experiments using nebulized HS showed a shorter rehydration time course than expected. The data presented here confirm that while CF hyperabsorption is ameliorated by the application of nebulized HS, the total duration of ASL rehydration is not overtly prolonged. Further studies will explore changes in ion transport, water flux, and mucus rheology as related to this phenomenon.

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MYOFIBROBLAST PROLIFERATION AND FIBROSIS IN CF LUNG DISEASE

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Background: End-stage human CF lung disease is associated with extensive pulmonary fibrosis. Myofibroblasts are TGF-beta stimulated profibrotic cells that respond to persistent tissue injury and mediate pulmonary remodeling. The role of myofibroblast differentiation in CF pathogenesis has not been investigated previously. In this study, we hypothesized that myofibroblast proliferation contributes to airway and vascular remodeling in human and porcine CF lungs.

Methods: WT and CF human and porcine lung tissues were evaluated for expression of TGF-beta, pSmad2 (a mediator of TGF-beta signaling), alpha-smooth muscle actin (a marker of myofibroblast differentiation), and Masson's Trichrome (collagen deposition). In early passage cell culture, TGF-beta release was measured using a TGF-beta bioassay and myofibroblast differentiation was quantified with FACS analysis. Tissue morphometry was quantified utilizing Bioquant (Nashville, TN) and MetaMorph (Sunnyvale, CA) image analysis software. Porcine lung tissue was provided courtesy of the University of Iowa Cystic Fibrosis Research Center.

Results: TGF-beta signaling was markedly elevated in human CF lungs and associated with increased myofibroblast differentiation and intense tissue fibrosis. Myofibroblast differentiation was increased 4-fold ($p < .005$) compared to normal lung tissue and approached levels observed in idiopathic pulmonary fibrosis (IPF). Lung specimens were evaluated from 2 WT (age 2 months and 3.5 months) and 3 CF (CFTR^{-/-}, CFTR^{ΔF508del}, ages 2 - 5.5 months) piglets. In WT and CF pigs with mild pulmonary pathology, fibrosis was minimal. In CF sections exhibiting severe disease, markedly fibrotic areas were identified that stained for collagen and alpha-smooth muscle actin fibers (demarcating myofibroblasts). Myofibroblast differentiation was increased 3-fold ($p < .01$) in CF piglets with severe disease. These myofibroblast-associated abnormalities extended from peripheral bronchioles and vascular adventitia, and obliterated surrounding lung parenchyma. In vitro studies of early passage porcine and human CF fibroblasts indicat-

ed increased TGF-beta production ($p < .05$) and increased myofibroblast differentiation ($p < .05$) in the CF derived samples compared to WT.

Discussion: Our studies implicate increased TGF-beta signaling and resulting myofibroblast hyperplasia as key contributors to CF pulmonary fibrosis with resultant collagen deposition and distortion of tissue architecture. The profibrotic pathway described here is a cause of lung injury and pulmonary decline in diseases such as IPF, obliterative bronchiolitis (OB) and scleroderma-associated ILD, but has not been reported previously in CF. We propose that this pathologic mechanism—which is strongly manifest in both human and porcine cystic fibrosis lungs—confers abnormalities in lung mechanics, gas exchange, and hemodynamics. Importantly, tissues from maturing CF piglets demonstrate pronounced airway and vascular remodeling with extensive myofibroblast proliferation in areas of obliterative fibrosis. The CF pig should therefore serve as an important model to investigate longitudinal fibrogenesis and causative myofibroblast proliferation, including changes that occur at the inception of cystic fibrosis lung injury.

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MICRO-RNA A KEY PATHWAY TO REGULATE ANO1 ACTIVITY IN CF BRONCHIAL EPITHELIAL CELLS

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ANO1 (TMEM16a) is a calcium-activated chloride channel (CaCC) expressed in secretory epithelia, as in lung epithelial cells. In this context, ANO1-targeted activators may be useful for drug therapy in cystic fibrosis to compensate CFTR chloride deficiency. We previously have demonstrated that mRNA, protein expression of ANO1 are down regulated in CF cell lines, in CF mice lung and in human bronchial tissues. More, we have also demonstrated that ANO1 activity is significantly decreased in CF cells.

To elucidate how ANO1 protein is regulated, we performed micro-RNA (miRNA) profiling because miRNA are an important class of regulators of gene expression. Thus, miRNA are predicted to regulate more than 50% of mammalian proteins. To date, several groups have examined the potential role of miRNA in molecular pathways involved in CF, including CFTR and inflammatory proteins; however, despite its potential value as a therapeutic target, miRNA regulation of ANO1 itself has not been investigated.

To analyze the impact of miRNA on ANO1 protein expression, we used two independent but complementary approaches.

1. Computational prediction of miRNA responsive elements within the 3'-UTR of ANO1 transcript was performed using common prediction databases (PicTac, miRANDA, miRBase, TargetScan and miRDB); and

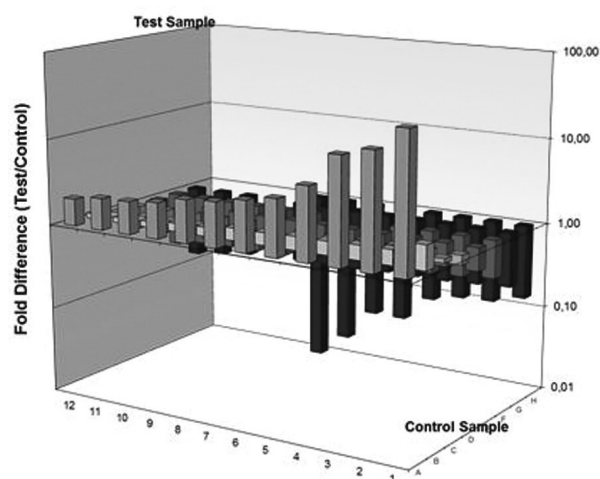
2. qPCR microRNA expression profiling (miRNome) comparing the miRNA expression between non-CF and CF cells (16HBE14o- vs. CFBE41o- and S9 vs. IB3-1) was accomplished.

Bioinformatic prediction use algorithms searching for sequences with perfect or nearly perfect pairing to the 3'-UTR sequence of the target protein. Overall, we registered more than 30 miRNA target predictions but only four were predicted by the five miRNA programs.

In parallel, the miRNome have highlighted a differential expression of some miRNA that could participate in ANO1 dysregulation. In these miRNome, the four miRNA predicted are present and involved.

To confirm this result, we decided to analyze by qPCR these four miRNA in different CF cell lines but also in tissue from CF and non-CF patients in different level of lung. The implication of these miRNA to confirm ANO1 regulation will be performed by functional experiments using mimic or inhibitor plasmid of each miRNA. We will assess the miRNA over-expression and inhibition on ANO1 expression and activity.

Investigating the expression and function of miRNA in CF will shed light on previously unidentified regulatory mechanisms controlling changes in gene expression and direct the development of future therapeutic strategies.



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TGF-BETA DOWNREGULATION OF CFTR AND TMEM16A THROUGH P38 MAPK IN HUMAN COLONOCYTES

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Background: TGF- β is a pleiotropic signaling molecule with important roles in tissue injury, inflammation and repair. It is a known genetic modifier of the CF phenotype, but the mechanism linking TGF- β activity to disease modification is unknown. Published studies have described TGF- β down regulation of CFTR expression and activity in T84 colonocytes through p38 MAP Kinase. TGF- β down-regulation of CFTR may be a means to limit epithelial chloride secretion, but the relevance of this finding to CF disease modification is unclear. We examined the influence of TGF- β on CFTR and TMEM16A expression and activity in T84 cells.

Methods: T84 cells were grown and seeded onto Costar 0.3 μ M permeable supports 5-7 days prior to studies in Ussing chambers. Cells were serum starved for 24 hours (0.1% FBS), and then exposed to experimental conditions for 48 hrs (control, 10 ng TGF-beta, TGF-beta + p38 MAPK inhibitor VIII). Inserts were then mounted and studied under voltage clamp conditions, warmed to 37°C and continuously gassed with 95%O₂/5%CO₂ in RPMI 1640 media + 25 mM HCO₃. Cells were treated with 10 μ M indomethacin (20 min), 100 μ M amiloride (10 min), 10 μ M forskolin + 100 μ M IBMX to activate CFTR or 2 μ M ionomycin to activate TMEM16A (10 min), 100 μ M carbachol (10 min), and 10 μ M CFTRinh172 (10 min). At the end of the ICM studies, cells were lysed for protein detection by IB or to monitor expression by RT-PCR. Cell calcium levels were monitored with Fluo3, and cAMP levels were measured by ELISA.

Results: ICM: Following treatment of monolayers with TGF- β (10 ng/mL 48 hrs), currents (forskolin/IBMX + carbachol) were reduced to 31.6% of control conditions (p=0.003), and similar reductions were seen following basolateral membrane permeabilization with nystatin (50 μ g/mL). TGF- β downregulated CFTR currents were partially restored by co-treatment with a p38 MAPK inhibitor (68.4% of control, p=0.04). TMEM16A currents following TGF- β treatment were reduced to 34% of control conditions (p=0.001), and similar reductions were seen following basolateral membrane permeabilization with nystatin (50 μ g/mL). Biochemistry: CFTR C Band was reduced to 28% of control conditions by TGF- β treatment, which was reversed by p38 MAPK inhibition (return to 83% of control, p=0.0012). TMEM16A protein was reduced to 30.2% of control conditions, which was partially restored by p38 MAPK inhibition (increase to 65% of control, p=0.04). RT-PCR: CFTR and TMEM16A transcript levels were reduced in a time dependent fashion over 24, 48, and 72 hours of TGF- β treatment (p<0.05). Calcium and cAMP: TGF- β treatment had no significant effects on cell calcium or cAMP levels following stimulation with forskolin/IBMX or ionomycin.

Conclusions: The results indicate that TGF- β potently downregulates CFTR and TMEM16A expression and Cl⁻ transport in T84 human colonocytes. The effects are reversible by co-treatment with a p38 MAPK inhibitor. The results provide a potential mechanism linking TGF- β to CF disease modification through TMEM16A, and provide a model by which enhanced TGF- β signaling could produce CF-like symptoms in CFTR-expressing epithelia.

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CARBACHOL-INDUCED INTERNALIZATION OF HUMAN MEMBRANE MUCIN MUC17 IS CONCOMITANT WITH CFTR EXTERNALIZATION IN ENTEROCYTES

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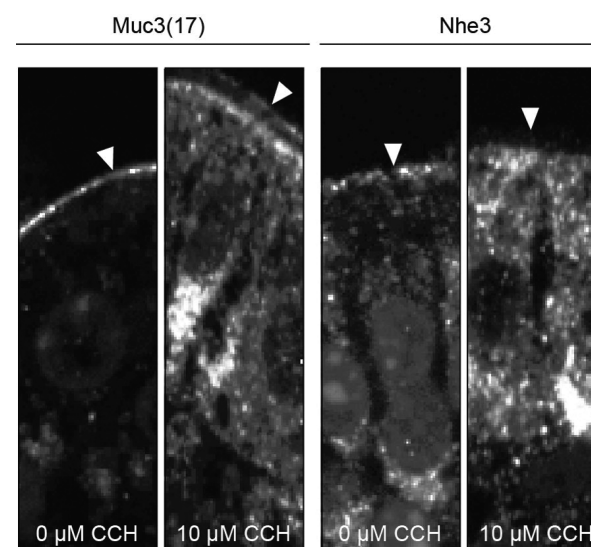
Transmembrane mucins are highly O-glycosylated, membrane-tethered proteins covering the apical surface of epithelial cells in luminal organs such as the lungs and the gastrointestinal tract.

We have previously reported that murine membrane mucin Muc3(17) is retained apically in enterocytes via an interaction with the protein Pdzk1. Furthermore, we proved that the human orthologue of Muc3(17), MUC17, binds PDZK1 *in vitro*. PDZK1 is a common adaptor protein for many ion channels. Both CFTR and NHE3 bind to and are partially regulated by PDZK1.

Using labeling techniques in cell cultures, mouse models and confocal imaging, we have investigated the interplay between membrane mucin MUC17, CFTR and NHE3. In mouse, expression of Cfr and Nhe3 on apical surfaces of enterocytes is regulated by the cholinergic agonist carbachol. Upon stimulation with carbachol, Cfr is mobilized to the outermost part of microvilli, while Nhe3 is internalized.

Here, we show that apically expressed MUC17 in Caco-2 cells and Muc3(17) in mouse duodenal enterocytes are internalized upon stimulation with carbachol, thus mimicking the response of NHE3/Nhe3 to carbachol. Our results reveal a well-orchestrated trafficking pattern where membrane mucins and ion channels that are crucial for intracellular and extracellular homeostasis, are co- and counter-regulated by carbachol.

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Muc3(17) and Nhe3 are internalized upon stimulation of enterocytes with carbachol. White arrows indicate the outermost part of microvilli.

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EXPRESSION OF PROLYL ENDOPEPTIDASE IN BRONCHIAL EPITHELIAL CELLS: IMPACT OF F508DEL CFTR

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CF airways are characterized by a persistent neutrophilic inflammatory response. Recently, our laboratory has described a novel neutrophil chemoattractant derived from collagen, proline-glycine-proline (PGP), as a critical regulator of this dysregulated inflammatory response. This peptide is generated by a discrete protease cascade; the terminal enzyme in the cascade, prolyl endopeptidase (PE), is expressed in neutrophils but it is unknown if lung structural cells express this enzyme. The purpose of this study was to examine expression of this enzyme in bronchial epithelial cells and to determine if cells expressing mutant F508del vs wildtype (WT) CFTR differentially express prolyl endopeptidase.

Initially, lysates from multiple cell lines (parental CFBE, Calu-3, 16-HBE) demonstrated variable PE expression by Western blot, RT-PCR, and PE activity assay. CFBE cells stably overexpressing WT or F508del also showed increased PE expression but F508del cells demonstrated increased expression compared to WT cells. Primary human airway epithelial (HBE) cells from F508del CF patients also demonstrated elevated active PE compared to primary HBE cells from non-lung disease controls with WT CFTR. Lysates from primary HBE cells co-incubated with collagen generated PGP, with increased PGP production observed with the F508del cells. The generation of PGP from primary HBE cells was inhibited by the addition of S-17092, a cell-permeant PE inhibitor.

In conclusion, PE is expressed in airway epithelial cells. Cells expressing F508del have increased PE expression. Lysates from primary airway epithelial cells are capable of generating PGP from collagen and inhibiting PE blocks PGP production. These findings offer additional insights into unique mechanisms by which airway epithelial cells may contribute to ongoing neutrophilic inflammation in CF lung disease.

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DIFFERENTIAL REGULATION OF INTRINSIC INFLAMMATION AND RESPONSE TO EXTRINSIC INFECTION IN CYSTIC FIBROSIS LUNG EPITHELIAL CELLS BY MIR-155 AND MIR-215

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Cystic fibrosis (CF) is due to mutations in the CFTR gene, which cause a massively proinflammatory phenotype in the CF airway. The chemical basis of the inflammation is hyper-production of interleukin-8 (IL-8) by CF airway epithelial cells, based on both an intrinsic mutation-dependent mechanism, and by infection. In infection-free, cultured CF lung epithelial cells, high levels of the microRNA (miR), miR-155 is responsible for hyper-expression of IL-8. However, whether infection-induced IL-8 expression in CF cells is also mediated by miR-155 is not known. We have hypothesized that miR-155 might be a general mediator of enhanced IL-8 expression in CF cells, either in response to other cytokine/chemokine mediators of inflammation, or following exposure to infectious agents. We have found that a reduction in miR-155 accompanies suppression of IL-8 by either the anti-inflammatory cytokine IL-10, or by inhibition of ambient IL-1 β with a neutralizing antibody. However, attempts to elevate IL-8 levels with either intact bacteria (viz. a mucoid strain of *Pseudomonas aeruginosa* (PA)), or lipopolysaccharide (LPS), were unable to elevate miR-155 above its intrinsically high level in the absence of these agents. Instead, in response to PA-infection, the CF cells modestly suppress the expression of miR-155. Instead, the cells express a novel set of miRs, including miR-215. The predicted module of mRNA targets focus on activation of the NF κ B signaling pathway, and on the pro-apoptotic p53 signaling pathway. These data indi-

cate that CF lung epithelial cells are at a serious survival disadvantage when confronted with *P. aeruginosa*, or bacterial cell products.

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INTERLEUKIN-13 INDUCES COMPARABLE GOBLET CELL HYPERPLASIA IN CYSTIC FIBROSIS AND NON-CYSTIC FIBROSIS AIRWAY EPITHELIAL CULTURES

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Respiratory disease in cystic fibrosis (CF) is characterized by chronic infection, mucopurulent obstruction and bronchiectasis. At the cellular level histopathology reveals marked goblet cell hyperplasia (GCH) in diseased CF human airway epithelia. Similar findings are observed in older CF pigs. In contrast, GCH is absent in the airway epithelia of newborn CF pigs. Interleukin-13 (IL-13) has been shown to induce GCH in cultured airway epithelia.

The goal of this research is to further understanding of how goblet-cell differentiation promoting effects of IL-13 are altered in the setting of CF. We hypothesized that IL-13 treatment would cause more GCH in CF airway epithelia cultures than non-CF airway epithelia cultures. Non-CF and CF airway epithelia cells were harvested from adult human donors and newborn pigs. Cells were cultured at the air-liquid interface and treated with IL-13 once they reached confluence. After 21 days epithelia cultures were histologically processed for periodic acid-Schiff (PAS) staining. Goblet, ciliated, and other epithelial cell populations were quantified.

IL-13 administration led to significant GCH in all experimental groups compared to control groups. The amounts of GCH induced in non-CF and CF epithelia were not significantly different. IL-13 administration also led to significant decreases in ciliated cell populations in all treated groups, with these changes again being similar between non-CF and CF epithelia. In summary, IL-13 induces similar GCH in both CF and non-CF airway epithelia. These findings suggest that the GCH found in CF is likely secondary to chronic infection and inflammation rather than a primary consequence of decreased CFTR function.

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ENHANCED AMILORIDE RESPONSE IN F508DEL MOUSE NASAL EPITHELIUM DOES NOT CORRELATE WITH PROGRESSIVE DEGENERATION OF OLFACTORY EPITHELIUM

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The CF mutant mouse nasal epithelium, excised and in situ, is frequently used as a model of CFTR deficient airway and in pharmacological and therapeutic gene correction studies. However, this tissue is highly complex, consisting of olfactory and respiratory epithelium, as well as an extensive subepithelial gland system covering an intricately septated nasal cavity. Prominent among the reported phenotypic characteristics of CFTR deficient (KO) mice are an enhanced amiloride response, and abnormal mucous blebbing in the nasal airway epithelium. Further, a distinct age dependent degeneration of the neuronal layer of olfactory epithelium is observed (Grubb, 2007; Grubb 2009; Hilliard 2008). It was suggested that the enhanced amiloride response would indicate enhanced ENaC dependent fluid resorption, and that this could explain the neuronal degeneration.

We have further studied this aspect in strains with the CFTR KO cam and F508del CFTR (Cfr-tm1eur) alleles backcrossed to the FVB genetic background. Skulls from CFTR deficient and normal mice were collected from animals aged 30-34 weeks (N= 5-10 per experimental group). After fixation and decalcification histological analysis was performed. We could confirm that in CFTR KO (FVB) olfactory epithelium a significant reduction of neuronal cell layer occurred (from average 8 to 3 nuclei, P< 0.01). However, no such degeneration was observed in F508del CFTR (FVB, C57Bl/6, 129/FVB) homozygous mice. We previously reported that excised

olfactory epithelium of both CFTR KO (FVB) and F508del CFTR (FVB) show a five to six fold enhanced amiloride response in Ussing chambers. Similar observations were made in F508del CFTR mice backcrossed to C57Bl/6 and in the 129/FVB outbred strain (Wilke, 2011). We can therefore rule out this parameter as a single cause of the neuronal degeneration. The lack of neuronal degeneration does correlate with a forskolin response of olfactory epithelium in F508del CFTR (FVB), presumably caused by residual activity of mutant CFTR in this tissue, which is absent in CFTR KO (FVB) (Wilke, 2011). This residual activity, expressed in a specific cell type (brush cell) in the mouse olfactory epithelium, apparently corrects neuronal degeneration. However, the residual CFTR activity was not sufficient to prevent mucous blebbing, which was observed in mutant nasal airway of all F508del CFTR strains, but not in wild-type littermates. Further, it did not prevent the enhanced amiloride response observed under Ussing chamber conditions.

We conclude that a high amiloride response, and presumed enhanced activity of ENaC driven resorption, may contribute to the degeneration of CF KO mouse olfactory epithelium, but that this degeneration can be rescued by residual F508del CFTR secretory activity in olfactory brush cells without reducing the enhanced amiloride response. The relevance of these data for human CF pathology remains to be investigated in the light of reported F508del CFTR expression in nasal airway epithelium.

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THE ROLE OF SHEDDASES IN CYSTIC FIBROSIS LUNG DISEASE

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We investigate the molecular pathways involved in CF lung disease to define new druggable targets. We previously observed that lung injury causes sustained induction of the EGFR agonist Amphiregulin (AREG) and IL-6 in F508del CFTR mutant mice (Cftr tm^{leu}) compared to normal. We hypothesise that these pathways contribute to abnormal resolution of injury (epithelial metaplasia, fibrosis) and inflammation in CF lung disease. Both AREG and the IL-6 co-receptor IL-6R are substrates of the membrane-bound metalloproteinase ("shedase") ADAM17, allowing trans-activation of neighbouring cells by cleavage of their active extracellular domains. IL-6R is produced as a shedase sensitive membrane bound form, and as a soluble mRNA splice variant (sIL-6R).

Here we report that in differentiated primary human bronchial epithelial cells grown on permeable supports (HBEC-ALI), IL-6R antigen is released to both apical and basolateral compartments. However, apical secretion is ten fold lower than basolateral in all four separate donor cultures (P<0.01). Basolateral but not apical secretion of IL-6R is partially inhibited (30-50%; n=8 filters from three donors P<0.02) by the specific ADAM17 inhibitor TMI-2 (Wyeth). The non-specific ADAM inhibitor TMI-1 inhibited IL-6R secretion to the same extent, ruling out a contribution of other ADAMs, and further suggesting that the residual secreted IL-6R antigen is the soluble mRNA splice product sIL-6R.

We conclude first that HBEC-ALI secrete IL-6R to two differentially regulated compartments separated by tight junctions. This has important consequences for the interpretation of data obtained with lavage fluids from patients and for attempts to modulate IL-6 activity using IL-6R decoys and therapeutic antibodies applied to the mucosa. Basolateral IL-6R shedding is partially sensitive to ADAM inhibitors, which could be effective in attenuating inflammation and tissue remodelling. We are presently investigating RNAi delivery to achieve further knockdown of IL-6R secretion. Delivery of stabilised IL-6R targeting siRNA (Ribox) to submerged HBEC on filters reduced IL-6R secretion for fifty to ninety percent for up to ten days.

Current studies are aimed at elucidating the effect of CFTR deficiency, tissue injury and oxidative stress on shedase activity towards the EGFR agonists and IL-6/IL6R, two pathways that converge on the transcription factor STAT3, a known genetic modulator of CF lung disease.

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IGE, IL-4 AND IL-8 INTERACT TO REGULATE AIRWAY SMOOTH MUSCLE (ASM) HYPERRESPONSIVENESS IN CF

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Background: CFTR has a range of cellular functions and is expressed in many cells, including human ASM (Michoud et al., *Am J Respir Cell Mol Biol* 2009;40:217). Recent evidence has linked over-expression of CFTR (Cohen et al., *Devel Dynamics* 2006;235:2736) or impaired expression (CF phenotype) to ASM contractile protein expression and signaling. IL-8, abundant in airways of CF patients and increased with infectious exacerbations, increases the phosphorylation of myosin light chain (MLC) in both normal and CF human ASM, but to a significantly greater extent in CF ASM grown in a contractile phenotype (Govindaraju et al., *Respir Res* 2008;9:76). Because many CF patients with airway hyperresponsiveness also have elevated serum IgE levels (atopy, multiple drug allergies, ABPA and related syndromes) we sought to examine the effect of polyclonal IgE on downstream contractile signaling in ASM and control IL-4 on cultured human ASM gene expression by PCR array targeting common inflammatory cytokines and receptors.

Methods: Human ASM primary cultures were prepared from tracheal or bronchial segments discarded from transplanted donor lungs. Isolated cells were seeded at a density of 5000 cells/cm² in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. To induce a contractile phenotype, confluent cells were grown for 7 days in serum-free Ham's F-12 medium. From both proliferative and contractile cells treated for 4 days with either vehicle, or 5 µg/mL IgE, or 10 ng/mL IL-4, mRNA was extracted and cDNA prepared by PCR. From cells grown in a synthetic phenotype we isolated cell protein and assayed for myosin light chain kinase by Western blot with chicken gizzard as standard control. A high molecular weight biotinylated marker was loaded in each gel to visualize the MLCK band based on molecular weight (Chitano et al., *Pediatr Pulmonol* 2004;38:456).

Results: Exposure of human ASM to 4 days of IgE without antigen resulted in a ~2-fold increase in MLCK protein expression. IgE induced the de novo expression of IL-8 in the proliferative phenotype and altered the expression in more than 10 genes in either proliferative (CXCL3 and CCR7) and contractile phenotype (C3 and TNF). Of note the increase in gene expression for IL-8 in the contractile phenotype was 2.5-fold (P < 0.01). In contrast, IL-4 altered the expression of more genes in the contractile phenotype (including IL-8).

Conclusions: We conclude that polyclonal IgE without antigen increases the protein expression of MLCK in the synthetic phenotype of human ASM and induces de novo the gene expression of IL-8. In the contractile phenotype of human ASM both IL-4 and IgE increase the gene expression of IL-8. Taken together, these data suggest links between increased circulating IgE, such as is seen with atopy, drug allergy and ABPA, and the ASM expression of MLCK, and a short circuit stimulation by IgE and IL-4 of gene expression of IL-8, known to increase the phosphorylation of MLC in CF ASM. Thus, IgE, IL-4 and IL-8 appear to work cooperatively and potentially synergistically to augment the phosphorylation of MLC in ASM and likely contribute to airway hyperresponsiveness in atopic states and with common bacterial infections in CF.

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CHANNEL ACTIVATING PROTEASES (CAPS): IDENTIFICATION OF NOVEL TARGETS FOR CORRECTING SODIUM CHANNEL DYSFUNCTION IN CYSTIC FIBROSIS

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Proteolytic cleavage of the endogenous α and γ subunits of ENaC (by channel activating proteases (CAPS)), is a key regulatory mechanism for ENaC channel activity. Excessive ENaC activity is a feature of CF which contributes to the depletion of the ASL (airway surface liquid) with resultant impairment of mucociliary function. Growing evidence suggests protease/protease inhibitor imbalance may underlie increased Na⁺ absorption in

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CF airway epithelia therefore inhibition of the CAPS-ENaC signalling axis represents a potential therapeutic target to restore mucociliary function.

Our group has developed a panel of active-site directed affinity probes which target and inhibit trypsin-like proteases (potential CAPS) including the broad-spectrum inhibitor QUB-TL1. QUB-TL1 selectively inhibits recombinant trypsin-like proteases (trypsin, prostatic, furin) without affecting non-trypsin like proteases (neutrophil elastase); and furthermore inhibits trypsin-like protease activity both in intact cells and in cell-conditioned media (CCM) retained from primary epithelial cell cultures. By targeting the biotin reporter group on QUB-TL1 with anti-streptavidin-HRP we can visualise several candidate CAPS in 1) intact non-CF and CF epithelia (differentiated primary nasal epithelial cells); and 2) CCM from these epithelial cell cultures. Experiments where we probe QUB-TL1 treated primary epithelial cells with an anti-prostatic (CAP1) antibody after an initial affinity purification step (to pull down the biotinylated protein fraction) indicates QUB-TL1 interacts with prostatic in these preparations. We have also sought to examine the effect of QUB-TL1 on ENaC γ subunit cleavage status in the apical membrane protein fraction of non-CF and CF differentiated primary bronchial epithelial cells. We find a number of differences between non-CF and CF epithelial cells in this set of experiments. (1) CF cells lack the presence of a full-length (likely inactive) high molecular weight form (~90kDa) of ENaC γ and (2) exhibit a much greater abundance of a lower (likely active) molecular weight form of the γ subunit (~60kDa). In cell cultures treated with QUB-TL1 the 60kDa fragment is largely reduced. The 90kDa species is not restored in CF cells and remains completely absent.

Conclusion: Our broad-spectrum affinity based probe detects and inhibits several trypsin-like species (including CAP1 (prostatic)) in non-CF and CF epithelial cell preparations. Moreover, our examination of the ENaC γ subunit status in the apical membrane of bronchial epithelial cells indicates key differences between non-CF and CF cells consistent with heightened ENaC channel activity (in CF). Treatment of these cultures with QUB-TL1 indicates a potential reduction in ENaC γ subunit cleavage; which may result in reduced ENaC channel activity. This work paves the way for further characterisation of these potential CAPS and a more detailed assessment of their effect on ENaC function.

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DETERMINATION OF ANO1 EXPRESSION, LOCALIZATION AND ACTIVITY IN CYSTIC FIBROSIS AIRWAYS

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Defective CFTR function in the airway epithelium is responsible for CF patient lung disease. In CF airways, mucus hypersecretion, infection and inflammatory processes contribute to airways degradation leading to a decreased lung function. ANO1 (Anoctamin 1) also called TMEM16a (Transmembrane protein 16 a) is a calcium-activated chloride channel (CaCC) recently described by three independent teams. This chloride (Cl⁻) channel is expressed in secretory epithelia as airway epithelium. Mice lacking ANO1 show a decrease in calcium-dependent Cl⁻ secretion associated with a reduced mucociliary clearance demonstrating the substantial physiological role of ANO1 in the airways. ANO1 is an eight transmembrane protein with two predicted sites for phosphorylation by extracellular regulated kinase 1,2 (ERK1,2) in intracellular C terminus.

Here we aim to characterize ANO1 in CF versus non-CF context by analyzing ANO1 expression, localization and activity, and determine whether ERK1,2 regulates ANO1 chloride activity.

For this study, we used different CF and non-CF models including bronchial epithelial cell lines CFBE41o- vs 16HBE14o-, lung explants from mice homozygous for the F508del mutation or KO for CFTR and their own littermates controls, and lung explants from CF and non-CF patients. ANO1 mRNA and protein expression were determined respectively by qPCR and Western blot. ANO1 localization was determined in bronchial epithelial cells by immunofluorescence after transient transfection with a plasmid containing human ANO1 gene coupled to the green fluorescent protein (GFP). We used fluorescent WGA (wheat germ agglutinin) as a plasma membrane label. ANO1 chloride activity was determined by halide sensor method

(Premo™ Halide Sensor). To investigate ERK1,2 regulation mechanism, we used PD98059 which is a MEK1 inhibitor.

Our results show that ANO1 activity is significantly decreased in CF compared to non-CF bronchial epithelial cells. ANO1 mRNA and protein expression are also significantly reduced in CF cell lines, and mice and human lung explants. Immunofluorescence shows that ANO1 colocalizes with fluorescent WGA in bronchial epithelial cells and that there is no difference in localization between CF and non-CF cells. Finally, we show that ANO1 activity is decreased in the presence of MEK1 inhibitor.

We conclude that the decrease of ANO1 activity in CF cells could be explained by the decrease of ANO1 mRNA and protein expression found in all tested CF models and may contribute to the worsening of ionic imbalance in CF patients. Here we confirm that ANO1 is expressed at the plasma membrane of bronchial epithelial cell lines. Moreover, ANO1 seems to be regulated by the ERK1,2 signaling pathway. All of these results lead us to think that this Cl⁻ channel could be potentialized to increase Cl⁻ efflux and counterbalance ionic transports in CF airways. Given these results, ANO1 might be a potential pharmacological target for the treatment of cystic fibrosis patients.

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EXPRESSION AND REGULATION OF IFRD1 IN NEUTROPHILS OF CYSTIC FIBROSIS PATIENTS

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Background: Interferon-related development regulator 1 (IFRD1), a protein highly expressed by neutrophils, was identified as a key modifier gene for cystic fibrosis (CF) lung disease in a genome wide association study. The expression levels and regulation of IFRD1 in neutrophils remained elusive. Here we investigated the protein expression and regulation of IFRD1 in peripheral blood and airway neutrophils from CF and healthy subjects.

Methods: IFRD1 expression was measured in peripheral blood and airway neutrophils from CF patients and healthy donors by flow cytometry. Where indicated, isolated neutrophils were stimulated prior to analyses. Isolated DNA from whole blood samples from CF patients were analyzed for IFRD1 single nucleotide polymorphisms (SNPs). Longitudinal lung function data and IFRD1 expression / regulation were correlated with IFRD1 SNPs.

Results: Peripheral blood neutrophils from CF patients expressed higher protein levels of IFRD1 compared to peripheral blood neutrophils from healthy subjects. In CF patients, IFRD1 protein levels in neutrophils were decreased in airway fluids compared to peripheral blood. In vitro studies showed that CXCL8 down-regulated IFRD1 expression in neutrophils, an effect that was abrogated with a CXCR2 inhibitor. Four IFRD1 SNPs modulated lung function in CF patients. Comparing different IFRD1 alleles showed that CXCL8 regulated IFRD1 expression depending on the genotype.

Conclusions: These studies demonstrate that (i) IFRD1 protein expression is upregulated in peripheral blood but not in airway CF neutrophils and (ii) CXCL8 modulates IFRD1 expression depending on the IFRD1 genotype.

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MARCKS MODULATES, BUT IS NOT ESSENTIAL FOR REGULATED EXOCYTIC SECRETION FROM GOBLET CELLS AND MAST CELLS

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MARCKS (Myristoylated alanine-rich C-kinase substrate) is a small protein hypothesized to be important in regulated exocytosis. By virtue of its myristoylated N-terminus and a Ser-rich hydrophobic domain, it associates

with the plasma membrane in resting cells, and following phosphorylation it translocates to the cytosol. MARCKS has been proposed to regulate exocytosis, variously, by tethering actin filaments to the plasma membrane (barrier function), sequestering PIP2, and/or tethering actin filaments to secretory granule membranes. The latter mechanism was suggested by the apparent inhibitory effects on regulated mucin secretion by the synthetic, myristoylated N-terminal, MANS, peptide. We sought to test the function of MARCKS in regulated secretion using human bronchial epithelial (HBE) primary cell cultures, and mast cells from the MARCKS null mouse.

Experiments with the MANS inhibitory peptide, and missense control (RNS) and non-myristoylated control (NONG) peptides on HBE cell cultures indicated that both MANS and RNS were apparently inhibitory against baseline and agonist stimulated mucin secretion, but the NONG peptide was without effect. Further analysis, however, revealed that the myristic acid residue of the peptides interfered in the ELISA assay for mucins, by blocking mucin binding to the plate, an effect mimicked by Tween 20. In experiments where mucins were assessed by a WGA sandwich ELISA, in which the mucins bind the lectin electrostatically, no effects of MANS, or the control, peptides were observed, for either baseline or agonist stimulated mucin secretion. Hence, the reported, inhibitory effects of the MARCKS derived MANS peptide appear to represent an artifact in the mucin assay used for the experiments.

Mouse mast cells were used to assess the role of MARCKS in regulated secretion, as null mice die perinatally. Mast cells were harvested from the livers of E14-18 embryos, matured in culture, and studied 24 h after adhering to fibronectin-coated plates. Secretion from DNP-IgE sensitized cells was determined as β -hexosaminidase (β Hex) release, following 30 m of stimulation with DNP-albumin. β Hex secretion in MARCKS deficient mast cells was elevated over WT controls at all concentrations tested, by 34 $\pm$ 5% and significantly so at the highest dose tested (3 ng/ml). β Hex secretion from mast cells deficient instead for the related, MARCKS-related protein (MRP) was unaffected, suggesting the stimulatory effects of MARCKS deficiency was specific. The data suggest that MARCKS is not essential for regulated exocytosis, but may instead play a negative modulatory role. To test this notion, the time course of β Hex release from WT and MARCKS deficient mast cells stimulated to secrete with 3 ng/mL DNP was examined. β Hex release from MARCKS deficient mast cells was complete just 3 min following stimulation, and was elevated over WT cells at 1, 2, and 3 m; secretion from WT cells was not complete until the 5 m time point. Hence, MARCKS plays a nonessential, modulatory role in regulated exocytosis.

These studies were supported by grants from the NIH and the North American Cystic Fibrosis Foundation.

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INSIGHTS INTO THE PATHOGENESIS OF EARLY CF LUNG DISEASE FROM LONGITUDINAL CULTURE, BRONCHOSCOPY, AND CHEST CT CLINICAL DATA

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Background/Objective: In addition to routine airway cultures, the clinical practice at the University of Iowa is to perform scheduled bronchoscopies and chest computed axial tomography (CT) scans on cystic fibrosis (CF) patients, roughly every two years, starting early in life. This provides a unique opportunity to investigate the temporal relationships between bacteria, lung inflammatory markers, and radiologic findings important in the pathogenesis of early CF lung disease.

Methods: Electronic medical records were reviewed to create a database of clinical information on 69 patients <10 years of age diagnosed with CF and seen between Aug 1996 and April 2012 at the University of Iowa. Data included bronchoscopies, chest CTs, and routine airway culture results. Descriptive statistics were then used to investigate the temporal relationships between microbiological, inflammatory, and radiological data. Because of substantial variability in early measurements of lung functions, initial lung functions were defined as a subject's highest FEV₁ measurement by age 8.

Results: We observed that the mean age for major events occur in the following order: 1st *Hemophilus influenzae* and 1st methicillin-sensitive *Staphylococcus aureus* infection before age 2; followed by lung inflammation as evidenced by elevated neutrophilia on bronchoscopy and 1st *Pseudomonas aeruginosa* infection by age 3; then air trapping diagnosed via

CT scan and 1st methicillin-resistant *Staphylococcus aureus* infection by age 4; and finally 1st mucoid *Pseudomonas aeruginosa* infection and bronchiectasis diagnosed via CT scan by age 5. The effect of age at onset of bacterial, inflammatory, and radiologic factors on starting lung function showed high variability and no statistically significant differences were noted in subjects' highest FEV₁ by age 8. However, only prior *Pseudomonas aeruginosa* infection and increased airway neutrophilia had a marked abnormal distribution with a negative skewness from patients with low starting FEV₁ values.

Conclusion: There is considerable variability in the timing of key events in the pathogenesis of CF lung disease. In general, these clinical data support the model that infection is the key first event that then leads to inflammation, which ultimately causes airway damage. *Hemophilus influenzae* and *Staphylococcus aureus* infections generally preceded *Pseudomonas aeruginosa* infection, supporting the "gateway hypothesis" that early infections pave the way for subsequent *Pseudomonas* acquisition. Only patients with a history of *Pseudomonas aeruginosa* acquisition or airway inflammation had abnormally low baseline best FEV₁ measurements.

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DEFECTIVE POST-SECRETORY PROCESSING OF MUC5B IN CF MUCUS

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The gel-forming mucins, MUC5AC and MUC5B, are the major contributors to rheological properties of airway mucus. These mucins are packaged intracellularly into secretory granules and a non-passive, post secretory process is essential to release the mucins from a highly compact, granular form to a linearized conformation that is vital to a transportable mucus in the lung. In CF, however, the absence or malfunction of CFTR in the membrane results in a volume depleted ASL, depleted bicarbonate levels, and a lower pH. Our hypothesis is that the impaired post-secretory conditions of CF modify the rate of Ca⁺⁺:Na⁺ exchange between the interstices of the expanding mucin granule and external environment, and consequently the proteolytic processing required for mucin expansion. The result is a final conformation which leaves mucin in a non-linear form, causing an aberrant mucus rheology and adhesion to epithelial surfaces.

Methods: We used saliva as a fresh source of MUC5B both from CF patients and normal. Saliva is an experimentally convenient model of mucin expansion, because it contains a single species of polymeric mucin, MUC5B. We utilized a sucrose density gradient and rate-zonal ultracentrifugation to capture and separate the different maturation form of the MUC5B. Fractions were analyzed by immuno-blotting, light scattering, and electron microscopy.

Results: In normal saliva (n=6), typically, 70% of the MUC5B population was recovered from the light side of the gradient (top, fractions 3-6) as the fully linearized form, while 25% of the MUC5B was recovered from the mid-density region (middle, fractions 7-10) as the semi-compact/semi-linear form. Less than 5% of the MUC5B was recovered from the bottom of the gradient as the compact form. In about half of the CF saliva samples (n=6), however, the MUC5B profile was abnormal. In these patients, on average, 40% of the MUC5B population was recovered as the linear form, while the rest of the MUC5B, ~60%, was found in the middle and bottom fractions in the semi-compact/semi-linear and compact forms. The other half of the CF saliva samples (n=6) exhibited a normal MUC5B distribution. An additional, unexpected discovery was the observation of an abnormal compact form in the CF saliva that featured large, irregularly shaped structures containing an unprocessed protein core which left the mucin more compact. These abnormal, compact mucin structures were observed in about 1/3 of the CF saliva samples (n=4) and none in the normal samples. The compact forms were not related to category of MUC5B profile in the sucrose density gradient.

Conclusion: The observations of an abnormal MUC5B distribution profile in a sucrose density gradient for CF saliva and the presence of an abnormal compact form indicated that the MUC5B mucin is slow to linearize and mature after granular secretion in CF mucus, possibly due either to a pH/bicarbonate or dehydration effect, or a combination of both. Why just

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half of the CF patients possessed an aberrant salivary MUC5B requires explanation, and will hopefully be elucidated in future studies involving more CF patients, who sampled at different times and with a consideration of their clinical status.

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DEFECTIVE POST-SECRETORY PROCESSING OF MUCINS IN CF-LIKE PIG AIRWAY MUCUS

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The gel-forming mucins of the lung, MUC5AC and MUC5B, are packaged in intracellular secretory granules and a post-secretory process is essential to mature the mucins from a highly compact, granular form to a linearized conformation that is vital to a transportable mucus in the lung. In CF, however, the absence or malfunction of CFTR in the membrane results in ASL with reduced volume, depleted bicarbonate, and low pH. We hypothesize that this impaired post-secretory environment in CF airways modifies the rate of $\text{Ca}^{++}:\text{Na}^{+}$ exchange in the secretory granules and consequently impairs the proteolytic processing required for the expansion of the mucins which leaves mucin in a non-linear compact form, causing an aberrant mucus rheology and adhesion to epithelial surfaces.

Methods: To test this hypothesis, ex vivo tracheas from pigs were treated with acetylcholine (100 μM) to stimulate secretion of mucus. Because CFTR normally mediates secretion of both Cl^{-} and bicarbonate, we evaluated the effects of inhibiting Cl^{-} and bicarbonate secretion individually and in combination. To block Cl^{-} secretion, tracheas were pretreated with bumetanide (100 μM). To block bicarbonate secretion, tracheas were bathed in bicarbonate-free Krebs with dimethylamiloride (DMA). To block secretion of both anions, another group of tracheas were pretreated with both bumetanide+DMA. Tracheas that received no pretreatment were used as controls. Mucus samples collected from the mucosal surface were immediately mixed with equal amount 8M GuHCl to preserve the mucin structure. Samples were subjected to 6-8M GuHCl gradient separation using rate zonal ultracentrifugation. Fractions were analyzed by immuno-blotting, light scattering, and electron microscopy.

Results: In control mucus, the majority of the Muc5b (70-90%) population was typically recovered in the light side of the gradient (top, fractions 2-5) as the linear form while only 5-10% of the Muc5b recovered at the medium density region (middle, fractions 6-9) as the semi-compact/semi-linear form and less than 3% of the Muc5b was recovered in the high density region of the gradient (bottom, fractions 10-12) as the compact form. In contrast, the mucus samples from bumetanide+DMA pretreated trachea showed an abnormal distribution of the Muc5b forms: 10-25% in the linear form, 20-30% in the semi-compact/semi-linear form and about 50% in the compact form. Analysis of the samples pretreated only with bicarbonate-free solution and DMA showed Muc5b distribution, top 30%, middle 30%, and bottom 40%. Analysis of the samples pretreated only with bumetanide in normal bicarbonate replete buffer showed the Muc5b distribution, top 20%, middle 30%, and bottom 50%. Electron microscopy analysis of the middle and the bottom regions indicated that the compact forms typically have large, irregularly shaped amorphous structures with unprocessed protein cores.

Conclusion: The observation of the abnormal Muc5b profile and the presence of the abnormal compact form in bumetanide+DMA pre-treated tissues, i.e. CF-like mucus, from pig trachea indicate that the mucin is slow to progress to the linear form after granular secretion in CF mucus, possibly due to either bicarbonate or dehydration effect or combination of both.

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ESTROGEN RECEPTOR ALPHA DEPHOSPHORYLATES STIM1 TO INHIBIT STORE-OPERATED CALCIUM ENTRY

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Cystic fibrosis is a genetic disease that affects multiple organ systems, including the airways, and is known to affect women more severely than

men. We have previously shown that 17β -estradiol (E2), acting through estrogen receptor α (ESR1), inhibits store-operated calcium entry (SOCE) in airway epithelia and have proposed that this leads to a reduction in Cl^{-} secretion. SOCE is regulated by STIM1, an endoplasmic reticulum (ER) Ca^{2+} -sensing protein. Depletion of ER- Ca^{2+} initiates translocation of STIM1 to the ER-plasma membrane junction where it aggregates and interacts with the store-operated Ca^{2+} channel Orai1, to induce Ca^{2+} influx. To better understand this process, we tested the hypothesis that ESR1 prevents Ca^{2+} influx by inhibiting STIM1 function. Immunofluorescence studies were performed on polarized human bronchial epithelial cultures (HBECs) to determine STIM1 localization. Diffuse STIM1 staining was visible under basal conditions. Upon ER- Ca^{2+} depletion, STIM1 translocated to the apical membrane. Pretreatment with E2 prevented STIM1 from translocating to the apical membrane, suggesting that STIM1 motility was inhibited by E2. Calcium imaging studies were also performed on HBECs. Because STIM1 localized to the apical membrane in HBECs we specifically monitored apical Ca^{2+} influx by placing the basolateral surface in a calcium free Ringer's solution. We observed a significant decrease in apical Ca^{2+} influx in HBECs that were pretreated with E2. This indicates that there is an apical Ca^{2+} channel that is inhibited by E2, which is most likely due to mislocalization of STIM1 following ER Ca^{2+} depletion. To better study the effects of E2 on STIM1, we utilized HEK293 cells that were expressing fluorescently tagged STIM1 constructs and ESR1-CFP. We found that E2 pretreatment reduced STIM1-STIM1 FRET to basal levels after ER- Ca^{2+} depletion, suggesting that E2 inhibits normal aggregation of STIM1. Fluorescent recovery after photobleaching (FRAP) studies revealed that E2 pretreatment significantly decreased STIM1 motility, without inducing puncta formation, and that depletion of ER- Ca^{2+} had no further effect. FRAP studies were also performed on a STIM1-YFP truncation mutant (570STOP), which removes 115 amino acids from the cytoplasmic region of STIM1. 570STOP was insensitive to E2, unlike WT STIM1, suggesting that STIM1's C-terminus is required for the inhibitory effect of E2. We next probed the phosphorylation status of STIM1-YFP and 570STOP $\pm$ E2 using a phosphoserine antibody. Under basal conditions, STIM1-YFP was phosphorylated. However, cells that were treated with E2 displayed a significant reduction in phosphorylation. We did not observe 570STOP phosphorylation basally or after E2 treatment, indicating that the C-terminus of STIM1 contains the phosphorylation sites. Overall, these data suggest that E2 inhibits basal phosphorylation of STIM1, which may disable STIM1 aggregation and trafficking to the apical membrane in HBECs.

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ARE MUCINS IMPORTANT DETERMINANTS OF THE PHYSICAL PROPERTIES OF CF SPUTUM?

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Background: Thick, tenacious airway secretions causing impaired mucociliary clearance and airway obstruction are a hallmark of CF lung disease. MUC5AC and MUC5B are the predominant gel-forming mucins in healthy airway mucus. CF sputum is a mixture of these along with DNA and bacterial and inflammatory cell products. There is conflicting evidence about the relative importance of mucins in determining physical properties of airway secretions. To investigate the influence of CF infection and inflammation on secreted mucins we have assessed the effects of endogenous proteolytic degradation at 37°C on sputum rheology and mucin concentrations.

Methods: Whole sputum was collected from CF patients during an exacerbation. Rheology was assessed on 100 μl aliquots using a controlled stress rheometer and 20mm/2° cone. Elasticity (storage modulus, G') and viscosity (loss modulus, G'') were calculated from a dynamic oscillatory test at 1Hz using a constant stress of 1Pa. Mucins were solubilised in guanidinium chloride, separated by agarose gel electrophoresis, identified after Western blotting using mucin-specific antibodies (MAN5AC-I and MAN5B-III) and their relative levels determined against solutions of known MUC5AC and MUC5B concentration run on the same gel.

In order to investigate effects of endogenous proteolytic degradation on mucins in sputum, rheology and mucin analysis were re-assessed after 60 min incubation at 37°C. Selected samples were also assessed after incubation with recombinant human DNase or with the mucin depolymerising agent dithiothreitol (DTT).

Results: Paired rheology data were obtained on samples from 12 patients (median age 22yrs, median FEV₁ 31%). After incubation, G' fell from a mean of 6.9 to 4.1 Pa s⁻¹ (40% decrease, p=0.01). Changes in G'' were more variable and non-significant (p=0.15). There were significant falls in detectable levels of both MUC5AC (6.0 to 3.7 µg/ml, p=0.008) and MUC5B (18 to 12 µg/ml, p=0.02) over the same period. With MUC5B in particular, there were alterations in the migration of mucins in the gel, with a shift to more mobile forms. There was no correlation between rheological parameters and MUC5AC or MUC5B levels, or between changes in these. DTT had a greater effect on elasticity than DNase.

Conclusions: MUC5B is the predominant mucin in CF sputum and mucins have an important role in determining sputum elasticity. There was significant loss of immunoreactivity to both MUC5AC and MUC5B antibodies in CF sputum samples over time, indicating degradation of mucins by proteases within sputum and supported by altered electrophoretic migration. In some subjects mucins are already significantly degraded in expectorated secretions. Secretions retained in the lung may be quite different from those expectorated, in terms both of rheology and biochemistry. This may lead to an under-estimation of the importance of mucins in determining physical properties of mucus in CF.

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NEUTROPHIL ELASTASE MODULATES CFTR CURRENT IN BRONCHIAL EPITHELIAL CELLS

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Acute and chronic neutrophilic infiltration underlies pathological changes in numerous disease states, including chronic bronchitis (CB) and cystic fibrosis (CF). Neutrophil elastase (NE) is a prominent neutrophil-derived serine protease found in abundance in lung disorders such as CB and CF. Recently, it has been suggested that neutrophilic inflammation may result in an acquired change of epithelial ion transport. For example, in chronic obstructive bronchitis and idiopathic bronchiectasis (two neutrophilic lung disorders) there is evidence for loss of CFTR activity. Chronic inflammation is also a well documented and principal cause of the progression of lung disease in CF. Further, neutrophilic infiltration may have a significant impact on attempts to restore CFTR activity with channel modulators.

Our laboratory has demonstrated a time-dependent inhibition of forskolin (fsk)-stimulated short-circuit current in monolayers of human CFBE14o- cells. CFBE14o- cells are derived from bronchial epithelial cells from a CF patient stably expressing complementary wild-type human CFTR. The purpose of the present study was to determine whether the down-regulation of CFTR-mediated currents observed in Ussing chamber experiments was due to a direct cellular effect or a disruption of the tight junctional complexes.

Whole cell recordings were obtained from CFBE14o- cells cultured on transwell filters using Amphotericin B perforated patch methodology. Experiments were performed at room temperature. Control experiments were performed in the presence of amiloride to eliminate ENaC mediated currents. Fsk induced CFTR(inh)172-sensitive currents consistent with functional expression of CFTR. Preincubation of cells with 0.01 to 1.0 µg/ml NE attenuated fsk-mediated CFTR activation demonstrating a cellular effect of NE rather than tight junctional disruption. Furthermore, following NE exposure, CFTR(inh)172 was ineffective thereby demonstrating the specificity of the NE attenuation of fsk stimulated CFTR currents.

In conclusion, NE leads to a reduction in CFTR mediated whole cell currents. Whether this is due to a direct or indirect effect, or a combination of the two, is unknown. Studies at the whole cell and single channel level are underway to define the mechanism of NE down-regulation of CFTR channel activity.

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INTRACELLULAR pH IS NORMAL IN CF GOBLET CELLS BUT NOT IN ADJACENT EPITHELIAL CELLS OF MURINE INTESTINAL ORGANIDS

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Goblet cell metaplasia and the secretion of tenacious, aggregated mucus are major pathological features of the epithelium in cystic fibrosis (CF)-affected organs such as the lung and intestine. Recent studies have demonstrated the importance of concurrent Cftr-mediated HCO₃⁻ secretion during mucus exocytosis/solubilization (Garcia MAS et al. *J Clin Invest* 119:2613-2622, 2009), but reports of Cftr expression in intestinal goblet cells are conflicting. Surprisingly little is known of goblet cell (GC) physiology, in particular, intracellular pH (pH_i) regulation in normal and CF epithelium. In these studies, the pH_i of well-differentiated GCs and adjacent enterocytes was measured in the crypt epithelium of primary, 3D gel cultures from wild-type (WT) and CF murine intestine (enteroids). Enteroids are epithelial structures possessing a central cavity with regenerating crypts that faithfully recapitulate intestinal epithelium (Liu J et al. *Am J Physiol*. doi:10.1152/ajpcell.00392.2011, 2012). Using confocal microscopy, pH_i was measured using the pH-sensitive dye SNARF 5F during superfusion of enteroid cultures with a CO₂/HCO₃⁻ buffered Ringer's. Goblet cells were identified by counter-staining mucin granules with quinacrine. Measurements were performed post-acquisition from Z-stack images using Imaris® 3D imaging software for the placement of measurement spheres within the cell borders of GCs and adjacent, non-secretory enterocytes. In studies of enteroids from six WT and CF gender-matched littermate paired mice, pH_i was not different between WT and CF GCs (WT= 7.40 ± 0.04; CF= 7.40 ± 0.04, ns, n = 2-3 GCs/culture in 2-3 enteroids/mouse). The pH_i of adjacent WT enterocytes was similar (7.44 ± 0.03, ns) to WT and CF GCs. In contrast, pH_i of CF adjacent enterocytes was significantly alkaline relative to WT GC, CF GC and WT enterocytes (pH= 7.57 ± 0.07, p=0.004). Preliminary studies were also performed to assess the relative pH within the GC thecae using the pH-sensitive dye Lysosensor®. In 2 WT and CF littermate mouse pairs, mean theca pH in CF GCs was slightly but not significantly reduced relative to WT (F0/F1 WT= 1.396 ± 0.115 vs. 1.219 ± 0.074, ns, 29 WT and 32 CF goblet cells). Sections of fixed WT and CF enteroids stained with PAS/alcan blue (pH 2.5) to assess acidic mucin content in GC thecae did not reveal a difference in the percentage of acidic glycoproteins (WT= 14.9 ± 4.1%; CF= 12.1 ± 2.5%, ns, n = 3 mouse pairs). We conclude that 1) the basal pH_i in CF GCs is not different from WT GCs, a finding consistent with the contention that Cftr is not expressed in intestinal GCs; 2) CF enterocytes adjacent to GC are significantly more alkaline, supporting the hypothesis that there is a failure of Cftr-mediated HCO₃⁻ efflux in the immediate vicinity during GC exocytosis; and 3) preliminary data indicate pH within the granular thecae is similar between CF and WT GCs in the absence of systemic elements (e.g., immune and mesenchymal cells). *Supported by NIH and CFF.*

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CHANGES IN NASAL MUCOSAL INFLAMMATORY CELL PHENOTYPE IN CYSTIC FIBROSIS (CF) MICE

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Background: Whether the CF airway is intrinsically pro-inflammatory, or exhibits an excessive inflammatory response to infection, is still debated. There is increased mucous cell density, thickened respiratory epithelium and increased clusters of lymphoid aggregates in the nasal mucosa of adult CF mice, apparently independent of infection.

Aims and Methods: We compared CF mice (CFTR^{tm1Unc}, n=6) and wild type (WT) littermates (n=6). The nasal mucosa was dissected and flow cytometry performed, staining for CD3, CD4, CD8, CD19, macrophage marker Moma2, IL-17, γδ T cell receptor, Natural Killer cell marker NK1.1 and neutrophil marker Ly-6G. Inflammatory cell populations obtained from lung digest and spleen were also compared. We measured IL-1β, IL-4, IL-

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9, IL-10, IL-13, IL-17, GM-CSF, IFN- γ , KC, MCP-1, MIP-1 β , RANTES, TNF- α , and MIP-2 (multiplex assay) in bronchoalveolar lavage fluid (BALF), nasal lavage (NL) and serum. Nasal and bronchoalveolar lavage was cultured to determine whether any differences found were secondary to infection.

Results: The nasal mucosa of the CF mouse had an increased percentage of B cells, neutrophils, $\gamma\delta$ cells, NK cells, and IL-17+ CD3, $\gamma\delta$, NK cells and neutrophils. (Table 1). These differences were not seen in the lung or spleen. Only 3 cytokines were detectable in NL. There was a trend towards an increase in KC levels in CF mice (55.0[24.6-111.4] vs 12.9[6.9-28.8], $p=0.06$ median[IQR]). There were no significant differences in the number of bacterial colony forming units nor the species of bacteria cultured in NL and BALF, and no differences in serum or splenic findings, between the 2 groups.

Conclusions: There are differences in nasal mucosal inflammatory cell populations between CF and WT mice, apparently independent of infection. These data suggest there may be a primary, CFTR related immune dysregulation in the airway mucosa.

	CF	WT	p value
Percentage B cells	1.7[1.1-3.2]	0.7[0.5-1.0]	0.009
Percentage Neutrophils	3.2[2.6-4.4]	1.9[1.3-2.6]	0.02
Percentage IL-17+ neutrophils	0.4[0.3-0.5]	0.2[0.2-0.3]	0.02
Percentage IL-17+ CD3 cells	1.5[1.3-2.9]	0.8[0.6-1.0]	0.004
Percentage $\gamma\delta$ cells	7.6[5.6-8.5]	3.7[3.2-4.5]	0.02
Percentage IL-17+ $\gamma\delta$ cells	2.5[1.9-2.8]	1.6[1.3-2.2]	0.03
Percentage NK cells	4.6[3.0-5.9]	2.3[1.7-2.8]	0.004
Percentage IL-17+ NK cells	1.6[1.2-1.9]	0.6[0.5-1.0]	0.02

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EXPRESSION OF DELTA-F508-CFTR LEADS TO DISRUPTION OF THE AIRWAY GLUCOSE BARRIER IN CF-RELATED DIABETES

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Cystic fibrosis-related diabetes (CFRD) impacts nearly half of the adult CF patients in the U.S. and can start at an early age. The pathological consequences of diabetes in CF patients are severe; CFRD patients have a significant increase in the frequency of pulmonary exacerbations and an accelerated decline in lung function resulting in dramatically shortened lifespan. Despite the significant frequency of CFRD, little is known about how the inability to control extracellular glucose levels impacts airway function in CF. Glucose provides an easily metabolized energy source for airway microbes, and high glucose induces oxidative stress, which further promotes an already unbalanced redox environment. In CFBE cells, 16HBE cells, and primary airway epithelial cells, we studied the impact of expression of Δ F508-CFTR on the two components that comprise the airway glucose barrier: tight junctions, which help limit the flux of glucose into the airway surface liquid, and epithelial cell glucose transporters, which work to remove glucose from the airway surface fluid. Airway epithelial cells express several glucose transporter genes and the insulin receptor gene, as shown by qRT-PCR; levels of several genes were altered in CF cells compared to WT cells. We found that the localization of the insulin-dependent glucose transporter, GLUT4, is altered in CF cells; GLUT4 is predominantly trapped in the ER and perinuclear compartments in CFBE cells expressing Δ F508-CFTR and does not traffic to the plasma membrane in response to insulin, while GLUT4 trafficked to the membrane efficiently in WT cells. CF cells also exhibited an increase in transepithelial permeability in response to TNF α and to high-glucose medium, while WT cells were able to recover tight junction function within 3 hours of exposure. High glucose also induced an increase in paracellular flux of calcein and Texas Red Dextran, and the rates were ~2-fold higher in CF cells compared to WT. Using a dual-luciferase-based reporter assay that measures the activation of transcription factors associated with ER stress and the unfolded protein response, we found that cells expressing Δ F508-CFTR exhibited ~3.5 fold

higher levels of ER stress than cells expressing WT-CFTR. In both cases, ER stress was amplified upon exposure to TNF α and to high-glucose medium, although the response was much larger in CF cells. These studies suggest that ER stress amplified by airway epithelial cell expression of Δ F508-CFTR impairs the airway glucose barrier and may predispose the lungs of CF patients to the detrimental consequences of systemic diabetes associated with CFRD. (Support: Center for CF Research.)

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ABNORMAL REGULATION OF AIRWAY SMOOTH MUSCLE FUNCTION IN NEWBORN CYSTIC FIBROSIS PIGS

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Airway smooth muscle dysfunction and remodeling are found in cystic fibrosis (CF). We have previously found that tracheal airway smooth muscle from newborn CF pigs has an impaired contractile response to acetylcholine and an enhanced relaxation response to isoproterenol. These findings, in addition to the narrowed airways and abnormal appearing airway smooth muscle bundles in newborn CF porcine tracheas, led us to hypothesize that CF airway smooth muscle is in a hyper-contracted state. To test this hypothesis, we first investigated airway relaxation in precision cut lung slices made from non-CF and CF newborn pig tracheal lobes. By using precision cut lung slices, we were able to study airway smooth muscle at its in situ length, smaller lumen airways, and under more in vivo conditions. Following isoproterenol treatment, CF airways tended to relax (increase lumen area) to a greater extent than non-CF airways suggesting that the CF airways are pre-contracted. We then isolated airway smooth muscle from non-CF and CF newborn pig tracheas to assess phosphorylation of the contractile protein myosin light chain, an indicator of airway smooth muscle contraction. Preliminary studies suggest that the relative ratio of phosphorylated myosin light chain to total myosin light chain was increased in CF compared to non-CF airway smooth muscle samples (35% vs. 18%). These findings suggest that CFTR plays an important role in the regulation of airway smooth muscle contraction and relaxation.

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STRUCTURAL DISRUPTION OF INNATE HUMORAL IMMUNE PROTEINS IN CYSTIC FIBROSIS SPUTUM

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Objective of the study: The most common cause of death in CF is respiratory failure secondary to pulmonary infection. Innate humoral defenses that help prevent respiratory infections include secretory IgA (S-IgA), pulmonary collectins [surfactant protein-A (SP-A), surfactant protein-D (SP-D)], and the complement cascade. The aim of this study was to evaluate the structural properties of innate humoral immune proteins in cystic fibrosis sputum compared to normal controls.

Methods: Expecterated sputum samples from patients with CF and healthy volunteers were analyzed with SDS-PAGE, Western blot and dot blot. S-IgA was probed using antibodies against secretory component and heavy chain. Polyclonal goat antibodies were used to probe SP-A, complement components C3 and C4. Monoclonal mouse antibody was used to probe SP-D. Sputum concentrations of C3 and C4 were measured by ELISA; S-IgA was measured with dot blot and optical densitometry was used to determine amount of intact SP-A and SP-D.

Results: We found degradation of secretory component and heavy chain of S-IgA in sputum of CF patients compared to healthy volunteer by West-

ern blot analysis. Secretory component of S-IgA was almost undetectable in sputum of CF patients. A high degree of degradation of SP-A and SP-D was also found in sputum of CF patients compared to healthy volunteer. Many low molecular weight bands of S-IgA, SP-A and SP-D were seen on Western blot analysis in sputum of CF patients suggestive of cleavage of these humoral immune proteins. There was significant degradation of critical complement components C3 and C4 in sputum of CF patients compared to sputum of healthy volunteer. Virtually no intact C3 or C4 were evident. These results suggest that there may be complement activation and then degradation of activated components, or degradation of intact components, or both in bronchoalveolar fluid (BAL) of CF patients. Total sputum concentrations of intact and fragmented C3 and C4 were reduced in CF patients by approximately 60% when compared to healthy volunteer (52.5 µg/mL vs. 140.15 µg/mL and 14.46 µg/mL vs. 41.32 µg/mL, respectively). Total sputum concentrations of intact and fragmented S-IgA, SP-A and SP-D were also reduced in CF patients compared to healthy volunteer.

Conclusions: Innate humoral immune proteins (S-IgA, SP-A, SP-D, complement components C3 and C4) are depleted in sputum of CF patients compared to healthy volunteer. More importantly these immune proteins are highly degraded in sputum of CF patients, such that only very small amounts of intact, and presumably functional, proteins are present. Given the structural stability of immunoglobulin, this was a striking finding suggesting potent proteolytic cleavage in the bronchoalveolar fluid of CF patients. These preliminary findings suggest that humoral immunity is significantly compromised in the CF lung, which may contribute to persistent colonization and infection with various respiratory pathogens. Our preliminary findings need to be confirmed in a larger sample of CF patients and healthy volunteers.

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AIRWAY SURFACE LIQUID ANTIMICROBIALS ARE pH-DEPENDENT

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Cystic fibrosis (CF) is a life-threatening disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This gene encodes an ion channel that conducts chloride and bicarbonate. Bacterial tracheobronchitis is one of the hallmarks of CF lung pathology; it occurs early in the disease course and contributes significantly to the pathophysiology. Prior data showed the presence of defective bicarbonate secretion in CF airway epithelia and reduced airway surface liquid (ASL) pH. We hypothesized that reduced pH impairs the activity of ASL antimicrobials. To test this hypothesis in vitro, we used bioluminescent reporter bacterial strains. We incubated *E. coli* in low nutrient medium (1% Tryptic Soy Broth, 10 mM HEPES) adjusted to a range of pH values from 6.8-8.0. We tested the ability of lysozyme and lactoferrin, the two most abundant antimicrobials in ASL, to kill *E. coli*. We measured luminescence to assess *E. coli* viability. We found that as pH values increased, lower concentrations of lysozyme and lactoferrin were required to reduce luminescence by 50% (IC50). We also incubated a luminescent *S. aureus*; a common pathogen found in CF, with lysozyme and lactoferrin. We found increased killing with higher pH values. To test if bacterial killing by ASL was also pH-dependent, we collected methacholine-stimulated ASL from newborn non-CF pigs and adjusted pH to a range from 6.8-8.0. We used gold grids coated with *S. aureus* and determined the percentage of live and dead bacteria with SYTO9/PI probes. We found that increasing pH promoted more bacterial killing. These data indicate that within the range of pH 6.8-8.0, lysozyme, lactoferrin and ASL are more effective in killing bacteria at higher pH values. These results may aid in understanding CF pathogenesis and suggest new therapeutic interventions.

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PURE, FACS-SORTED, LIVE NEUTROPHILS FROM CF AIRWAYS DISPLAY MARKED CHANGES IN THEIR TRANSCRIPTIONAL PROFILE, CONSISTENT WITH REPROGRAMMING

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Background: Single-cell analysis has recently demonstrated the presence of at least two subsets among live neutrophils that are homing to the airways of cystic fibrosis (CF) patients. In comparison to their blood counterparts, CF airway neutrophils display pronounced changes in function (e.g., active elastase release) and signaling (e.g., activation of cAMP response element binding protein -CREB- and mammalian target of rapamycin -mTOR- pathways). Thus, live CF airway neutrophils may be key contributors to disease and attractive targets for new therapies. Thus far, live CF airway neutrophils have only been analyzed using immunophenotyping methods. Based on the presence of active CREB and mTOR signaling in these cells, it is conceivable that significant transcriptional changes also occur in these cells, which the present study seeks to identify.

Methods: Live airway neutrophils were FACS-sorted from induced sputum collected from 7 CF patients. A comparative hybridization using Agilent Technologies 2 color gene expression microarray was performed on CF airway neutrophils against blood neutrophils collected at the same visit, to reveal any differentially expressed genes. Seven experiments were performed on total live airway fractions and three experiments were performed on sorted CD16^{hi}CD63^{lo} (elastase non-releasing) and CD16^{lo}CD63^{hi} (elastase-releasing) subsets.

Results: Out of 4,210 genes interrogated, 220 were upregulated in sputum neutrophils (~5.2%) in comparison to blood neutrophils, and 120 were downregulated (~2.9%). In a gene ontology analysis of the upregulated genes, biological functions involving translational elongation and termination, viral regulated responses, regulation of cell death, cellular component disassembly, and endocrine system development were significantly enriched. In contrast, the downregulated genes were enriched for negative regulation of nucleotide metabolism (suggesting increased nucleotide metabolism) and for cellular macromolecular complex assembly. In sorted CD16^{hi}CD63^{lo} and CD16^{lo}CD63^{hi} subsets, regulation of plasminogen activation was significantly enriched. In addition, the CD16^{lo}CD63^{hi} (elastase-releasing) subset is distinguished by significant enrichment of RAGE receptor binding.

Conclusions: This pilot study corroborates previous studies by our groups suggesting that neutrophils homing to CF airways undergo significant changes that include transcriptional modulation. Our results, based on highly pure populations of live CF airway neutrophils, are consistent with recent studies in diseases other than CF, and challenge the conventional paradigm of mature neutrophils being unable to undergo transcriptional reprogramming. Finally, airway neutrophils sorted into discrete functional subsets show further evidence of transcriptional differentiation. Taken together, our results support the notion that specific functional and transcriptional pathways are at play in live airway neutrophils homing to CF airways.

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IMPAIRED NEUTROPHIL BACTERIAL KILLING IN CYSTIC FIBROSIS WITH PARTICULAR REFERENCE TO ACTIVATION OF PROTEOLYTIC ENZYMES BY VACUOLAR PH AND POTASSIUM FLUX

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Cystic fibrosis (CF) is characterised by chronic bacterial infection of the lung and destruction of lung tissue eventually leading to respiratory failure. CF neutrophils are recruited to the site of infection, yet paradoxically fail to eradicate pathogens. Previous studies have shown that the CFTR is expressed in resting neutrophils and was also detected in phagocytic vacuoles formed after bacterial engulfment. Reported reasons for impaired vacuolar killing include inadequate hypochlorous acid formation and chlorination of phagocytosed bacteria. However, both the generation of reactive oxy-

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gen species and activation of antibacterial proteases are essential for adequate vacuolar killing. After phagocytosis of microbes in healthy cells, components of the electron transport chain pump electrons across the membrane of the phagocytic vacuole to form superoxide and hydrogen peroxide in the lumen. The movement of electrons is compensated by K^+ and H^+ resulting in a hypertonic environment of elevated pH (7.8), optimal for granule protease activation. The aim of this project was to evaluate dysregulated K^+ influx and inadequate alkalization of the phagocytic vacuole as a cause for impaired bacterial killing in CF.

Circulating neutrophils were purified from blood of stable patients with CF (n=5) homozygous for the $\Delta F508$ mutation. K^+ concentrations inside the phagocytic vacuole and cytoplasm were measured by X-ray microprobe analysis on snap frozen, freeze dried cells. Neutrophils were mixed with IgG opsonised *Staphylococcus aureus* (*S. aureus*) at 37°C for 3 min and cryofixed. Sections were analysed using a Tecnai T12 electron microscope fitted with an EDAX X-ray detector. Vacuolar pH was determined using IgG coated *S. aureus* labelled with the ratiometric dye BCECF. Ethical approval was obtained from Beaumont Hospital Ethics Committee.

A comparison of the K^+ concentration in the vacuoles with those in the corresponding cytosol was made and revealed a mean concentration of K^+ in vacuoles of healthy control cells of 631mmol/g (200-300mM). Moreover, control cells illustrated an increase in peak fluorescence corresponding to a rise of intravacuolar pH from 7.4-7.8 within the first 2 min and thereafter fell to approximately 7.0 after 8 min. An alteration in the alkalization and hypertonicity of the CF phagocytic vacuole due to changes in K^+ flux is explored as a cause for impaired microbial killing.

The novel findings of this study highlight the regulation of intravacuolar pH in CF neutrophils as a potential therapeutic target to enhance bacterial killing.

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LOW LEVELS OF IGF-1 CONTRIBUTE TO IMPAIRED ALVEOLAR MACROPHAGE FUNCTION IN ADULTS WITH CYSTIC FIBROSIS

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Background: Alveolar macrophages are major contributors to the innate immune function of the lung environment. Although alveolar macrophages isolated from CFTR-/- mice have impaired ability to kill phagocytosed bacteria, no prior study has investigated primary alveolar macrophage function in adult patients with cystic fibrosis (CF). Patients with CF are known to have low levels of serum insulin-like growth factor 1 (IGF-1), and our prior studies demonstrate a relationship between low levels of serum IGF-1 and macrophage dysfunction in murine sepsis. It is unknown whether a relationship exists between reduced IGF-1 and alveolar macrophage function in patients with CF. Our central hypothesis is that low levels of IGF-1 in adult patients with CF lead to impaired alveolar macrophage function with resultant chronic bacterial infections.

Methods: Serum and bronchoalveolar lavage (BAL) samples were obtained from six subjects with CF with FEV1 > 50% and from 8 healthy subjects. Macrophages were isolated from BAL fluid after neutrophil depletion. Serum and BAL IGF-1 levels were determined by ELISA. We measured the ability of alveolar macrophages to effectively kill the clinical *Pseudomonas aeruginosa* strain DH1135. Subsequently, macrophages were incubated with high (400ng/mL) or low (150ng/mL) concentrations of IGF-1 prior to inoculation with bacteria to determine the effect of IGF-1 on bacterial killing.

Results: We demonstrated a significant difference in bacterial killing by CF alveolar macrophages (3.78 ± 1.03 log CFU remaining) compared to control (1.665 ± 0.7 log CFU remaining) with $p < 0.001$. CF subjects had lower serum IGF-1 at baseline (348.5 ± 88 ng/mL) compared to healthy controls (623.9 ± 121.5 ng/mL), $p < 0.001$. BAL IGF-1 levels from CF subjects were significantly lower (110.1 ± 36.13 ng/mL) than healthy controls (404.7 ± 107 ng/mL), $p < 0.001$. Exposure to a high concentration of IGF-1 enhanced healthy alveolar macrophage bacterial killing compared to cells exposed to a low concentration of IGF-1 ($p = 0.005$) and no IGF-1 ($p =$

0.002). Increased bacterial killing was also observed in CF alveolar macrophages exposed to a high concentration of IGF-1 compared to low-concentration IGF-1 ($p = 0.014$) and no IGF-1 ($p = 0.0062$). Finally, treating healthy alveolar macrophages with 10% CF BAL fluid decreased bacterial killing compared to healthy BAL fluid ($p < 0.001$). The addition of IGF-1 to CF BAL fluid improved bacterial killing from 5.99 ± 1.12 log CFU remaining to 2.823 ± 0.68 log CFU remaining ($p < 0.001$).

Conclusions: Alveolar macrophage function is impaired in adult patients with CF. Reductions in IGF-1 levels in CF contribute to the development of impaired alveolar macrophage function. Exposure to increased concentrations of IGF-1 ex vivo, results in improved function of CF alveolar macrophages. Further studies are needed to determine whether alveolar macrophage function can be enhanced in vivo with IGF-1 treatment.

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THE ARGININE DECARBOXYLASE PATHWAY IN PRO- AND EUKARYOTES; CONVERGING IN THE CF LUNG

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Cystic fibrosis (CF) lung disease is characterized by chronic airways infections that are nearly impossible to eradicate once established. The diseased CF airway hosts a complex milieu of pathogens, inflammatory cells, and a number of complex metabolites and structural molecules many of which have been liberated by inflamed or necrosing neutrophils. These metabolites make sputum a rich but toxic environment for bacterial colonizers which must adapt quickly or perish. *P. aeruginosa* adapts to this environment, in part, by forming a biofilm. While nutrient limitation and surface contact are typical initiators of a biofilm, many of the triggers of a biofilm in the CF lung have yet to be determined.

We have discovered that the arginine derivative agmatine is one of these triggers. Agmatine is the product of the arginine decarboxylase pathway present in both bacteria and mammals. In mammals agmatine acts as a neurotransmitter, a regulator of nitric oxide and a ligand of the α_2 -adrenoreceptor, which is implicated in lung inflammation. We have previously shown that agmatine in CF sputum is positively correlated with inflammatory markers and that agmatine is capable of NF- κ B mediated inflammatory signaling in both inflammatory cell culture and in mouse lungs. Furthermore agmatine greatly augments LPS induced NF- κ B expression in cell culture and in lung injury experiments. As agmatine is produced by inflammatory cells in the presence of LPS, it is possible that agmatine serves as a cytokine, overlapping the role of catecholamines in immune modulation. While these discoveries are the first to describe a physiologic role for agmatine in the lung, its detection and response by *P. aeruginosa* shed light into a complex host-pathogen interaction.

In most bacteria, agmatine serves as the major intermediate in the production of polyamines, which are essential for bacterial growth. In a separate line of work we discovered a novel operon that reverses the *P. aeruginosa* response to agmatine from biofilm dissolution to biofilm enhancement. Given a rapid response and metabolism of agmatine by *P. aeruginosa* we believe the agmatine found in CF sputum is of neutrophil source, not bacterial source. Furthermore, we have created a biologic sensor of agmatine using a *P. aeruginosa* devoid of all forms of agmatine metabolism, but luminescent in the presence of agmatine, to show bacterial detection of lung agmatine. Finally mutants of both agmatine production and metabolism show different outcomes in an acute pneumonia model. Taken together these two lines of work implicate agmatine as an important biologic mediator to both the host and pathogen in the CF lung. While it is an excellent nutrient source for *P. aeruginosa*, its role as a cytokine may serve as the danger signal to form a biofilm, and its rapid removal may lessen inflammation.

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ROLE OF INHIBITOR OF APOPTOSIS (IAP) IN CF AIRWAY INFLAMMATION - MODIFICATION BY SMAC MIMETICS

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Introduction: In CF *S. aureus* and *P. aeruginosa* activate TLR2/4 resulting in constitutive NF-kappaB activation, decreased apoptosis and chronic inflammation. Recently, Inhibitors of Apoptosis (IAPs) have been described, which enhance inflammation through activation of TRAF3/6, but are controlled through an endogenous antagonist, SMAC. Pharmacological depletion of IAP1/2 inhibits inflammatory mediator release. IAP antagonists (Smac mimetics), are in clinical trials for the treatment of solid cancer but have not been tested in CF airways inflammation. We therefore aimed to investigate the expression of IAPs in CF and non-CF epithelial cells and the effect of a Smac mimetic on LPS-induced inflammation.

Methods: Experiments used 16HBE41o- and CFBE41o- cell lines and primary nasal epithelial cells (NECs) obtained from patients homozygous for F508del (CF NECs) and age-matched controls (non-CF NECs). Cells were grown in submersion, but primary cells were also differentiated at air-liquid interface. All cells were stimulated with LPS (*P. aeruginosa*, Sigma) for 0-24h. To investigate the effect of the Smac mimetic SM-164 on the inflammatory response, 16HBE41o- and CFBE41o- cells were pre-treated with SM-164 0-100 nM for 2h and then exposed to LPS for 0-24h. The effect of SM-164 on cell viability was assessed by MTT test. Release of IL-8 into cell supernatants was determined by ELISA (PeproTech). The mRNA expression of cIAP2, p65 was assessed in cell lines and primary cells by qPCR. Protein expression of cIAP2 was determined by Western blotting using an anti-cIAP2 specific antibody (Cell Signalling).

Results and Conclusion: In both, CF NECs (n=6) and CFBE41o-, the expression of cIAP2 was significantly increased basally compared to non-CF cells (Non-CF NECs and 16HBE41o- {p<0.05}). In cell lines this was confirmed at protein level. The increase in cIAP2 was associated with a persistent up-regulation of NF-kappaB (p65) in nuclear extracts and mRNA.

Incubation of NECs and cell lines with SM-164 did not significantly affect cell viability, but did reduce cIAP2 protein expression in a dose dependent manner. Pre-incubation with SM-164 (1-100 nM) significantly reduced LPS induced IL-8 release (24h) in CFBE41o-, but not in 16HBE41o- cells. Similar to the IL-8 release, SM-164 pre-treatment dose-dependently reduced p65 mRNA. Lower concentrations of SM-164 had no effect on LPS induced IL-8 release or p65 expression.

Here we show for the first time the expression of cIAP2 in CF epithelial cells (CF NECs / CFBE41o-) and its association with NF-kB (p65) expression and subsequent IL-8 release. Pre-treatment with a Smac mimetic significantly reduced LPS- and NF-kappaB-induced IL-8 release in CF cells through reduction of p65 expression, suggesting that Smac mimetics may be potent anti-inflammatory drugs.

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THE ROLE OF MTOR IN CFTR-DEPENDENT MICROTUBULE STABILITY AND FUNCTION

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Cystic fibrosis (CF) epithelial cells are characterized by decreased transepithelial chloride transport, increased perinuclear cholesterol accumulation, and impaired organelle trafficking. The alteration of organelle trafficking has been recently demonstrated to include the inhibition of endosomal trafficking (Manson ME, et al. 2012). We have shown that the inhibition of endosomal trafficking is due to an alteration in cytoskeletal features, where the decrease in acetylated alpha tubulin (AAT) is most pronounced; however, it is unknown how the loss of CFTR function and trafficking are mechanistically connected. To determine whether the decrease in AAT has functional consequences linked to CF and trafficking, acetylation was reestablished with 10 μ M tubastatin, an HDAC6 inhibitor. HDAC6 is a

cytosolic histone deacetylase along with being a deacetylase of alpha-tubulin. Inhibition of HDAC6 reestablishes AAT levels in CF epithelial cells. Live-cell imaging of CF cells with endosomes labeled with NPC1-mCherry reveals that tubastatin treatment restores wild type (wt) levels of endosomal movement.

In order to elucidate the mechanisms leading to reduced AAT in CF cells and tissues, it was hypothesized that endoplasmic reticulum (ER) stress triggered by CFTR dysfunction and/or misfolding inhibits the mTOR pathway and plays a prominent role in cytoskeleton alterations. To test this hypothesis, wt epithelial (9/HTEo-) cells were treated with 100 nM thapsigargin, an ER stress inducer, for 72 hours. Microtubule acetylation was reduced 67.4 $\pm$ 3.4% as determined quantitatively by western blot analysis and qualitatively by immunofluorescence. Further, CFTR-corrected S9 cells were treated with proteasome inhibitor MG132 to examine the impact of another inducer of ER stress; S9+ 2 μ M MG132 results indicated a 50% reduction of AAT comparable to the inherent decrease of AAT in CF epithelial (IB3) cells. To test the potential role of mTOR signaling in microtubule acetylation control, S9 cells were treated with rapamycin, a direct inhibitor of mTOR, and western blot analysis was performed. S9 cells exhibit a decrease of 31.8 $\pm$ 2.1% in AAT. These data are consistent with ER stress induced inhibition of mTOR signaling as a mechanism leading to reduced microtubule acetylation and resulting trafficking issues in CF cells.

These data suggest the importance of the mTOR pathway in cytoskeletal rearrangement in CF epithelial cells. Dysfunctional CFTR protein and mutant CFTR misfolding have been noted to lead to ER stress induction, an upstream inhibitor of the mTOR pathway (Rab A, et al. 2006). AMP Kinase (AMPK) activation also leads to mTOR inhibition. Previous reports of increased AMPK activation in CF cells are consistent with the reduction of mTOR signaling seen here (Hallows KR, et al. 2005). Further understanding of mTOR regulation and its relation to HDAC6 function in CF cells can lead to new sites of therapeutic intervention to address cellular changes brought about by the absence of CFTR function in epithelial cells.

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LEVELS OF ANTIMICROBIAL PEPTIDES IN THE AIRWAY OF CHILDREN WITH CYSTIC FIBROSIS ARE NOT RELATED TO SERUM VITAMIN D CONCENTRATION

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Introduction: There are increasing data on the role of vitamin D as an immunomodulatory agent and it is thought, from *in-vitro* data, that this may be via induction of the antimicrobial peptides, LL37 and H β D-2. There is convincing evidence of the clinical health benefits of adequate vitamin D level in many diseases including TB and COPD but the evidence in cystic fibrosis (CF) is unclear and mixed findings are reported. We hypothesised that levels of antimicrobial peptides would be increased in children with adequate vitamin D and that this could account for reported improvements in lung function via improved airway defence.

Aims and Methods: The main aim of this study was to establish if a relationship exists between vitamin D and the airway antimicrobial peptides LL37 and H β D-2 in children with CF. We also sought to identify whether any clinically beneficial effects of adequate vitamin D exist in this population. After informed consent, blood and bronchoalveolar fluid (BALF) were collected from children undergoing clinically indicated fiberoptic bronchoscopy. BALF supernatant levels of LL37 and H β D-2 were by measured by ELISA. Serum levels of 25(OH)D2 were measured using mass spectrometry coupled with high-performance liquid chromatography.

Results: Samples were collected from 120 children with CF (58% female); median age (range) 6.9 (0.1-17.6) years. Most samples were performed in singlicate due to resources but high level of agreement was seen in those performed in duplicate. Median 25(OH)D2 level was 57 nmol/L (range 7-191 nmol/L). LL37 ranged from 0.1-21.9 ng/mL with median value of 0.49 ng/mL and H β D-2 from <15.6 - >1000 pg/ml, median 150 pg/ml. Thirty-four percent of patients had vitamin D insufficiency (<50 nmol/L).

LL37 was significantly correlated with serum neutrophils ($r = 0.4$, $P < 0.0001$), BALF total cell count ($r = 0.7$, $P < 0.0001$), and BALF neutrophil differential count ($r = 0.5$, $P < 0.0001$). Interestingly, these relationships were

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not seen with H β D-2. However, contrary to our hypothesis neither LL37 nor H β D-2 correlated with vitamin D and no differences were seen between vitamin D “adequate” and “insufficient” patients. In addition, in our cohort, there was no association seen between vitamin D and FEV₁ ($r^2=0.01$, $p=0.4$).

Conclusions: Our results demonstrate that there is not a relationship between serum levels of 25(OH)D2 and BALF levels of either H β D-2 or LL37. If vitamin D is involved in the induction of such defence peptides *in vivo*, the impact of this on protein levels may be limited in the degradative environment of the inflamed airway. In addition, we found no clinical or physiological effects of vitamin D deficiency. If any beneficial effect of vitamin D on respiratory health does exist in CF, it is small and not mediated via the antimicrobial pathway.

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IMINOSUGAR-BASED INHIBITORS OF CERAMIDE GLUCOSYL-TRANSFERASE (GLCCERT) AND NON-LYSOSOMAL GLUCOSYLCERAMIDASE (GBA2) REDUCE THE INFLAMMATORY RESPONSE TO *P. AERUGINOSA* IN CF BRONCHIAL EPITHELIAL CELLS
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Strategies aimed to limit the excessive inflammatory response are a relevant approach for CF patients. Recent findings suggest that the pathophysiology associated with CF may be at least partly corrected by interfering with sphingolipid (SL) metabolism, already known to play a crucial role in the pathogenesis of several lung diseases (Yang and Uhling, 2011).

Iminosugars represent the most promising class of sugar analogues as therapeutic agents (Beutler, 2001). The most famous iminosugar, N-butyl deoxynojirimycin (NB-DNJ, miglustat) is an inhibitor of the synthesis of glycosphingolipids (GSLs) which produces an anti-inflammatory effect *in vitro* and *in vivo* and reduces the *P. aeruginosa* induced immunoreactive ceramide expression (Dehecchi 2011). Considering that miglustat inhibits ceramide glucosyl-transferase (GlcCerT) and non-lysosomal glucosylceramidase (GBA2), it could affect the host response to *P. aeruginosa* through one or more of these pathways of the SL metabolism. We extended the investigation to other iminosugar-based inhibitors of GlcCerT and GBA2: the galactose analog of miglustat N-butyl deoxygalactonojirimycin (NB-DGJ) and Genz-529468, a very selective inhibitor of GBA2. Bronchial cells were treated with different doses of miglustat and in parallel with NB-DGJ or Genz-529468, before infection and the expression of IL-8 mRNA was measured 4 hrs later. We found that all these inhibitors have an anti-inflammatory effect, with IC50 values 22 μ M (miglustat), 12 μ M (NB-DGJ) and 7nM (Genz-529468) in IB3-1 cells and 23 μ M (miglustat), 5 μ M (NB-DGJ) and 7nM (Genz-529468) in CuFi-1 cells, indicating that Genz-529468 is very potent in reducing the *P. aeruginosa*-dependent transcription of IL-8. These results suggest that GBA2 could be at least one of the targets of the anti-inflammatory effect of these iminosugars. Therefore, we transiently transfected CuFi-1 cells with specific siRNA for human GBA2 or control duplex scrambled RNA before infection with *P. aeruginosa*. We obtained a significant reduction of GBA2 expression by about 60% and, in parallel, a significant decrease of transcription of IL-8 by about 50%. These data indicate that GBA2 could play a role in the SL metabolism linked to ceramide-mediated signaling processes. Our results further strengthen the hypothesis that the pharmacological modulation of SL metabolism, that can intercept the ceramide metabolic pathway at many levels, may be an effective approach for the treatment of CF lung inflammation. In this respect iminosugar-based compounds could provide novel therapeutic options to reduce the exaggerated inflammatory response in CF lungs.

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DIFFERENTIAL EXPRESSION OF THE IMMUNOMODULATORY CLEC12A AND MELANOCORTIN-1 RECEPTORS IN NEUTROPHILS HOMING TO CF AIRWAYS

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Background: Cystic fibrosis (CF) inflammation is dominated by the massive recruitment of blood neutrophils into airways. We have previously demonstrated that CF airway neutrophils are not subject to rapid necrosis, as previously thought, but rather undergo complex immunomodulation that involves, among other mechanisms, the active release of elastase-rich granules. While neutrophils respond to an exceptionally diverse and redundant array of activating receptors, less is known about the identity of inhibitory receptors that may modulate these cells. Here, we report the first results from an ongoing large study, in which we are investigating the expression in CF blood and airway neutrophils of two potentially inhibitory receptors. These are the melanocortin-1 receptor (MC1R), a G α_i (and G α_s -)-protein coupled receptor, and the C-type lectin domain family 12 member A (CLEC12A), an ITIM-associated membrane receptor.

Methods: We used flow cytometry to compare expression in gated live neutrophils from paired samples of whole blood and expectorated sputum of MC1R (N=14) and CLEC12A (N=6). Neutrophils from expectorated sputum were obtained by rapid mechanical dissociation with saline, on ice, followed by 800G centrifugation.

Results: MC1R expression was unimodal on CF airway and blood neutrophils and was increased 5-fold on the latter, as compared to the former (median fluorescence intensity: 1925 $\pm$ 206 vs. 359 $\pm$ 44, $P<10^{-4}$). There was no significant difference in MC1R expression between previously described CD16^{hi}CD63^{lo} and CD16^{lo}CD63^{hi} (elastase non-releasing and elastase-releasing, respectively) subsets. In contrast with MC1R, CLEC12A expression was unimodal on blood neutrophils but bimodal on airway neutrophils. CLEC12A^{hi} and CLEC12A^{lo} airway subsets, showed 4-fold difference in CLEC12A expression among each other, with respectively higher and lower expression levels than in blood neutrophils. This large difference may be accounted for by divergent regulation of this immunomodulatory molecule among neutrophils immediately upon entering the CF airway environment, or by a post-activation downregulation process. To address this question, we compared activated, elastase-releasing CD16^{lo}CD63^{hi} airway neutrophils to their CD16^{hi}CD63^{lo} counterparts. Consistent with a post-activation downregulation of CLEC12A, the former showed significantly lower expression than the latter (1847 $\pm$ 363 vs. 5969 $\pm$ 1308, $P=.02$). However, there was no direct correspondence between subsets defined based on CD16/CD63 and CLEC12A expression levels, suggesting that CLEC12A modulation and elastase-granule release in CF airway neutrophils occur through uncoordinated, independent sets of events.

Conclusions: This study identifies MC1R and CLEC12A as two attractive targets for inhibitory regulation of CF airway neutrophils. While MC1R is expressed equally among all CF airway neutrophils, CLEC12A displays a bimodal pattern that opens an opportunity for subset-specific modulation among CF airway neutrophils.

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ROLE OF MUCUS ADHESION AND COHESION IN MUCUS CLEARANCE

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Since the lung is exposed to the outside environment, it must defend itself against bacteria and noxious materials that deposit on the airway surface during normal breathing. The protective airway surface layer involves two parts: an upper mucus layer in contact with air and a lower periciliary layer (PCL) with densely grafted cilia lining the surface of epithelial cells.

The mucus layer traps any inhaled matter and it is transported to the throat by the beating cilia; the whole defensive process is called mucus clearance. This defensive mechanism works efficiently for healthy people; it is impaired, however, for patients with chronic bronchitis such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF).

The clearance of mucus involves formation of cracks between mucus and the PCL and the sliding of mucus layer over the PCL. To form a crack one needs to: 1) create new mucus-air and PCL-air interfaces; and 2) break possible bonds crossing the two layers. We hypothesize that the abnormal mucus clearance for patients with chronic lung disease is due to both the higher adhesion strength between diseased mucus and epithelial cells as well as the higher cohesion strength of the diseased mucus compared with that for normal (healthy) condition. We have developed peel test and cavitation rheology to quantify the mucus-PCL adhesion strength as well as the mucus cohesion strength. It was found that both the adhesion and cohesion strengths increase at higher mucus concentration corresponding to diseased conditions. Such increase of the adhesion and cohesion strengths could be due to the increase of the density of broken bonds at higher mucus concentration. Moreover, the mucus-PCL adhesion strength increases with the separation rate between mucus and cells, which might be due to the increase of energy dissipated into mucus at higher deformation rate. Our findings suggest that rehydrating mucus might reduce both mucus-PCL adhesion strength and mucus cohesion strength, and therefore, restore effective mucus clearance.

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SURFACTANT PROTEIN A (SP-A): STRUCTURE AND FUNCTION IN CYSTIC FIBROSIS COMPARED TO CHRONIC BRONCHITIS

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Background: Surfactant protein A (SP-A) is the most abundant pulmonary surfactant protein and belongs to the family of innate host defence proteins. SP-A binds calcium dependent to carbohydrate structures on pathogens via its carbohydrate recognition domain (CRD) and enhances their clearance by phagocytes. SP-A recognizes DNA via its collagen like region and CRD.

Cystic fibrosis (CF) lung disease is characterized by chronic inflammatory and proteolytic processes in the airways, leading to destruction of the lung and early death. The goal of this study was to assess the oligomeric organization and function of lung SP-A of CF patients in comparison to patients with chronic bronchitis and asthma.

Methods: The bronchoalveolar lavage fluid (BALF) from 31 clinically stable patients with CF and 14 patients with chronic bronchitis was analysed. Gel chromatography was used to investigate the macromolecular organization of SP-A. SP-A is eluted as three major peaks: the first peak shows very complex oligomeric forms (18-mer and larger forms), the second peak 6- to 12-mer forms and the third peak shows di- and trimer forms. The SP-A amount was determined by Slot-Blot.

Sybr green assay was used to analyse DNA concentrations. The functional capability of SP-A was measured by carbohydrate-binding assay on fucose-sepharose columns. When calcium is chelated by EDTA, SP-A can no longer bind to carbohydrates and is released from the affinity column. Therefore any SP-A protein recovered in the calcium wash fractions was unable to bind to fucose (SP-A non-binding). SP-A protein recovered in the EDTA wash bound to fucose (SP-A binding).

Results: CF BALF had an increased percentage of neutrophils. The distribution of SP-A oligomeric forms differed between the CF BALF and chronic bronchitis: in CF the large oligomeric forms were predominant whereas the small forms were diminished. In chronic bronchitis the large and small oligomeric forms were found in even distribution. However only CF BALF showed a fourth peak corresponding to SP-A fragments.

The carbohydrate-binding characteristic of SP-A in CF was significantly diminished compared to controls. The macromolecular organization of SP-A non-binding and SP-A binding did not differ between CF and controls. BALF DNA concentration was significantly higher in CF BALF. A higher DNA concentration of the BALF was associated with a higher percentage of neutrophils in BALF.

Conclusions: CF BALF had a significantly different distribution of oligomeric forms of SP-A compared to chronic bronchitis. Even in clinically stable patients with CF SP-A function was significantly diminished. The

CRD of SP-A may be impaired due to proteolysis and high DNA binding in CF BALF. These changes contribute to an impaired function of SP-A associated with innate functions in patients with CF.

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CONTRIBUTION OF THE TH17 PATHWAY IN LUNG INFLAMMATORY RESPONSES IN F508DEL-CFTR MICE

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Objectives: The exuberant lung inflammatory response in CF has long been recognized as a neutrophil-dominated process. More recently, attention has been drawn to the contribution of other immune cell populations into the progressive lung tissue damage in CF e.g. macrophages. However, little is known about the role played by lymphocytes. The recent observation of increased levels of the proinflammatory IL-17, secreted by T helper 17 (Th17) cells, in the bronchoalveolar lavage (BAL) of CF patients undergoing pulmonary exacerbation, raised the assumption that Th17 pathway may play a central role in CF lung disease. This work aimed at investigating the contribution of Th-17 in lung inflammatory responses in the CF mouse model.

Methods: We characterized, by qRT-PCR and ELISA, the in-vivo molecular inflammatory environment in lungs of normal and homozygous F508del-CFTR mice in basal conditions and after endotracheal instillation of lipopolysaccharide from *Pseudomonas aeruginosa* (LPS).

We showed here that basal mRNA expression levels of IL-17A, IL-17F and IL-22 were barely detected in lungs from CF and non-CF mice, with, as expected, no detectable levels of the corresponding interleukins in BAL. Interestingly, IL-17A, IL-17F and IL-22 mRNA after LPS instillation were found to be higher in the CF group than in the normal group. These data were in agreement with those monitored at the protein level, as IL-17A and IL-22 responses were detected in BAL from CF but barely from normal. Th-17-related cytokines (IL-1 β , IL-6, IL-23, GM-CSF) were similarly induced by LPS in both genotypes.

Conclusion: Our results showed that responses to LPS of Th-17-related factors are exaggerated in CF mice.

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ASSESSING ACTIVITY OF CF AND NON-CF SECRETIONS AGAINST PSEUDOMONAS AERUGINOSA WITH A MULTI-WELL BIOLUMINESCENCE IMAGING ASSAY

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Rationale: Bacterial airways infections are the most important cause of death from CF. Our central hypothesis is that antimicrobial activity of glandular secretions from CF subjects is less effective. We are using saliva as a surrogate for airway mucus because they share many antimicrobials and MUC5B, but whereas airway gland mucus samples are scant and rare, saliva can be easily, safely, and repeatedly collected in large volumes. Furthermore, prior studies detected *Pseudomonas aeruginosa* (PA) in CF but not in non-CF saliva.

Methodology: We used a bioluminescence assay to obtain a rapid read-out of bacterial number over time. *Pseudomonas aeruginosa* (Xan5), stably transfected with luciferase, emit light only when alive and light output (photons/sec) is correlated ($r > 0.95$) with the number of colony forming units (Kadurugamuwa et al. Infect. Immun. 2003). Bacterial number over time and condition was tracked by measuring emitted light at 30 min intervals in each well of a 96-well microtiter plate (typically starting population = 1×10^5 PA/well). Lysozyme was used to calibrate the assay; it killed PA in a dose and time dependent fashion; for example with 500 μ g/mL, PA luminescence was reduced to 8.2% by 1 hr ($n=9$). We obtained basally secreted (Bs) and stimulated saliva (Stim, by chewing); these originate mainly from sub-

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mandibular and parotid glands, respectively but produced similar effects. Saliva was sterilized (^{137}Cs γ radiation) before use.

Results: We tested saliva from 3 CF and 3 non-CF subjects. Saliva (3 non-CF) initially inhibited and then stimulated PA growth when the nutrient level was very low ($< 0.1\%$ Luria Bertani broth, LB). It only inhibited PA growth when nutrients were more abundant (50% LB). Saliva from 2 of 3 CF subjects stimulated PA growth more than non-CF saliva with 1% LB condition; by 700-1200% for CF#1 and by 200-250% for CF#3. CF#2 was equivalent to non-CF saliva (70-100%). The inhibitory action of non-CF saliva on PA in 50% LB was greatly reduced by brief boiling ($n=2$), whereas boiling CF saliva had little effect on PA growth in 1% LB ($n=2$).

Conclusions/Discussion: Human saliva contains both nutrients and antimicrobials so that its effects on bacterial growth can be + or - depending upon starting nutrient levels. Heating appears to inactivate the antimicrobial effect but leave the nutrient effect. A parametric comparison across conditions will be needed to confirm these initial results that suggest a compromised antimicrobial activity in the saliva of some CF subjects. This approach may help clarify the basis for an antimicrobial defect of CF mucus. Moreover, because aspiration of infected saliva could contribute to airway infections, the results also suggest that oral hygiene might decrease CF lung infections.

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THE ALARMIN IL-33 IS ELEVATED IN CYSTIC FIBROSIS EPITHELIA: AN INFLAMMATORY TIME BOMB THAT CAN SUSTAIN NEUTROPHILIC INFLAMMATION?

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Pulmonary disease in CF is the result of severe airway inflammation characterized by the presence of numerous neutrophils. Both chronic infection and the ensuing tissue destruction contribute to inflammation and lead to fatal respiratory failure. Multiple inflammatory pathways have been investigated in CF but the underlying reason why the neutrophilic response is deregulated in the CF lung remains largely unknown. Tissue injury contributes to inflammation when cell death occurs by necrosis leading to the release of pro-inflammatory factors known as alarmins. Alarmins are related to pathogen associated molecular patterns (PAMPs) molecules, and promote inflammation through their interaction with members of the IL-1R/Toll-Like Receptor family. The objective of this study was to determine whether alarmins are expressed and play a role in CF, which has not been extensively studied. Using immuno-histochemistry on tissue sections from explanted CF lungs, we discovered the striking presence of the alarmin IL-33 in the nuclei of airway epithelial cells (AECs). IL-33 is a recently identified member of the IL-1 family. Following exposure of AECs to *Pseudomonas aeruginosa* diffusible material (PsaDM) a strong interaction was found between IL-33 mRNA expression and the absence of CFTR using two-way ANOVA. No interaction was detected for three other alarmins tested (S100A9, HMBG1 and IL-1 α). In addition, using immunofluorescence, IL-33 was detected in the nuclei of CF but not non-CF AECs exposed to PsaDM. Based on these results, we focused our investigation on IL-33 as a likely candidate linked to CF pathology. IL-33 binds the ST2 receptor, which we observed on infiltrating leukocytes in tissue sections from CF lungs. Moreover, we found that ST2 was mostly expressed on neutrophils following isolation of circulating leukocytes from CF patients. These results support the notion that neutrophils are targets of IL-33. We next determined whether IL-33, once released, could contribute to neutrophilic inflammation, based on the recent finding that IL-33 promoted neutrophil recruitment and survival during sepsis (1). Pre-treating neutrophils with IL-33 enhanced their recruitment by bacterial-stimulated AECs media, although IL-33 was not directly chemotactic towards neutrophils. Based on these results, we propose that IL-33 is up regulated in CF lung disease and that the presence of IL-33 in the nucleus of CF AECs act as an inflammatory "time bomb" if released by tissue injury to increase neutrophilic airway inflammation. Therefore preventing IL-33 action in CF epithelia may decrease inflammation without fully impairing host defence mechanisms.

1. J. C. Alves-Filho et al., Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nature Medicine* 16, 708 (Jun, 2010).

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GLOBAL QUANTITATIVE PROTEOME PROFILING OF THE IN VITRO CYSTIC FIBROSIS RESPIRATORY EPITHELIAL SECRETOME

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Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) which transports chloride ions and glutathione. It is not clear how defective CFTR transport results in the viscous, dehydrated airway surface liquid (ASL) that impairs mucociliary function and facilitates bacterial adherence in CF airways. Emerging evidence suggests the downstream consequences of mutated CFTR impact the ability of the innate immune system to maintain homeostasis in CF airways. Host antimicrobial activity is compromised in CF lungs and there is significantly higher survival of bacteria in CF ASL in vitro. Since CF mucus has decreased microbicidal activity, we hypothesized that expression and secretion of immune proteins is altered in CF ASL. To test the hypothesis, global quantitative proteomics of in vitro CF and non-CF ASL was undertaken with a focus on immune proteins. ASL was collected in vitro from three sets of passage twelve life-extended CF (ΔF508/ΔF508) and non-CF human bronchial epithelial cell lines (Fulcher ML et al. 2009) differentiated at the air liquid interface for fourteen days. Prior to seeding, cells were labeled by stable isotope labeling with amino acids in cell culture (SILAC) by feeding CF cells with "heavy" isotopes of arginine and lysine ($^{13}\text{C}_6$ -Arg and $^{13}\text{C}_6$, $^{15}\text{N}_2$ -Lys) and control cells with the common "light" arginine and lysine isotopes ($^{12}\text{C}_6$ -Arg and $^{12}\text{C}_6$, $^{14}\text{N}_2$ -Lys). Equal volumes of CF and control apical secretions were combined, separated by one-dimensional gel electrophoresis, in-gel digested with trypsin, analyzed by LC-MS-MS/MS, and identified proteins were quantified as the ratio of CF over non-CF. Proteins up-regulated in CF were involved in protein degradation, glutathione synthesis, heat shock, and inflammation. Specific up-regulated immune proteins in CF included proteasome members, annexins, S100A protein, IL-1, and IL-8. Proteins down-regulated in CF were involved in protease inhibition, innate immunity, cell death, chaperone pathways, sodium and chloride transport, immune cell migration, and complement activation. Specific down-regulated immune proteins included clusterin, LPLUNC1, TGF β 2, and the proteinase inhibitors neutrophil gelatinase, alpha-1-antichymotrypsin, antileukoproteinase, and metalloproteinase inhibitor 1. The advantage of the in vitro secretion model is the ability to study uninfected non-inflammatory conditions. Many of the dysregulated immune proteins reported here are opposite to those reported in secretions from chronically infected CF lungs, indicating that the downstream consequences of CFTR in CF airway cells set the stage for chronic inflammation and infection. Differentially expressed proteins are currently being validated by Western blot of apical secretions from independent CF and non-CF ALI cultures.

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NEONATAL ALVEOLAR MACROPHAGE ARE IMPAIRED IN THE CFTR MUTATED MOUSE PUP

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Although cystic fibrosis (CF) is hallmarked by neutrophilic infiltration and inflammation in the lung, there is growing evidence that the resident alveolar macrophage (AM) may modulate the initiation and progression of pulmonary disease. With the advent of early identification of the CF newborn, the potential to intervene to modify inflammation and infection before respiratory symptoms occur drives the need for such investigations. We hypothesized that cfr mutation increases oxidant stress and impairs phagocytosis in the developing AM and that the availability of the antioxidant glutathione (GSH) modulates these changes. We evaluated the newborn offspring of C57Bl/6 cfr knockout (S489X) (KO) gut corrected mice at P7-10

days of life and their wild type littermates (WT). A bronchoalveolar lavage was performed and epithelial lining fluid (ELF) analyzed via HPLC for mixed disulfides (MD), a measurement reflective of glutathione bound to cysteine due to oxidation, and GSSG, the oxidized portion of GSH. In the ELF, MD concentration was >4 fold higher in KO compared to WT ($p=0.053$) while GSSG was increased three fold in KO compared to WT. There were no differences in the percentage of AM isolated from the ELF between groups (WT- 87%, KO-87.5%). However, 60% fewer AM were retrieved in cftr KO pups compared to WT ($p<0.01$). KO AM lacked cftr immunostaining by confocal fluorescent microscopy. Phagocytosis of FITC-labeled *Staphylococcus aureus* was assessed via confocal fluorescent microscopy. Phagocytosis was dramatically impaired by more than 60% in KO compared to WT AM ($p<0.05$). Exogenous GSH (200 μ M) treatment normalized phagocytosis in KO AM to WT levels ($p<0.05$ vs without GSH, $p=NS$ vs WT). These data suggest that at baseline in the asymptomatic newborn mouse, GSH oxidation in the ELF was accompanied by impaired function of the cftr KO AM that was modulated by GSH availability. We postulate that although the neonatal CF lung does not demonstrate overt signs of pathology, newborn AM are altered by cftr mutation and may silently contribute to the development of pathology in the CF lung.

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LUNG ANTIGEN PRESENTING CELLS ARE IMPAIRED AND AFFECTED BY ALTERED PULMONARY SPHINGOLIPID COMPOSITION

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Dysfunctional CFTR leads to chronic inflammation and infection of the respiratory tract. The role of CFTR in pulmonary immune cells is only partially understood. Pulmonary antigen presenting cells (APC) orchestrate the innate and adaptive immune responses in the lung. The present study analyzes the phenotype and immune stimulatory capacity of APC from CFTR knockout (CF) mice and the effect of altered sphingolipid composition in CF on APC. Total numbers of lung APC were lower in CF mice compared to wild type (WT) controls. Expression levels of the maturation markers MHCII, CD40, and CD86 and of the pattern-recognition receptors toll-like receptors (TLR) TLR1, TLR4 and TLR5 on lung APC were decreased in CF APC. The T cell-stimulatory capacity of CF APC was impaired at baseline and following pulmonary infection with respiratory syncytial virus. The level of sphingosine-1-phosphate (S1P), a mediator of immune cell migration and activation, was decreased in the bronchoalveolar lavage fluid (BALF) of CF mice compared to WT controls. Whereas, increased expression of S1P receptors on CF APC was observed in CF APC. Culture of CF lung APC with BALF from WT mice normalized expression of MHCII, CD40, and CD86. Supplementation of CF BALF with S1P rescued the reduced expression of MHCII and CD40 in WT lung APC. These findings suggest that APC are impaired in the CF lung and that low S1P affect lung APC function. This provides a link of defective CFTR and pulmonary innate immune dysfunction in CF.

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CF AIRWAY EPITHELIAL CELLS LACK THE ANTI-INFLAMMATORY DOMAIN OF THE NF- κ B INHIBITOR A20

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Introduction: A20 inhibits TLR-induced NF- κ B signalling at the level of TRAF6. However, this function is reliant on complex formation with a TRAF6 binding protein called TAX1BP1 (Shembade *et al.* 2009, EMBO J). We have previously shown that although A20 is expressed in CF airway

epithelial cells, basal and LPS-stimulated expression is reduced compared with control cells (Kelly *et al.* 2010, Ped Pulmonol). Furthermore, A20 did not interact with TRAF6 in CF, but this interaction was highly evident in control cells at all time points examined (Kelly *et al.* 2010, Ped Pulmonol). Gibberellin (GA) is a plant diterpenoid involved in the regulation of plant development via induction of A20/ZnF proteins (Liu *et al.* 2011, J Plant Physiol). GA has previously been shown to have anti-inflammatory properties in diabetic mice (Davis *et al.* 1989, J Am Podiatr Med Assoc).

We hypothesized that induction of A20 by GA would compensate for the loss of normal A20 function in CF and would result in reduced airways inflammation.

Methods: Cell lines (16HBE14o- and CFBE41o-) and nasal epithelial cells (NECs) from patients (F508del homozygous) and age-matched controls were stimulated with LPS (*P. aeruginosa*, Sigma, 10 μ g/mL) for 0-24h. A20 (full length and C-terminal) and TAX1BP1 (full length) were assessed by Western blotting. IL-8 release was measured by ELISA. In an attempt to induce A20, cells were pre-incubated with GA (30 μ M) 1h prior to addition of LPS. GA-mediated induction of A20 was confirmed by qPCR.

Results and Discussion: In the absence of GA, A20 mRNA was maximally induced in 16HBE14o- 1h after LPS stimulation. Thereafter, A20 expression fell back to basal levels. However, in 16HBE14o- pretreated with GA, A20 remained at maximal levels 24h after LPS stimulation. This increase in A20 expression was accompanied by reduced IL-8 release in GA treated cells. The anti-inflammatory effects of GA pretreatment were confirmed in primary control NECs where significant induction of A20 ($p<0.01$) and reduction of IL-8 release (34%, $p<0.05$) was observed 24h after LPS stimulation. However, this anti-inflammatory effect was not maintained in CF cells (cell lines or primary NECs).

The anti-inflammatory effect of A20 is dependent on the C-terminal domain, which facilitates interaction with TAX1BP1 and TRAF6 (Shembade *et al.* 2010, Science). However, western blots showed that CFBE41o- cells poorly expressed the C-terminus. Additionally, TAX1BP1 protein expression was abolished in CFBE41o- cells following LPS stimulation. These findings show significant variations in the normal expression and function of A20 and TAX1BP1 in CF epithelial cells. It is unclear at present as to whether these defects are inherent or acquired over time. Importantly, GA treatment is not able to reverse these defects. The absence of the C-terminal domain of A20 likely explains the inability to interact with TRAF6 and may contribute to chronic and persistent NF- κ B driven inflammation in the CF epithelium.

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MODULATION OF SMALL MATRIKINES BY REACTIVE ALDEHYDES IN CF

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A proximate cause of airway remodeling, bronchiectasis, and mortality in cystic fibrosis (CF) is inflammation, produced in part by high level recruitment of neutrophils (PMNs) and their products. We have demonstrated an increasing role for the matrikine PGP as a mediator of PMN dependent inflammation in CF lung disease. We hypothesize that as a result of chronic inflammation in CF airways, there is endogenous production of reactive aldehydes that enhance the activity of chemotactic matrikines such as PGP and the elastin fragment VGVAPG.

A mass spectrometry (MS) method for detecting VGVAPG and Acetyl-VGVAPG was developed and combined with PGP measurements in order to simultaneously track these activators of PMN migration. A significant increase of VGVAPG and Acetyl-VGVAPG in CF sputum versus normal was quantified by MS, similar to that observed for neutrophil chemoattractants PGP and Acetyl-PGP. Acrolein-2, 4-dinitrophenylhydrazine (DNP) and acetaldehyde-DNP derivatives were synthesized and purified. Multiple reaction monitoring MS methods were developed for quantitation. We found a five-fold increase in both acrolein and acetaldehyde upon derivation of CF secretions. Western blot analysis of CF sputum also revealed increased acrolein modified proteins. Matrikines and reactive aldehydes at these lev-

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els are likely contributors to airway inflammation in CF lung in vivo and may also serve as biomarkers of the underlying inflammatory process.

The same acrolein dependent mechanism likely contributes to lung remodeling and inflammation in CF airways. PMNs from donors were isolated and treated with acrolein overnight. Supernatants were collected and monitored for enzymatic activity by commercially available kits (myeloperoxidase (MPO), human neutrophil elastase (HNE), and matrix metalloproteinase-9 (MMP-9)) or a protocol developed in our laboratory (for prolylendopeptidase (PE)). An ex vivo collagen generation assay was used to measure Ac-PGP and PGP production. MMP-9 activity increased in a dose-dependent manner at concentrations up to 100 μ M acrolein. PE activity increased significantly above control at all concentrations of acrolein ($p < 0.05$ for treatments 1 μ M to 1 mM). MPO activity increased approximately 7-fold (131.9 ng/mL $\pm$ 1.75, $p = 0.00001$) at 100 μ M acrolein. Ten μ M acrolein also produced a significant increase in human neutrophil elastase (HNE) compared to control; 44.2 ng/mL $\pm$ 1.52 compared to 28.4 ng/mL $\pm$ 1.06, ($p = 0.006$) respectively. PGP production from collagen by PMN supernatants was found to increase with acrolein treatment. Leukotriene A4 hydrolase (LTA4H) was detectable in the supernatants of PMNs incubated with nM and low μ M acrolein. The ability of LTA4H to cleave the N-terminal proline from PGP was blunted by acrolein, a finding expected to further enhance PMN chemotaxis. In conclusion, reactive aldehydes at levels present in disease sputum can perpetuate a feed forward mechanism contributing to long-term inflammation. This mechanism would be expected to elicit airway remodeling, loss of lung function, and increased morbidity and mortality in CF. Supported by CFF R464-CR11-Component II.

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MUCUS: MEMORY AND MESH

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The increased viscoelastic nature of dehydrated mucus in the airways of cystic fibrosis (CF) patients correlates to decreased mucus clearance, increased infection, and poor overall respiratory health. While the bulk viscoelastic properties of mucus, such as viscosity and elasticity, have been studied at length over the past several decades, they do not provide a description of mucus that is sufficient to account for key phenomena observed in mucus: namely requirement of elasticity for mucus clearance and the dependency of diffusive particles on their particle size and surface chemistry. Here we examine two further biophysical properties, the mesh size and memory of mucus as a function of mucus concentration, and relate these both to mucus clearance and the diffusive motion of micron and nanometer sized particles in mucus.

Generally, it is accepted that the polymeric mucin content of mucus is centrally responsible for the overall viscoelastic properties of the fluid. When macroscopically deformed, the fluid will recover towards its initial, unstrained position. Interestingly, the recovery of the fluid does not follow a single exponential decay, but rather demonstrates a fast recovery time of order 0.1 sec, and a slower relaxation characterized by timescale on the order of minutes. Microscopically, when particles are diffusing within a viscoelastic gel such as mucus, their steps are not independent from one another as they would be in a viscous fluid like water. The net result is that if a diffusing particle makes a random step right, it is more likely that its next step will be left, or anti-correlated. We demonstrate that the degree to which the diffusive path of a particle is anti-correlated increases with mucus concentration and that the time scale over which the microscopic memory is present in mucus is similar to the fastest relaxation times observed in our macroscopic experiments. These initial results suggest that the fast relaxation times of mucus are correlated to the ciliary beat frequency and may be central to clearance.

Using microrheological particle tracking methods, we have established a strong correlation between the overall mucin concentration of mucus and the mesh size of the mucus gel. While our results are consistent with polymer physics predictions of the spacing between molecules, they demonstrate sensitivity to the tracer particles' surface chemistry. By incorporating experiments at multiple, controlled mucus concentrations we are able to explore the effect of particle size and surface chemistry and develop these results

into a context relevant for drug delivery, mucus clearance, and correction of the hyper-concentrated mucus typical in CF.

Taken together, the memory and relaxation time of mucus gives us new insights into the nature of the fluid that will effect cilia driven clearance, the spread of infection within a mucus layer, and drug delivery.

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PENDRIN EXPRESSION IS INCREASED IN BOTH GRAM POSITIVE AND GRAM NEGATIVE PNEUMONIA IN MICE

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The innate defense of the lung against extracellular pathogens includes both physical and immunological mechanisms that are dependent upon ion transport. The primary physical defense is mucociliary clearance. When mucociliary clearance fails, as in cystic fibrosis (CF), there is a complex immunological response that protects the host. This includes the production of antimicrobial peptides and chemicals by epithelial cells as well as the generation of cytokines that orchestrate the host defense response. We previously demonstrated that interleukin-17A (IL-17), which is critical in the host response against extracellular bacteria, altered airway surface pH in vitro and increased Pendrin mRNA expression in HBE cells treated with IL-17A up to 48 h. Using immunoblotting and confocal immunofluorescence, we confirmed that Pendrin protein expression is increased in IL-17 treated HBE cells and that it is primarily localized to the mucosal surface of the cells. Functional studies using live cell fluorescence microscopy confirmed a novel Cl/HCO₃⁻ exchange in normal HBE cells treated with IL-17A that was significantly reduced in the presence of siRNA against Pendrin. To study the expression of Pendrin in vivo we used a pneumonia model in mice. Wild type mice were infected with *Klebsiella* and *Staphylococcus pneumoniae*, and sacrificed at 6, 18, 24, 48 and 72 hours. Pendrin expression was significantly increased in the lungs of infected mice as compared to vehicle mice at each time point. To establish a role for Pendrin in bacterial pneumonia, Pendrin null mice were compared to wild type littermate controls after being infected with *Staphylococcus aureus* or *Klebsiella pneumoniae*. Despite data in wild-type mice, there were no significant differences between Pendrin null mice and wild type littermate controls at 24 h following *Staphylococcus* infection or 48 h following *Klebsiella* infection. Further experiments are underway to study more time points following infection.

Taken together, our data strongly suggest that IL-17A increases the expression and function of Pendrin in epithelial cells as a part of the immune response against bacterial lung infections. However, the exact role of Pendrin remains elusive when studied in mice.

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ROLE OF THE TUMOR PROGRESSION LOCUS 2 (TPL-2) IN THE INNATE IMMUNE RESPONSE OF LUNG EPITHELIAL CELLS DURING PSEUDOMONAS AERUGINOSA INFECTION

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Introduction and Objective: Loss of CFTR activity causes mucus hypersecretion, excessive inflammation of lung tissue, reduced lung function and chronic infection with *Pseudomonas aeruginosa* (Psa). Our group recently showed that incubation of airway epithelial cells in presence of Psa diffusible material (PsaDM) activates the ERK1/2 kinase, an enzyme in the pathway of the MAP kinases (Berube J, et al., JBC 2010). Activation of ERK1/2 by phosphorylation leads to an increased secretion of interleukin (IL) -6 and -8 in normal and CF cells. Moreover, loss of CFTR enhances activation of ERK1/2 and the immune response during incubation with PsaDM. This increased immune response leads to increased neutrophils recruitment by CF cells versus normal cells. Implication of CFTR in this mechanism comes from the fact that its re-expression in CF cells corrects the immune response amplification, making them identical to non-CF cells. Phosphorylation of ERK1/2 is controlled by two upstream kinases, MEK1 and MEK2. The latter are activated by kinases Raf-1 and/or Tpl-2 which respectively derive their signals from growth factors or pathogen recognition

receptors. Our hypothesis is that the inflammation caused by Psa may be reduced by neutralization of the signaling pathways upstream of ERK1/2. Our objective is to demonstrate that Tpl-2 kinase is responsible for inflammatory effects triggered by Psa and targeting it represents a very promising therapeutic approach to counteract the devastating effects of inflammation induced by Psa in CF.

Methods: We propose to use the pharmacological approach and RNA interference (RNAi) to assess the role of Tpl-2 in our cell models. Together, these two techniques will allow us to measure the rate of phosphorylation of ERK1/2, expression and secretion of IL-6/8 and the neutrophils recruitment following incubation of bronchial epithelial cells with the PsaDM. The epithelial cell models available are derived from normal (BEAS-2B, NuLi, UNC-N) and CF lungs (CuFi, UNC-CF).

Results: Our preliminary results are extremely encouraging. Indeed, we recently discovered that translational inhibition of Tpl-2 by specific siRNA led to decreased sensitivity to PsaDM, as seen by lowered phosphorylation levels of ERK1/2 and a 60% decreased IL-6 production. We also found that during stimulation of BEAS-2B cells by PsaDM, pharmacological inhibition of Tpl-2 blocks the phosphorylation of ERK1/2 and reduced production of IL-6 by 82%. The pharmacological inhibition of Tpl-2 in CF and non-CF cells causes marked decreases (~70%) in IL-6 production during stimulation with PsaDM.

Conclusion: Identification of new therapeutic targets is a significant benefit for CF patients. Tpl-2 kinase emerged as a new target and specifically targeting it preserves the signaling pathway originated from growth factors. Its role in inflammation and its separation from other MAP kinases pathways makes its pharmacological modulation possible in a long-term treatment. Moreover, this is the first demonstration of the role of Tpl-2 in the innate immune response of lung epithelial cells following bacterial infection.

Supported by Cystic Fibrosis Canada.

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OVEREXPRESSION OF PRO-INFLAMMATORY EICOSANOID METABOLIC ENZYMES IN CYSTIC FIBROSIS AIRWAY CELLS IN RESPONSE TO INFLAMMATORY STIMULUS

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Objectives: Progressive pulmonary disease is the primary cause of morbidity and mortality in cystic fibrosis (CF). This is a consequence of a self-perpetuating cycle of airway obstruction, infection, and inflammation. In particular, excessive inflammation that is disproportionate to the infectious stimulus plays a critical role in the tissue destruction leading to pulmonary failure. Eicosanoids, including prostaglandins (PGs) and leukotrienes (LTs), are bioactive lipids derived from arachidonic acid (AA) that function to stimulate acute inflammation. Their production is controlled by the activity of metabolic enzymes in a tightly regulated temporal program. The objective of this study is to determine whether there are alterations in the expression of these enzymes in CF versus wild-type cells in response to an inflammatory stimulus.

Methods: Experiments were performed in a cell culture model of CF. CuFi cells are immortalized airway epithelial cells derived from a patient with CF due to a homozygous $\Delta F508$ mutation. They are compared with a similarly developed cell line, NuLi, derived from the airway of a normal patient. These cell lines are particularly useful, as they are low passage, and retain the morphologic and physiologic features of airway epithelial cells. To assess the response to inflammatory stimulation, we incubated the cells with a filtrate of *Pseudomonas aeruginosa* culture supernatant (PaF), a substance known to generate an inflammatory cytokine profile from these cells, for varying lengths of time. Expression of metabolic enzymes was measured using qRT-PCR. These enzymes included cPLA2, which catalyzes the release of AA from phospholipids so that it can be metabolized to eicosanoids, 5-LO, the rate-limiting enzyme in LT synthesis, COX-2, the rate-limiting enzyme in PG synthesis, and mPGES-1, the enzyme responsible for further metabolism of PG precursors to PGE2.

Results: cPLA2 expression was stimulated by PaF in NuLi cells, with a plateau of 4- to 6-fold induction from 8-24 hrs. However, it was stimulated 8-fold in CuFi cells at 8 hours, and continued to rise with a plateau at 16-fold induction at 18-24 hrs. COX-2 showed sustained induction in both cells, but

the stimulation was more than 2-fold higher in CuFi than NuLi cells throughout the time course. mPGES-1 exhibited minimal stimulation in NuLi cells that was more than 10 times greater in CuFi cells. 5-LO expression was significantly higher in CuFi cells at baseline, and was suppressed by incubation of cells with PaF. However, this suppression was significantly greater in NuLi cells, resulting in greater expression in CuFi cells at all time points.

Conclusions: CF cells exhibit alterations in both magnitude and time course of the expression of eicosanoid metabolic enzymes in response to an inflammatory stimulus. At all time points this leads to greater expression of these enzymes in CF versus wild-type cells. We predict that this will result in greater production of pro-inflammatory eicosanoids, particularly LTB4 and PGE2, from CF airway epithelium. These changes may play a role in the hyperactive inflammatory response of CF.

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TYPE I IFN AND THE INNATE IMMUNE RESPONSE IN A MURINE MODEL OF *P. AERUGINOSA* AIRWAY INFECTION

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Type I IFNs have long been recognized as an important mediator of host defense against viral pathogens. In cystic fibrosis (CF), viral infection is associated with acquisition of *P. aeruginosa* and it has been observed that patients infected with *P. aeruginosa* have worsened morbidity with subsequent viral infection compared to those without preceding bacterial infection. Previously, groups have proposed that Type I IFN therapy might be an appropriate therapy for viral infection in the setting of CF. A more fundamental question is whether *P. aeruginosa* induces Type I IFN production in vivo and whether this Type I IFN production contributes to host defense or is detrimental. We infected C57Bl/6 mice with *P. aeruginosa* and demonstrated significant IFN β induction as early as 1 hour post infection. In subsequent studies comparing infected C57Bl/6 and IFNARKO mice, we demonstrated that there was significantly more inflammation in the wild type during the innate immune response. Furthermore, there were significantly lower levels of inflammatory cytokines and chemokines in the IFNARKO mice compared to wild type mice. We conducted additional studies in wild type mice that had been pre-treated with Type I IFN and these mice demonstrated increased neutrophil recruitment and inflammatory cytokine/chemokine production when compared to wild type mice that were not pretreated. There was no significant difference in bacterial load in the lung or bacterial dissemination when comparing the infected IFNARKO mice to wild type or the Type I IFN-pretreated wild type mice to those that had not been pretreated. These data demonstrate that Type I IFNs are produced during *P. aeruginosa* pulmonary infection, contribute to airways inflammation during the innate immune response and do not appear to contribute to host defense in a murine model of *P. aeruginosa* pulmonary infection.

GENETICS

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EXOME SEQUENCING OF EXTREME PHENOTYPES IDENTIFIES *DCTN4* AS A MODIFIER OF CHRONIC *PSEUDOMONAS AERUGINOSA* INFECTION IN CYSTIC FIBROSIS

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Background: Recent evidence indicates that genetic factors beyond *CFTR* genotype have a strong influence on age at onset of *Pseudomonas aeruginosa* (Pa) infection among individuals with CF.

Aim: The objective of this study was to use whole exome sequencing to identify genetic variants associated with differential risk for chronic Pa infection.

Methods: Study participants comprised individuals from the US EPIC Observational Study. Individuals in the early and late extremes for age at onset of chronic Pa infection were selected for exome sequencing (n=48 at each extreme), balancing on sex and *CFTR* risk group. Chronic Pa was defined as two or more 3-month periods with positive Pa cultures in any one year period. The number of rare, nonsynonymous variants in each gene was tabulated for each individual for whom sequencing was successful (43/48 early Pa; 48/48 never Pa). For each gene, we tested for a significant difference between phenotypic extremes in the number of rare variants while adjusting for *CFTR* risk group and population structure. Validation of significant associations from this exome discovery phase was performed in remaining EPIC OBS individuals.

Results: In the discovery phase, one gene, *DCTN4* that encodes for the protein dynactin 4, was found to be significantly associated with phenotype with $p < 0.05$ after correction for multiple testing (naïve $p = 2.2 \times 10^{-6}$; adjusted $p = 0.025$). Twelve individuals carried rare variants at either of two sites in the early chronic extreme (rs11954652 or rs35772018) compared to none in the late extreme. We then sequenced *DCTN4* in 667 remaining EPIC OBS individuals who had been enrolled under the criterion of no prior history of Pa positive cultures. We used Cox regression to test for association between age at onset of chronic Pa infection and presence of either *DCTN4* variant identified in the discovery study. Presence of either *DCTN4* variant was associated with a significant increase in the hazard for age at onset of chronic Pa: HR = 1.9, 95% CI=(1.2, 2.7), $p=0.001$. Individuals who were homozygous for rs11954652 (n=9) or heterozygous for rs35772018 (n=13) were at even higher risk: HR=3.7, (1.3, 10.2), $p=0.02$; and HR = 8.6, (2.4, 30), $p=0.0009$, respectively. There was no detectable interaction between *DCTN4* variants and *CFTR* genotype. Individuals with these *DCTN4* variants had a higher frequency of Pa positive cultures than those without ($p=0.02$).

Conclusion: Dynactin 4 is a component of the dynein-dependent motor involved in autophagy, a cellular quality control process to degrade damaged proteins and microbes. These results provide strong evidence that *DCTN4* is a genetic modifier of Pa susceptibility in CF and illustrate the utility of exome sequencing as a tool for identifying novel genetic modifiers of this complex phenotype.

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GENES OTHER THAN *CFTR* AFFECT SWEAT CHLORIDE IN CYSTIC FIBROSIS

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Introduction: The gold standard for diagnosis of cystic fibrosis is measurement of sweat chloride levels through pilocarpine iontophoresis. Recent work through the *CFTR2* project has suggested that some of the observed variation in sweat chloride measurement is a function of specific *CFTR* mutations. To test the extent to which genes other than *CFTR* may also be involved, we estimate the heritability of the sweat chloride phenotype in a family-based study.

Methods: Heritability was estimated using data from the CF Twin and Sibling Study, supplemented by the CF Foundation Patient Registry. Measurements were collected for 1191 individuals, (67 pairs of monozygous twins (MZ), 18 pairs of dizygous twins (DZ), 473 families with 2 siblings, and 75 families with 3 siblings). In each family, all subjects with CF had the same *CFTR* genotype. If a subject had more than one measurement of sweat chloride, the minimum value was used. Heritability estimates were derived from twice the difference of correlation coefficients for MZ twins and DZ twins [$h^2 = 2(MZr - DZr)$]. Confidence intervals were generated by bootstrap method using 10^6 pairs randomly sampled from the study sample. As limited numbers of pairs of DZ twins with CF exist, heritability was also estimated using a group of 155 pairs of same-sex DZ twins and siblings born within 3 years of each other (DZ/Sib).

Results: The mean sweat chloride value for MZ twins was 92.8 ± 20.5 mmol/L (n=134); DZ twins, 100.4 ± 10.1 (n=36); and siblings 93.8 ± 20.8 (n=1021). MZ twins had an average of 1.6 ± 0.8 sweat chloride tests performed, DZ twins 1.6 ± 0.5 tests, and siblings 1.5 ± 0.7 tests. For most subjects age at the time of testing was documented and the mean age for MZ twins was 2.9 ± 5.7 years (n=130); DZ twins, 2.3 ± 7.1 (n=36); and siblings 3.2 ± 6.5 (n=965). Using ANOVA, there was no difference in sweat chloride values ($p=0.13$), age at the time of tests ($p=0.67$), or total number of tests performed ($p=0.82$) between MZ twins, DZ twins, and siblings. The heritability estimate for sweat chloride using MZ and DZ twins was 1.00 (95%CI: 0.46, 1.00; n=85 pairs) suggesting a strong genetic component for this trait. The estimates were similar when restricting the analysis to subjects with severe exocrine pancreatic insufficiency (1.00; 95%CI: 0.15, 1.00; n=76 pairs) or to subjects homozygous for the *CFTR* mutation F508del (0.94; 95%CI: 0.00, 1.00; n=51 pairs). The heritability estimate using the MZ and DZ/Sib groups was 0.77 (95%CI: 0.28, 1.00; n=222 pairs). Again, this was similarly high for pancreatic insufficient subjects (1.00; 95%CI: 0.61, 1.00; n=187 pairs) and F508del homozygotes (0.96; 95%CI: 0.28, 1.00; n=112 pairs) using the MZ and DZ/Sib groups.

Conclusions: Using data from a large family-based study of twins and siblings with CF, we have demonstrated that genes beyond *CFTR* (i.e., genetic modifiers) contribute substantially to the variability in sweat chloride. Understanding genetic factors which influence sweat chloride can increase this measurement's utility in clinical studies. Genetic modifiers of sweat chloride may influence *CFTR* function or affect chloride transport independently of *CFTR*.

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DISSECTION OF THE GENETIC ARCHITECTURE OF CF-RELATED DIABETES

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Background: Cystic fibrosis-related diabetes (CFRD) occurs in 10-15% of adolescents and 25-40% of adults with CF. Lung function and nutritional status are worsened by CFRD and improved by its treatment. The cause of CFRD is unknown, but genes (both *CFTR* and other genes) play an important role. Having family members with type 2 diabetes (T2D) is a risk factor for CFRD, and three genetic modifiers of CFRD (*TCF7L2* (ref 1), *CDKALI*, and *CDKN2A/B*) were identified because of their role in T2D. However, other genes, not involved with T2D, could also be important.

Objective: We performed a genome-wide association study to identify novel genetic modifiers of CFRD.

Design/Methods: Diabetes phenotype information was obtained for 3,899 participants in the CF Twin and Sibling study, Canadian Consortium for CF Genetic Studies, and CF Gene Modifiers Study. All had pancreatic insufficiency, and diabetes was present in 698 (18%). Age of onset of CFRD was treated as a survival trait (event = CFRD diagnosis; censor = last negative test). Genotypes were measured using the Illumina 610-Quad array with 564,637 single nucleotide polymorphisms (SNPs) analyzed. Study-specific P-values were combined by meta-analysis as was done previously (ref 2). A replication sample of 704 individuals was genotyped using TaqMan.

Results: Genome-wide significant evidence for association with CFRD was found for SNPs in *SLC26A9* (HR=1.37 [95% CI: 1.23-1.54]; P=3.9E-08; n=3060). Effect size (hazard ratio) was similar in each study and in patients homozygous for F508del mutations in *CFTR*. There was no evidence for allelic heterogeneity. The same alleles associated with CFRD in the replication sample (HR=1.48 [1.12-1.95]; P=0.006; n=704). *SLC26A9* encodes a chloride/bicarbonate transporter which is known to interact functionally with *CFTR*. Interestingly, the CFRD risk alleles also increased the risk for meconium ileus in CF (ref 3).

We also follow-up on evidence for linkage to CFRD at chromosome 2q35-2q36.1 (LOD = 3.8, study-wide significant) and chromosome 12p12 (LOD = 2.24, suggestive). Region-wide significant evidence for association was found for a noncoding SNP at 12p12 (P=1.5E-05; 1205 SNPs tested). These loci harbor genes which could play roles either in multiple forms of diabetes or specific to CFRD. A pilot sequencing project has identified candidate SNPs to be prioritized for further testing.

Conclusions: Risk for diabetes in CF appears to be conferred in part by a combination of common variants involved in T2D (*TCF7L2*, *CDKALI*, and *CDKN2A/B*), common variants in CF-specific modifier genes (*SLC26A9*) and rare variants in other modifiers (chr 2q35-36.1).

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EXPLORING EPIGENETIC MODULATION IN CYSTIC FIBROSIS: RELATIONSHIP BETWEEN DNA METHYLATION AND INFLAMMATORY GENE EXPRESSION IN EPITHELIAL CELLS

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Cystic fibrosis (CF) is a recessive autosomal disease that affects the respiratory, gastrointestinal and reproductive systems. The most common

mutation, $\Delta F508$, causes incorrect folding of the protein, which induces the misregulation of mucociliary clearance, chronic respiratory infection and lung dysfunction. Genotype/phenotype studies have demonstrated significant variation in the severity of the disease. This variability could be related to epigenetic modifications, such as DNA methylation, of gene expression. The objective of this study was to explore the relationship between DNA methylation and gene regulation in CF epithelial cells. A combination of immunoprecipitation of methylated DNA and microarray screening covering all the promoter regions of the human genome was used to study the methylation pattern of CF (CuFi-1/CFBE ($\Delta F508$ homozygous)) and non-CF (NuLi-1) or CFTR-corrected (corrCFBE) epithelial cell lines. We observed first an overall hypomethylation of $\Delta F508$ cell lines with however some hypermethylated regions. Interestingly, when CFTR is corrected in CF cells, the methylation profile of 160 promoters returned to the level observed in non-CF cells. These results suggest the presence of a relationship between CFTR expression and methylation profiles of epithelial cells. Using a redundancy analysis (RDA) we identified 56 regions where 60% of the variance of the methylation profiles was explained by $\Delta F508$ mutation, and less than 20% by the cell type. In order to link promoter methylation profile to gene expression, the transcription level of genes involved in inflammatory processes, present in the 56 regions previously selected by RDA, was validated by qPCR on CF and non-CF cell lines as well as on bronchial epithelial cells isolated from $\Delta F508$ homozygous patients and non-CF patients. A strong correlation between DNA methylation and gene expression was found for 3 genes that were downregulated by 50% in CF patients: RGS2 (a regulator of G-protein involved in cytokine release), DHRS9 (dehydrogenase/reductase that promotes insulin resistance) and TULP4 (a cytoplasmic protein that mediates the degradation of target proteins). These results demonstrate that, in a homozygote $\Delta F508$ background, changes in the DNA methylation profile have an impact on gene regulation in CF patients.

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SPONTANEOUS DOMINANT GENETIC ALTERATION IN INBRED C57BL/6 $\Delta F508/\Delta F508$ MICE PROTECTS FROM DEATH AT WEANING

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Meconium ileus presents at birth in ~15% of infants with cystic fibrosis. Later in life, CF patients also suffer from a range of primary and second complications from intestinal malfunction. While CF mice typically do not die at birth from intestinal complication, they have varying extents of intestinal obstruction at weaning depending on the genetic strain. This strain-to-strain variation in weaning-induced intestinal obstruction in CF mice has strong genetic components. Potential intestinal modifier genes in CFTR-knockout mice on mixed genetic backgrounds have been mapped to chromosome 1, 3, 7, 9, 10, and 14. Most of the associated chromosomal locations are either positively or negatively correlated with intestinal obstruction and appear to be inherited in a recessive fashion. Intestinal obstruction at weaning onto solid chow is typically very severe and uniformly lethal in inbred C57BL/6J CFTR-KO and $\Delta F508/\Delta F508$ -CFTR mice. In a $\Delta F/\Delta F$ -CFTR C57BL/6J colony at University of Iowa, we observed the emergence of $\Delta F/\Delta F$ -CFTR mice that survived weaning. For many years, CF mice in this colony uniformly died within 1-4 days following weaning onto solid chow. Through selective inbreeding, a sub-colony was established for which all $\Delta F/\Delta F$ -CFTR mice survive weaning and males, but not female, CF animals remained fertile. The purity of the C57BL/6J background in six surviving $\Delta F/\Delta F$ -CFTR mice was confirmed by genome wide SNP analysis using 334 SNP markers informative for the C57BL/6J and 129S1/SvImJ genetic backgrounds and found to be >99.7% C57BL/6J. Defined breedings of two fertile $\Delta F/\Delta F$ males with wild type C57BL/6J females was used to evaluate the inheritance pattern of the survival locus in F2 $\Delta F508/\Delta F508$ offspring derived from F1 WT/ ΔF intercrosses. From a cohort of 346 F2 offspring (of which ~25% were $\Delta F/\Delta F$) the pattern of inheritance supports a single dominant gene survival locus (29% of F2 $\Delta F/\Delta F$ mice died at weaning and 71% survived). This is extremely close to the 75/25 percent expected distribution of a dominant trait and thus suggestive of a Mendelian inheritance of a monogenic survival locus. Preliminary SNP analysis for C57BL/6J and C57BL/10J markers on a small subset (20 survivors and 5 non-survivors) of the F2 cohort derived from intercrossed F1 C57BL/6 x

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C57BL/10J mice identify two candidate loci associated with survival (LOD score >2.98 and >2.37). Additional SNP analysis is underway on a larger cohort of CF animals. Both these chromosomal regions appear to be unique from previously identified locations associated with weaning survival in CF mice. Interestingly, short circuit current analysis of intestine segments from surviving mice did not demonstrate cAMP-mediated Cl⁻ current, suggesting that the genetic survival trait does not restore $\Delta F508$ -CFTR function or alter Cl⁻ conductance. In summary, we believe we have captured a novel spontaneous genetic alteration that behaves as a dominant modifier gene to prevent intestinal obstruction in an inbred C57BL/6J background. Identification of this gene and its function may yield useful information on CF intestinal pathophysiology.

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DEVELOPMENT OF A PORCINE MODEL OF CYSTIC FIBROSIS WITH INDUCIBLE CFTR EXPRESSION

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Cystic fibrosis (CF) is an autosomal recessive disease that affects approximately 30,000 children and adults in the United States. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), which encodes a phosphorylation and ATP regulated anion channel expressed on the apical membrane of epithelia. The most common CF-causing mutation, $\Delta F508$, has defects in processing and reduced channel function. Recently, our lab has developed CFTR^{-/-} and CFTR $\Delta F508/\Delta F508$ pig models that recapitulate human CF disease phenotypes. *In vitro* and *in vivo* studies suggest that gene therapy and pharmacological approaches can restore channel function, though the current methods have limitations and many questions remain unanswered. In this work we aim to determine when CFTR expression is required to alter the CF phenotype and whether restoring CFTR to CF animals will prevent, forestall or reverse disease phenotypes. To answer these questions we are generating CFTR $\Delta F508/\Delta F508$ pigs that express inducible CFTR in affected epithelia (CFTR $\Delta F3Tg$). We are using a tet system that includes the doxycycline-inducible reverse tetracycline transactivator (rtTA) and a tetracycline-operator (tet-O) to drive inducible expression of CFTR. Cells bearing all the transgenes have been used as donors for somatic cell nuclear transfer to pig oocytes, and the first several litters have produced viable piglets with variable levels of correction of CF newborn phenotypes. This new porcine model should be valuable in directing future approaches to gene therapy and pharmacological therapies and in further elucidating the pathophysiology of the disease.

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GENERATION OF A CFTR-KNOCKOUT RAT MODEL FOR CYSTIC FIBROSIS

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Modeling cystic fibrosis (CF) in animals has presented a challenge due to species and strain-dependent variations in phenotype. Developing a CF rat model has many potential advantages: 1) larger size (versus mice) allows for the evaluation of multiple organs, surgical procedures, and measurement of parameters that are otherwise difficult to obtain in mice; 2) rats are a traditional animal model for pharmacologic and toxicologic studies, an increasing priority for analysis of CFTR modulators; 3) rats have an abundance of submucosal glands and mucous goblet cells in the trachea and lobar bronchi compared to mice, increasing the potential for a pulmonary CF phenotype; and 4) antibodies and other reagents are readily available for protein-based studies in rat versus larger animal models. Importance of alternatives to a CF mouse is further indicated by the recent development of genetically modified porcine and ferret models of the disease. In the present

study, we applied zinc finger endonuclease (ZFN) technology towards development of a CF rat. We describe the generation of the first CFTR knockout rat on a Sprague-Dawley background via pronuclear microinjection of ZFNs, which bind and cleave double strand DNA in a sequence-specific manner, leading to insertions/deletions following nonhomologous end joining pathway-mediated repair. The CFTR knockout rats carry a 16 base pair deletion in exon 3, creating a frameshift and a premature termination codon in exon 4. Initial breeding of CFTR heterozygous rats resulted in approximately 27% wild-type pups, 45% heterozygous pups, and 27% homozygous null pups, indicating that the mutation is not embryonically lethal. Measurements of intestinal short-circuit current, analysis of airway surface liquid depth, ciliary beating and mucus clearance rates in excised trachea, together with histopathological analysis of lung, intestine, pancreas and other tissues are planned in order to confirm the null phenotype. Supported by the Cystic Fibrosis Foundation Grant R464-CR07.

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A NOVEL INTERPLAY BETWEEN THE NMD MECHANISM AND ER STRESS RESPONSE AFFECTING CELLULAR HOMEOSTASIS UNDER NORMAL CONDITIONS AND IN HUMAN DISEASES

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Introduction: Recently an effort is being made to develop treatment for premature termination codons (PTCs) causing human genetic diseases, to promote translational readthrough of the PTC leading to full-length functional proteins. We have previously found that the response to readthrough treatment is correlated with the CFTR transcript levels. One mechanism regulating the level of transcripts carrying PTCs is the nonsense mediated mRNA decay (NMD). The NMD mechanism degrades transcripts carrying PTCs, preventing truncated protein accumulation. The NMD has a crucial post-transcriptional regulatory role that affects the expression of many physiologic transcripts. We have previously found variability in NMD efficiency among different cell lines, indicating that NMD efficiency is an inherent character of cells that can modulate the response to readthrough treatment. NMD substrates encoding membrane or secreted proteins are processed in the endoplasmic reticulum (ER). An imbalance between ER protein folding capacity and the level of proteins that cannot be correctly folded activates the Unfolded Protein Response (UPR). UPR alters the transcriptional and translational cellular programs to enable the cells to cope with the stress.

We hypothesize that: 1. Inefficient NMD leads to accumulation of proteins in the ER and activates the UPR. 2. The UPR further inhibits the NMD mechanism (which is translational dependent). Hence, the interplay between these homeostasis mechanisms can regulate the level of many physiologic transcripts including transcripts carrying disease-causing PTCs.

Results: NMD down-regulation induced the UPR, as indicated by increased levels of UPR markers. Importantly, UPR activation inhibited the NMD as revealed by increased levels of physiological NMD substrates as well as the level of CFTR transcripts carrying PTCs. We further show that NMD inhibition together with UPR activation enhances both the ER stress and the NMD inhibition indicating a novel interplay between these two homeostatic mechanisms. Subsequently, we have studied two sisters, homozygous for a PTC in the CFTR gene, who differ in response to the read-through treatment and CFTR transcript levels. Using SILAC we compared the entire proteome between cells derived from these sisters. Out of 6000 analyzed proteins, 440 showed a significantly different level between the cell lines. At least 44 were ER proteins functioning in the UPR. Strikingly, a ~2 fold higher level of each of these proteins was found in the responding sister, indicating a higher UPR activation.

Conclusion: Our results revealed a new positive feedback loop between two homeostasis mechanisms, the UPR and NMD under normal conditions and in human diseases. This novel interplay regulates the level of CFTR transcripts carrying PTCs and might affect the response of patients to read-through treatments.

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IMPAIRED CFTR FUNCTION IN MILD CF ASSOCIATED WITH THE S977F/T5TG12 COMPLEX ALLELE

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The S977F mutation (c.2930C>T) in the CFTR (CFTR/ABCC7) gene is extremely rare. We describe in this study the case of an adult patient carrying the complex allele S977F/T5TG12 in trans with the F508del mutation. Mild respiratory manifestations arose in adulthood associated with azoospermia, single episodes of acute pancreatitis and minor hemoptysis. Sweat test Cl⁻ levels were ranging from 40 to 42 mEq/L; it was performed according to the Gibson and Cooke method. Measurements of nasal potential differences (NPD) were abnormal consistent with CF features, compatible with the diagnosis of a clinically mild form of cystic fibrosis. Wilschanski indexes (Wilschanski, et al., Eur Respir J 2001) in all three NPD measurements were above 1.2 while non CF values are < 0.8 in our Center. This diagnosis was established after NPD measurement following CFTR genetic analysis by denaturing high pressure liquid chromatography (DHPLC), supporting the relevance of both approaches for diagnosis of CF in specialized centres.

We wondered whether a newly described approach, that utilizes peripheral blood monocytes and reports an index named "CF index" potentially useful to identify CF subjects when the value scores below 0 (Sorio, et al., PLoS One, 2011), could support the diagnosis in this subject.

We calculated a CF index with negative values, in keeping with the established diagnosis of CF. A healthy carrier (F508del mutation) having a CF index with positive values was considered as reference. Depolarization of membrane potential, dependent on chloride efflux, was measured by single-cell fluorescent imaging using the potential-sensitive probe bis-(1,3-diethylthiobarbituric acid) trimethine oxonol (bis-oxonol, Molecular Probes) before and after activation of CFTR with 8-Br-cAMP, forskolin and IBMX in the absence of extracellular chloride, as previously described for epithelial cells (Renier et al. Hum Gene Ther, 1995).

The combined application of DHPLC and NPD analysis in the algorithm for CF diagnosis (De Boeck et al. Thorax, 2006) appeared useful for the management of similar cases. Our findings are consistent with the previously reported case of a subject carrying the CFTR genotype G542X / S977F presented with clinical evidence of CF (Férec, et al., 1997 <http://www.genet.sickkids.on.ca/resource/nl/CFnewslet.69.html>). In addition, the development and validation of novel functional assays is expected to improve our diagnostic capability and lead to the possibility of improved counseling and treatment of these challenging clinical cases, including the use of CFTR-targeted treatments now in sight.

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FROM THE BASIC DEFECT TO THERAPY: FUNCTIONAL CLASSIFICATION OF CFTR MUTATIONS BASED ON MRNA, PROTEIN AND FUNCTIONAL ANALYSES

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Any of the ~1,900 reported CFTR mutations[1] disrupting or diminishing the efficiency of mRNA processing will have an impact on disease manifestation. Besides nonsense mutations leading to mRNA nonsense-mediated decay (NMD) and nucleotide changes at consensus splicing sites, addi-

tional variants may impact the splicing process and/or induce transcript degradation. As some small-molecule CFTR modulators (VX-809, VX-770) start hitting the clinic and other RNA modulators (ataluren, AON, Kinetin, EGCG) increase their therapeutic potential, correct characterization of CFTR mutations becomes crucial to decide on the most adequate therapeutic strategy[2].

Our goal here is to understand the functional impact of CFTR mutations regarding mRNA processing, stability and translatability. CFTR transcripts were analysed by RT-PCR[3] upon extraction from native tissues of CF patients with F508del in one allele and one of the following in the other: 711+1G>T(IVS5), 1716+18672A>G(IVS10), 1717-1G>A(IVS11), 1812-1G>A(IVS11), 1898+1G>T(IVS12), 3120+1G>A(IVS16); or missense mutations: G85E(ex3), R334W(ex7), S549R(ex11), A561E(ex12), and I1234V(ex19). Comparing the transcripts' relative levels from both alleles (reference-F508del levels), our data show that only 1717-1G>A, 1812-1G>A, 3120+1G>A and 1716+18672A>G induce transcript degradation (63-80% of degradation) and the following mutations have an impact on splicing: 711+1G>T-Ex5 skipping (in-frame); I1234V-partial ex19 skipping (last 18nts); whereas 3120+1G>A, 1812-1G>A, 1717-1G>A and 1716+18672A>G alter splicing resulting in frameshift thus PTC introduction and mRNA NMD. Transcripts from two siblings genotype 1609delCA(ex10)/1898+1G>T(IVS12);TG13T5(IVS8) showed almost no 1609delCA-transcripts (3.5±1.2%) and 88%±2.8% from 1898+1G>T-transcripts lack ex9/ex12 simultaneously. Minigenes containing one or more introns (IVS4art+IVS5; IVS14a+IVS14b; IVS17a and IVS19art) into full-length CFTR cDNA were constructed to further characterize this abnormal mRNA processing. HEK cell lines stably expressing these wt mini-genes were produced. By RT-PCR and Western blot analysis, correct splicing of transcripts and processing of the CFTR protein was confirmed. Next, we used them to introduce the following CF-related mutations: 711+1G>T, 711+3A>G, 711+5G>A (IVS5); 2789+5G>A (IVS14b); 3272-26A>G (IVS17a) and 3832A>G (IVS19). All these mutant minigenes originated alternative transcripts resulting in complete absence of or misprocessed CFTR protein.

Our analysis of CFTR mutations suggests the following functional classification [2]: Class I/splicing-711+1G>T, 1812-1G>A, 1898+1G>T; Class II/NMD-1717-1G>A, 3120+1G>A; Class III - G85E, A561E; Class IV - R334W; Class V - S549R; Class V- I1234V and 1716+18672A>G. These data are of high relevance for personalized medicine through mutation-specific therapies.

Work supported by PTDC/SAU-GMG/122299/2010, PIC/IC/83103/2007, BioFIG (PEst-OE/BIA/UI4046/2011) grants (FCT, Portugal).

[1] <http://www.genet.sickkids.on.ca/app>

[2] Amaral MD (2011) Curr Drug Ther 12, 683-93

[3] Ramalho AS et al (2011) Methods Mol Biol 741,115-35.

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DECREASED EXPRESSION OF THE SPLUNC1 GENE IS ASSOCIATED WITH INCREASED PULMONARY DISEASE SEVERITY IN CYSTIC FIBROSIS PATIENTS

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Rationale: Although CFTR has been identified as the gene responsible for cystic fibrosis (CF), there is significant variability in lung function which can be partially explained by non-CFTR genetic factors. This has led to interest in identifying non-CFTR genes which are modifiers of lung disease severity in CF.

A recent genome wide association study (GWAS) of modifier genes in CF identified the rs1078761 (I84V) polymorphism in the 20q11 region as a potential marker associated with lung disease severity in CF patients (p=1.3x10⁻⁴). The region contains two genes, LPLUNC1 (BPIFB1) and SPLUNC1 (BPIFA1), either of which could be responsible for the association. LPLUNC1 and SPLUNC1 are two of the most highly expressed genes in the upper airways, and are structurally similar to the innate immune molecules bactericidal permeability increasing protein (BPI) and LPS binding

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protein (LBP). Although the rs1078761 polymorphism is an amino acid changing polymorphism located in LPLUNC1, it is a conservative change not predicted to functionally impact LPLUNC1 protein conformation or function. The rs1078761 polymorphism is highly associated with another polymorphism, rs4911311, which is particularly interesting due to its location in the promoter region of LPLUNC1.

Hypothesis: Alleles of the rs1078761 and rs4911311 polymorphisms which are associated with more severe disease modulate the expression of the SPLUNC1 and/or LPLUNC1 genes.

Methods: To test if the rs1078761 and/or rs4911311 polymorphisms are associated with mRNA expression levels of LPLUNC1 and SPLUNC1, RNA and DNA were extracted from peripheral lung tissue samples from 91 non-CF and 9 CF patients. Samples were genotyped for the two polymorphisms and TaqMan gene expression assays (Applied Biosystems) were used to measure mRNA levels. To investigate whether protein levels correspond to mRNA levels according to genotype, total protein was extracted from a subset of the same peripheral lung tissue samples, and LPLUNC1 protein levels were measured with Western blotting.

Results: The C allele of rs4911311 and the G allele of rs1078761 were associated with lower levels of SPLUNC1 mRNA in non-CF lung ($p=0.0084$ and 0.0086 , respectively). However, neither polymorphism was associated with LPLUNC1 mRNA levels ($p=0.0711$ and 0.2829) or LPLUNC1 protein levels ($p=0.8057$ and 0.5607). In addition, both LPLUNC1 and SPLUNC1 have 6 and 30 fold higher levels of mRNA expression, respectively, in CF lung samples when compared to non-CF samples ($p=0.005$ and $p<0.0001$).

Conclusion: The G allele of the rs1078761 polymorphism, which is associated with increased severity of lung disease in CF patients, and the corresponding C allele of the rs4911311 polymorphism, are associated with decreased levels of SPLUNC1 but not LPLUNC1 expression. These data suggest that low levels of SPLUNC1 may be detrimental to pulmonary health in CF.

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IMPACT OF POLYMORPHIC -794 CATT REPEATS OF MACROPHAGE MIGRATION INHIBITORY FACTOR PROMOTER ON SEVERITY OF CF

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Macrophage migration inhibitory factor (MIF) is a pro-inflammatory molecule able to sustain an exaggerated Gram negative inflammatory response by inducing Toll-like receptor-4 expression. MIF knockout mice display a reduced inflammatory response to *Pseudomonas aeruginosa* in comparison with wild-type offspring.

A regulatory role for MIF in CF was suggested by the demonstration that the functional polymorphism 5-CATT repeat (MIF5) displaying a lower promoter activity was associated with a milder disease phenotype in CF patients with different CFTR genotypes (Baugh JA et al., Genes & Immunity, 2002).

We aimed at assessing the association of MIF5 polymorphism with disease severity in a group of CF patients homozygous for F508del CFTR mutation. Allele frequencies were determined in 134 non-CF and 189 CF subjects.

DNA was amplified by polymerase chain reaction using forward and reverse primers as previously described (Baugh JA et al., Genes & Immunity, 2002). Results were analyzed using GenescanView 1.2 software.

In this multicenter study, key clinical features of CF patients were recorded. In comparison with those carrying other polymorphic alleles, patients with MIF5 displayed concordant trends to better nutritional status,

lower diabetes incidence and slower decrease of FEV1. Quartile analysis based on Kulich's normalized FEV1 showed that MIF5 is associated with a better lung function expressed as FEV1 ($p = 0.03$). Kaplan-Meier analysis showed that MIF5 patients display a delay in first measurement of FEV1 $< 60\%$ ($p = 0.03$).

Relevant roles of macrophages in CF have been recently recognized, consistent with our findings. The potential role of MIF in CF deserves further study.

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EVALUATION OF THE MELANOCORTIN-3-RECEPTOR AS A POTENTIAL MODIFIER OF CF LUNG DISEASE

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CF is a genetic disorder with a varying degree of disease severity. Twin and sibling analysis indicates that modifier genes independent of CFTR play a significant role in variation in lung disease. Linkage analysis of affected siblings revealed a region on 20q13.2 (LOD=5.03) that harbors a modifier of lung function in CF. The melanocortin-3-receptor (MC3R) is a compelling biologic candidate for the modifier effect within the region of maximal linkage. MC3R encodes a G-protein coupled receptor for melanocyte-stimulating hormone that has been shown to modulate lung inflammation in mice. Furthermore, variants in the coding region of MC3R have been associated with risk for childhood obesity.

To investigate whether genetic variation in MC3R may account for the modifier effect, the coding region of this gene was sequenced in 48 sibpairs with CF: 35 pairs inherited the same variants in 20q13.2 from each parent (IBD=2) and had similar lung function, and 13 pairs inherited different variants (IBD=0) and had dissimilar lung function. Eight IBD=2 pairs had the V81I variant (rs3827103). One of those 8 pairs had an additional R257S variant (rs61735259). There were no variants found in IBD=0 pairs. These results provided preliminary evidence suggesting that common variation within MC3R may be involved in CF lung function variability. As it is unknown whether MC3R is expressed in tissues relevant to CF pathology, we analyzed its expression and localization pattern in epithelial and other cell lines.

MC3R mRNA is present in normal human bronchial epithelial (HBE) cells and in CF bronchial epithelial (CFBE) cells homozygous for the $\Delta F508$ CFTR mutation by RT-PCR. An alternative transcription start site was discovered in both cell lines by 5' RACE, which identified one upstream exon and an intron with splicing consensus sequences. Inclusion of the upstream exon extended the length of 5' UTR but did not alter the ORF. RACE confirmed that there is no additional downstream exon at the 3' end. Translation of MC3R is predicted to encode a 36kD peptide of 323 residues. MC3R is detected as a 40kD protein in primary airway epithelial cells, human brain tissue and chronic myelogenous leukemia cells (K562). Transfection of K562 cells with MC3R siRNA reduced expression of the 40kD band by $68.8 \pm 1.4\%$, thereby confirming the 40kD band as MC3R. MC3R siRNA did not reduce expression of a distinct 60kD band detected by anti-MC3R antibodies in HBE and CFBE cell lysates.

Localization of N' HA-tagged MC3R and its effect on CFTR expression were investigated by confocal microscopy. MC3R showed a diffuse cytoplasmic distribution in polarized Madin Darby Canine Kidney cells that stably express WT or $\Delta F508$ GFP-CFTR. Previous studies have demonstrated that N' GFP or HA-MC3R is expressed in the plasma membrane and the cytoplasm in HEK293 and COS7 cells. Studies are currently underway to assess if the distribution of native MC3R differs from the tagged versions. MC3R co-expression did not change the apical membrane localization of WT-CFTR or cytoplasmic localization of $\Delta F508$ -CFTR.

While other members of the melanocortin receptor family are primarily expressed in the CNS, MC3R also displays expression in airway epithelia consistent with a possible modifier role in CF lung disease.

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USE OF SERUM INDUCED TRANSCRIPTIONAL SIGNATURES AS A PREDICTIVE DISEASE SPECIFIC MARKER FOR CYSTIC FIBROSIS

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CF is a genetic disorder caused by mutations in the CFTR gene in which lung disease is characterized by chronic infection by *Pseudomonas aeruginosa* (Pa), the major cause of morbidity and mortality among CF patients. A challenge of guiding treatment based on oropharyngeal cultures in young children is the test's poor specificity for lower airway infection. We used a novel genomics approach to generate genome-wide expression profiles with the purpose of identifying gene expression markers for CF severity. Transcription was induced in healthy unrelated peripheral blood mononuclear cells (PBMCs) through co-culture with autologous serum (self-baseline control), allogeneic serums (healthy unrelated controls), and CF patient serums.

Methodology: All CF diagnoses were confirmed to have sweat chloride levels >60 mEq/L and/or two CFTR mutations. ELISAs of 192 serum samples from the Wisconsin Newborn Screening Program, representing 43 individuals sampled over 15 years and comparative cultures, indicate that with initial seroconversion the host response to Pa infection defined by crude Pa cell lysate, Pa Exotoxin A levels, and the levels of the Pa type III virulence factors PopB (transporter protein) and ExoS (cytotoxin) increased over time, suggesting that serology to Pa may help determine the initial pathologic airway infection. Concurrently, we completed expression profiling of RNA derived from co-culture of PBMCs with the serum of CF patients, who possessed increasing degrees of colonization status with Pa. RNA was amplified and labelled using the GeneChip 3' IVT Express Kit (Affymetrix, Santa Clara, CA) and hybridized to Affymetrix HGU133plus2.0 array. Image data were quantified with Affymetrix Expression Console Software and normalized with Robust Multichip Analysis to determine signal intensity using the autologous signal as the baseline. ANOVA, false discovery rates and principal components analysis plots were conducted using Partek Genomics Suite version 6.5. Differentially expressed probe sets were defined as those possessing an FDR <10% and log2 ratio >0.5 among comparison groups. Hierarchical clustering was performed using Genesis.

Results: Comparison of those array signatures performed prior to seroconversion and afterwards, identified several hundred unique genes. Pathway analysis using OntoExpress and DAVID indicates that the most significant differences in gene expression include those involved with airway defense, antigen presentation, and transcription.

Conclusion: Our methodology, utilizing serum induced transcription in PBMCs functioning as reporter cells, consistently identified a small number of genes that are unique to CF, and to CF patients with initial Pa infection conversion, as targets for further study.

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MUC5AC REFERENCE GENOMIC AND MRNA STRUCTURES FROM HIGH-THROUGHPUT SEQUENCING DATA

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The gel forming mucin, MUC5AC, has long been suspected to play important roles in airway innate immunity and CF disease progression, and has recently been shown to be associated with disease severity (Guo, et al, Mucin variable number tandem repeat polymorphisms and severity of cystic fibrosis lung disease: significant association with MUC5AC. *PLoS One*.

2011;6(10):e25452. Epub 2011 Oct 6.). To advance our understanding of MUC5AC function in CF and potential targeted intervention, it is critical to have complete and accurate genomic and mRNA sequence information. The current reference and alternative human genome assemblies are incomplete at the MUC5AC locus with an estimated 50 kb gap in the middle of the gene, largely due to the presence of Variable Number of Tandem Repeat (VNTR) sequences. We have constructed a complete genomic and mRNA structure of MUC5AC from working draft genome sequence, partial mRNA nucleotide sequences, and the reference genome. Independent validation of the genomic construct was obtained by comparing the *de novo* assembly of long sequence reads from 3rd-generation PacBio sequencing of human fosmid clones mapped to the reference genome through end-sequencing. The PacBio long sequence reads (up to 10kb) enabled us to perform successfully *de novo* assembly across the VNTRs. The MUC5AC exon structure and putative mRNA coding sequence were deduced from the draft genomic sequence by comparing to the longest protein sequence from Uniprot database. The draft exon structure and mRNA sequence were largely verified and extended at both ends by next-gen Illumina sequencing of mRNAs from a number of CF patients' nasal scrape samples. We believe the complete draft genomic sequence of MUC5AC will provide a useful tool to study CF, as well as other respiratory diseases, and general MUC5AC biology.

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IDENTIFICATION OF CFTR AND FLUID SECRETION REGULATORS IN ZEBRAFISH

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CFTR is a primary mediator of chloride transport in vertebrates and is required for proper fluid secretion in many organ systems. CFTR mutations cause CF, a disease characterized by decreased hydration of mucus on epithelial surfaces that ultimately causes destruction of the lungs, liver, pancreas, intestine and vas deferens. Although CF inheritance is monogenic, the severity of disease symptoms varies significantly, even in patients with identical CFTR mutations. Thus, identification of the genetic pathways controlling fluid secretion and/or CFTR activity will be an important step towards discovering disease modifier genes and new therapeutic targets for the treatment of CF. Previously, our lab has used forward genetics in the zebrafish system to uncover a conserved regulator of Cfr and characterize fluid secretion in the intestine. We have now identified two new mutants with excess fluid accumulation in the gut. Our preliminary results indicate that one of these mutants shows Cfr-dependent fluid accumulation and analyses are underway to determine if the second mutant displays Cfr-dependent fluid accumulation. Using a combination of positional cloning and candidate gene approaches, the objective of this study is to isolate the mutated loci responsible for the fluid accumulation phenotype in each mutant. We will also characterize the development of other endodermally derived organs (e.g. pancreas, liver) that express *cfr*. Identification of new regulators of fluid secretion will be the first step towards the development of new pharmacological agents for therapeutic intervention to improve the quality of life for CF patients.

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INTERLEUKIN 8 (IL8) EXPRESSION IN CYSTIC FIBROSIS (CF) NASAL EPITHELIA IS CORRELATED WITH A GENETIC VARIANT IN THE IL8 PROMOTER ASSOCIATED WITH CF LUNG DISEASE SEVERITY

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Background: Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by mutations in *CFTR*, with the leading cause of mortality

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being pulmonary disease. There exists a broad range of lung disease severity, even amongst F508del homozygotes, and non-CFTR modifier genes are expected to account for much of this variability (heritability 0.54-0.89; Vanscoy et al, 2007 *AJRCCM*; Mekus et al, 2000 *Twin Res*). Candidate-gene modifier studies demonstrate that a single nucleotide polymorphism (SNP; rs4073) in the *IL-8* promoter is associated with CF lung disease severity: In 1,112 CF patients, 2 copies of the rs4073 risk allele (TT) carried a significant association with lung disease severity ($p=0.02$). This effect was more pronounced in males ($n=608$; $p=0.001$). A luciferase reporter assay showed differential *IL-8* expression associated with rs4073 genotype in a human airway cell line (9HTEo) (Hillian et al, 2008 *Genes & Immun*). We hypothesize that differential expression of *IL-8* is correlated with rs4073 genotype *in vivo*.

Methods: A multicenter collaboration (University of North Carolina, Case Western Reserve University, Johns Hopkins University, University of Toronto) was developed with a goal of obtaining nasal epithelial samples from 150 CF subjects. In 119 subjects, our techniques have yielded high-quality RNA, and we have begun to measure *IL-8* expression via real time RT-PCR, testing correlations with rs4073 in an initial 54 CF patients (27 males).

Results: Subjects with 2 copies of the risk allele (TT; $n=15$) demonstrate a 2.82 fold increase in *IL-8* expression over patients with 1 copy (AT; $n=26$) and a 2.11 fold increase over patients with no copies (AA; $n=13$) ($p=0.012$, $p=0.0053$). Analysis of males showed 2 copies of the risk allele (TT; $n=8$) is associated with a 4.24 increase in *IL-8* expression over males with 1 copy (AT; $n=13$), and 6.47 fold increase over males with no copies (AA; $n=6$) ($p=0.011$, $p=0.003$).

Conclusions: These findings validate previous SNP association and *in vitro* expression studies (Hillian et al, 2008) by demonstrating that increased *IL-8* expression occurs *in vivo* in association with risk genotype in rs4073, particularly in males. Understanding the association of genetic variation and differential *IL-8* expression may provide further insight into mechanism, add ability to predict underlying non-CFTR-related genetic risk, and aid in development of therapeutic strategies. The North American CF Gene Modifier Consortium has performed a genome wide association and linkage study in 3467 CF patients (Wright et al., 2011 *Nat Genet*), and gene expression studies of directly harvested nasal airway epithelia are expected to provide further mechanistic insight into newly identified modifiers of CF lung disease.

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MODEL SYSTEMS

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INVESTIGATING THE ROLE OF THE PANCREATIC BETA-CELL IN THE DEVELOPMENT OF CF-RELATED DIABETES USING PSEUDOISLET MODELS

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Introduction: CF-related diabetes (CFRD) is the largest co-morbidity in CF. The development of hyperglycemia promotes bacterial colonization and accelerates lung decline. The etiology of CFRD is complex and poorly understood. However, pancreatic beta-cells have been shown to express CFTR, and functional CFTR channel activity is needed for normal patterns of insulin secretion (Edlund et al. 2010, *Acta Physiologica*). Moreover, pancreatic islets from CF ferrets are smaller than normal, and insulin-positive cells progressively decrease after birth (Olivier et al. 2011, *Ped Pulmonol*). Additionally, beta-cell distribution is altered in neonatal CF pigs (Meyerholtz et al. 2011, *Ped Pulmonol*). These findings suggest that early changes in the endocrine pancreas may take place before the destruction of the exocrine tissue in CF.

This study aimed to determine the role of the pancreatic beta-cell in the development of CFRD using cell-based 3D pseudoislet models expressing different CFTR mutations. Close cellular proximity and the anatomical 3D structure of native islets are needed to achieve normal patterns of insulin secretion. Pseudoislet models have previously been shown to mimic the behaviour of native islets and remove the significant animal burden posed by islet isolation for initial mechanistic studies.

Methods: The insulin-secreting, MIN6, beta-cell line was used for all experiments. We confirmed the expression of CFTR in wild-type MIN6 cells by Western blot. MIN6 cells were transfected with CFTR shRNA (CFTR deficient), R117H cDNA or F508del cDNA. All cells were configured as 3D pseudoislets as previously described (Kelly et al. 2010, *Pancreas*). The size and number of cells present in each pseudoislet was assessed. Cellular viability was investigated by MTT assay. Pseudoislets were exposed to rising concentrations of glucose and other insulinotropic secretagogues for 0-24h, and insulin secretion into the surrounding medium measured by ELISA. Calcium influx is an essential step in insulin exocytosis. We therefore examined calcium influx into pseudoislet cells by fluorescent microfluorimetry using FURA 2AM loading dye.

Results and Discussion: No difference in size, cell number, morphology or viability was observed between wild-type MIN6 pseudoislets and CFTR deficient or R117H pseudoislets. However, F508del pseudoislets were smaller ($<100\mu\text{M}$), contained fewer cells and had a significant reduction ($p<0.01$) in cellular viability compared with other pseudoislets. Compared with wild-type pseudoislets, insulin secretion in response to all stimuli, except basal (1.1mM) glucose concentrations, was significantly reduced ($p<0.05-0.001$) in CFTR deficient, R117H and F508del pseudoislets. The most pronounced reductions in insulin secretion were observed in F508del pseudoislets ($p<0.001$), which may reflect the lesser number of viable cells in this model. In addition, CFTR deficient, R117H and F508del pseudoislets showed reduced calcium influx following glucose challenge. Overall, these findings suggest that functional CFTR is important in insulin secretion and that the pancreatic beta-cell may have an important role to play in the development of CFRD.

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MYCOBACTERIUM ABSCESSUS COMPLEX: HOST RESPONSE IN A CF MURINE MODEL

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Introduction: *Mycobacterium abscessus* is a rapidly growing nontuberculous mycobacterium (NTM), considered by many to be the most pathogenic NTM in cystic fibrosis (CF) patients. However, clinical effects of *M. abscessus* infection in CF patients are highly variable, ranging from asymptomatic to dramatic declines in FEV1. Treatment regimens consist of months of multiple antibiotics often complicated by significant toxicity. Thus, prognostic factors to help guide treatment decisions are needed. *M. abscessus* exists in two morphotypes: smooth and rough. Differentiating between the morphotypes in clinical laboratories is not routine, and it is not known if morphotype identification would have prognostic value for CF patients. There is evidence that the rough morphotype is more pathogenic than the smooth, but prior animal models have had limited applicability to human CF airways disease due to dominating systemic disease or use of immunocompromised animals. We used a novel immunocompetent murine model of CF *M. abscessus* pulmonary infection to test our hypothesis that the rough morphotype is more pathogenic than the smooth morphotype.

Methods: Known quantities of *M. abscessus* rough and smooth morphotypes isolated from CF patients were suspended in thrombin and fibrinogen solutions and sequentially instilled into the tracheas of wild-type (WT) and CF (S489X with hCFTR gut correction) mice to retain the bacteria in distal airway plugs. At euthanasia, bronchoalveolar lavage (BAL), lung, and spleen tissues were collected and cultured on appropriate media. Weight trends were measured and airway leukocytes and cytokines were quantified.

Results: Persistent pulmonary infection with minimal systemic dissemination was achieved at high rates as far as 28 days post infection. CF mice had greater maximum weight loss than WT mice, a finding most evident with rough morphotype. CF mice had greater levels of KC (IL-8 analogue) and TNF- α in BAL fluid than WT mice. Rough morphotype infection resulted in a greater number of BAL neutrophils by day 14 in all mice. Thirty percent of animals infected with smooth morphotype had some spontaneous colony conversion to rough morphotype *in vivo*; these animals with rough conversion had greater maximal weight loss and slower weight gain, a finding most pronounced in the CF mice.

Conclusion: In an improved murine model of CF *M. abscessus* pulmonary infection the rough morphotype induced a greater host inflammatory response compared to the smooth morphotype. This trend was more pronounced in the CF compared to the WT mice, emphasizing the need for a CF

specific model to study host response in NTM infections. Future studies will further evaluate the potential relationship of morphotype to the clinical variability seen in CF patients infected with *M. abscessus*, as well as the host factors that may influence conversion from the smooth to the rough morphotype. Ongoing studies with this model are also examining the differences in host response between the species of *M. abscessus* complex (*M. abscessus*, *M. massiliense*, *M. bolletii*). These results may provide important prognostic data that could influence clinical laboratory reporting practices to better guide clinical treatment decisions.

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CFTR BIOMARKERS IN HUMAN AND MURINE COLONIC TISSUE: SIMILARITIES AND DIFFERENCES IN ION TRANSPORT AND PROTEIN DETECTION

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Background: CFTR is expressed in the murine and human large intestine, where it contributes to disease morbidity and mortality from bowel obstruction. Expression and function of CFTR in the murine intestine has been utilized to examine CFTR modulators that have entered clinical trials and thus can serve as a preclinical model system to test CFTR-active therapies. In the current study, we compared CFTR-dependent ion transport characteristics, CFTR detection by immunoblot and by immunohistochemistry in human rectal biopsies and murine rectal tissue using common reagents and techniques.

Methods: Human rectal biopsy tissue (n=15, all non-CF) and murine rectal tissue (n=47 adult mice – C57Bl6J) were dissected, and immediately mounted and studied under voltage clamp conditions. Tissues were warmed to 37°C by a circulating pump and continuously gassed in RPMI 1640 media + 25 mM HCO₃. Tissue was treated with 10 μM indomethacin, then treated with amiloride (100 μM), 10 μM forskolin + 100 μM IBMX (10 min), followed by carbachol (100 μM), and bumetanide (100 μM – 10 min). At the end of the ICM studies, tissue was either lysed for CFTR detection by IB or IHC. For IB, 20 μg protein was loaded into 6% gels. Mouse anti-CFTR antibody and rabbit anti-mouse-HRP with ECL and densitometry were used for protein detection and quantification. For IHC, paraffin sections were blocked with 10% normal goat serum and peroxidase blocker. Mouse anti-CFTR was incubated with sections and then incubated with ImmPRESS anti-mouse IgG for 30 min. DAB was used to detect CFTR protein.

Results: ICM - The mean (SD) amiloride-sensitive currents (μA/cm²) in human biopsies vs murine tissue were -22.03 (26.65) and -25.21 (47.28). The mean (SD) forskolin/IBMX-stimulated currents in human biopsies vs murine tissue were 95.34 (49.77) and 127.67 (126.34). The mean (SD) CCh-stimulated currents in human biopsies vs murine tissue were 55.42 (38.38) and 74.87 (102). The mean (SD) forskolin/IBMX + CCh-stimulated currents in human biopsies vs murine tissue were 150.76 (77.53) and 202.54 (182.08). The mean (SD) bumetanide-sensitive currents in human biopsies vs murine tissue were -24.63 (25.26) and -45.86 (54.46). None of these Isc differences were statistically significant.

CFTR biochemistry measures - C Band CFTR was readily detected by our protocol with the mouse anti-CFTR 596 antibody in both human and murine rectal tissue (input=20 μg). In addition, our IHC protocol detected both human and murine CFTR in colonic tissue sections, with predominant staining in glandular cells within the intestine.

Conclusions: The results indicate that the biochemical and functional detection of murine and human CFTR protein in tissues can be accomplished with common and parallel techniques. The murine colonic tissue demonstrated greater cAMP-dependent Cl⁻ transport than the human colonic biopsies, while the human tissue demonstrated greater CCh currents relative to the murine tissues. The combined CFTR currents in response to cAMP and CCh were similar across species, as were CFTR detection by IB and IHC utilizing the 596 anti-CFTR antibody.

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ACQUIRED CFTR AND TMEM16A DYSFUNCTION IN VIVO ASSOCIATED WITH TGF-BETA ACTIVATION

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Background: Loss of CFTR function in Cfr knockout mice produces bowel obstruction at weaning, despite normal pancreatic function. These observations suggest that ion and fluid transport in the murine intestine are responsible for bowel obstruction and the lethal Cfr knockout phenotype. Stool retention and DIOS is a common source of morbidity in CF, but it is unclear what tissue-specific signals may contribute to this clinical problem. In this study, we asked whether the induction of stool retention and constipation was sufficient to alter Cl⁻ transport in the murine colon, focusing on CFTR and TMEM16A expression and function. As recent results from our laboratory indicate that TGF-β potentially downregulates CFTR and TMEM16A expression and activity in T84 cells, we also explored whether stool retention increased TGF-β signaling in vivo.

Methods: C57Bl6J mice were treated with twice daily loperamide at a dose of 1.5mg/kg for 14 days. Stool output was monitored, and at day 14 mice were sacrificed. Rectal tissues were then mounted and studied under voltage clamp conditions, warmed to 37°C and continuously gassed with 95%O₂/5%CO₂ in RPMI 1640 media + 25 mM HCO₃. Cells were treated with 10 μM indomethacin (20 min), 100 μM amiloride (10 min), 10 μM forskolin + 100 μM IBMX to activate CFTR or 2 μM ionomycin to activate TMEM16A (10 min), 100 μM carbachol (CCh - 10 min), and 10 μM CFTRinh172 (10 min). Tissues were lysed and probed for CFTR and TMEM16A expression by immunoblot using mouse anti-CFTR 596 antibody and rabbit anti-TMEM16A antibody. Tissues were also fixed in paraffin blocks, stained for markers of TGF-β activation (pSMAD2 and p38), and assessed by immunohistochemistry. Paired T tests were used to compare Isc responses across conditions.

Results: Mice treated with loperamide demonstrated reduced stool output (0.69gm/mouse/day in control vs 0.34gm/mouse/day in loperamide treated, p<0.001). CFTR currents assessed by intestinal current measurements (ICM) were reduced in loperamide-treated mice compared with controls, with forskolin/IBMX + CCh = 24.96 μA/cm² (SD=8.86) compared with 85.8 μA/cm² (SD=37.75) in controls (p=0.04). TMEM16A currents were also reduced during loperamide treatment, with ionomycin + CCh = 6.2 μA/cm² (SD=2.03) in the loperamide-treated mice compared with 17.1 μA/cm² (SD=6.6) in controls (p=0.05). CFTR detection by IB was reduced in loperamide-treated mice compared with controls by 59% (SD=4.34, p=0.004). TMEM16A detection by IB was reduced in loperamide-treated mice compared with controls by 58.8% (SD=20.56, p=0.014). IHC of rectal tissue in loperamide-treated mice demonstrated enhanced detection of TGF-β markers, including pSMAD2 and p38 relative to untreated controls in colonic glandular enterocytes.

Conclusions: Pharmacologic constipation reduced the expression and activity of both CFTR and TMEM16A in mice, and increased TGF-β signaling in vivo. The results indicate that stool retention is sufficient to broadly inhibit Cl⁻ transport via two pathways, and provide a potential mechanism to link loss of Cl⁻ transport through activation of TGF-beta signaling.

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DISTINCT TRANSDUCTION DIFFERENCE BETWEEN ADENO-ASSOCIATED VIRUS TYPE 1 AND TYPE 6 VECTORS IN HUMAN POLARIZED AIRWAY EPITHELIA

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Of the many biologically isolated AAV serotypes, AAV1 and AAV6 share the highest degree of sequence homology, with only six different capsid residues. Both have been reported to infect airway epithelium efficiently from apical membrane, but it remains unclear if the two serotypes behave identically in terms of airway transduction. We compared the transduction

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efficiencies of rAAV1 and rAAV6 in primary polarized human airway epithelia (HAE) and found significant differences in their abilities to transduce epithelia from the apical and basolateral membranes. rAAV1 transduction was ~10-fold higher than rAAV6 following apical infection, while rAAV6 transduction was ~10-fold higher than rAAV1 following basolateral infection. Furthermore, rAAV6 demonstrated significant polarity of transduction (100-fold; basolateral>>apical), while rAAV1 transduced from both membranes with equal efficiency. To evaluate capsid residues responsible for the observed serotype differences, we mutated the six divergent amino acids either alone or in combination. Results from these studies demonstrated that capsid residues 418 and 513 most significantly controlled membrane polarity differences in transduction between serotypes, with the rAAV6-D418E/K513E mutant demonstrating decreased (~10-fold) basolateral transduction and the rAAV1-E418D/E513K mutant demonstrating a transduction polarity identical to rAAV6-WT. However, none of the rAAV6 mutants obtained apical transduction efficiencies of rAAV1-WT, suggesting that all six divergent capsid residues in AAV1 act in concert to improve apical transduction of HAE and thus rAAV1 may be an ideal choice for gene therapy for airway diseases, such as cystic fibrosis.

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ENDOGENOUS LUNG EPITHELIAL STEM/PROGENITOR CELL COMPARTMENTS DIFFER IN CYSTIC FIBROSIS AND NORMAL MICE

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Background: We have recently described a FACS sorting strategy and clonogenic assay to identify and prospectively isolate a rare EpCAMpos α 6-integrinpos β 4-integrinposCD24low multipotent epithelial stem/progenitor cells (EpiSPC) in adult mouse airways which can self-renew and give rise to lineage-restricted airway and alveolar progenitor cells when co-cultured with lung stromal cells and cytokines (McQualter et al., PNAS 107:1414). In this study, we have utilised this approach to ask whether there were differences in the incidence and proliferative potential of this candidate EpiSPC population in CF and normal mice.

Methods: We excised and disaggregated the lungs and conducting airways from heterozygous (normal), CF (unc), and CF(FABP) mice. Sorted CD45negCD31negEpCAMposSca-1low α 6-integrinpos β 4-integrinposCD24low cells from the three groups of mice were then cultured in our matrigel-based clonogenic assay to quantify EpiSPC.

Results: We detected a 5.2-fold and a 2.4-fold increase in the incidence of EpiSPC in the tracheal epithelia of CF(unc) and CF(FABP) mice respectively, compared to heterozygous (normal) tracheal epithelium.

Conclusions: These findings are consistent with the notion that expanded and dysregulated airway EpiSPC compartment could contribute to CF airway remodelling and mucous cell hyperplasia in progression of disease in CF lungs. These methods may have application to CF pigs and CF ferret lung tissues to understand CF lung pathogenesis and disease in models more similar to humans and able to recapitulate CF airway disease development more closely.

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ABNORMAL GLYCEMIC REGULATION IN CYSTIC FIBROSIS PIGS IS NOT ASSOCIATED WITH DEFICIENT IMMUNOHISTOCHEMICAL EXPRESSION OF INSULIN OR GLUCAGON IN THE PANCREAS

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People with cystic fibrosis (CF) develop diabetes mellitus as they age: ~50% of patients over 30 years of age have cystic fibrosis related diabetes (CFRD). Prior to CFRD onset, first phase insulin secretion from CF islets is impaired despite capacity for insulin secretion at later points following glucose challenge. After CFRD onset, structural damage to islets is partial and insufficient alone to cause diabetes. The mechanisms of these structural and functional impairments are not known. Utilizing the porcine CF model (both homozygous null and deltaF508 mutations), we studied fetal (day 54, days 83-90), newborn (pig gestation is ~114 days) and postnatal pig pancreas (2-12 months) for structural changes in the islets using insulin and glucagon immunostaining compared to non-CF. We also measured the fasting blood glucose, plasma insulin and glucagon (by ELISA) in newborn CF and non-CF pigs. Random blood glucose and hemoglobin A1c (HbA1c) were measured in postnatal pigs (mean age 4 months). Results are reported as mean $\pm$ SEM. Whole pancreas sections from CF and non-CF fetal and neonatal pigs showed no difference in alpha (glucagon) or beta (insulin) immunostaining area density. However, when the cellular density was measured within the remnant lobular tissue of neonatal pigs, there was a significant increase in the islet tissue area density of both alpha and beta cells. The area density of insulin and glucagon immunostaining in postnatal pigs was similar to neonates (no difference in the whole pancreas, increased density within the lobular parenchyma) and not different between CF and non-CF pigs. Fasting blood glucose and glucagon levels in CF newborn pigs were not significantly different from non-CF pigs, but the plasma insulin levels were significantly higher in CF newborns (1.5 $\pm$ 0.04 μ U/mL n=5 for non-CF, 2.2 $\pm$ 0.2 μ U/mL n=7 for CF, p<0.05). In postnatal randomly fed CF pigs, the blood glucose levels were significantly higher compared to non-CF (92 $\pm$ 9 mg/dL n=10 for non-CF, 168 $\pm$ 28 mg/dL n=6 for CF; p<0.01) with no difference in HbA1c. In conclusion, postnatal CF pigs develop hyperglycemia over time, but the abnormal glycemic regulation in CF pigs is not accompanied by reductions in islet mass. These findings in CF pigs suggest functional abnormalities in CF islets, similar to functional abnormalities postulated in CFRD patients, and/or deficiency in insulin action. Additional studies of these animals may help understanding the pathogenesis of CFRD.

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PATHOGENESIS OF MECONIUM ILEUS IN CF PIGS

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Meconium ileus is a life-threatening complication of cystic fibrosis that is present in about 10-20% of CF infants at birth. The CF pig, a large animal model of CF that closely replicates other human CF phenotypes, is born with meconium ileus that is remarkably similar to the human disease. Both porcine models of CF, *CFTR*^{-/-} and *CFTR*^{ΔF508/ΔF508}, show 100% penetrance of meconium ileus at birth and thus represent a feasible model to study the pathogenesis of meconium ileus. At birth, *CFTR* mRNA expression was highest in the proximal porcine small intestine and decreased distally. We

detected no CFTR mRNA in the CFTR null porcine intestine. Using confocal immunocytochemistry, we detected CFTR primarily in the crypts of the wild-type, but not CF, newborn intestine. Consistent with CFTR expression, we observed forskolin/IBMX-stimulated short circuit current in excised small intestinal and colonic segments bathed in a media containing both HCO_3^- and Cl^- and less current in the absence of Cl^- , in agreement with CFTR's role in HCO_3^- transport. cAMP-stimulated currents were absent or much reduced in intestinal segments from CFTR null animals. Consistent with CFTR's role in fluid transport, we observed fluid secretion in excised ileal segments of wild-type, but not CF piglets. Because meconium ileus develops during fetal development it has been difficult to understand its origins in humans. To understand the pathogenesis of meconium ileus, we asked if there was a correlation between onset of meconium ileus and CFTR in fetal piglets. Preliminary studies revealed that CFTR mRNA is present as early as 41 d gestation, a period that also corresponds to expression of CFTR in both human and mouse intestine. Likewise, we measured forskolin/IBMX-stimulated current in excised intestine from 35 d fetal wild-type piglets. This evidence of CFTR expression and CFTR-mediated anion transport in piglets in the first trimester of gestation precedes the first visible detection of meconium-like luminal contents at 54 d gestation. These findings suggest that absence of CFTR expression and function contribute to the pathogenesis of meconium ileus in CFTR null piglets.

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FROG PALATE EPITHELIAL MODEL TO EVALUATE OSMOTIC RESTORATION OF SURFACE LIQUID UNDER FLUID-DEPLETED CONDITIONS

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Several model systems have been developed to study airway epithelial cells under normal (N) and fluid-depleted (FD) conditions. Considerable variability in such factors as airway surface liquid (ASL) depth and composition were found, but there was good evidence that ASL decreased in CF and was attributed to Na^+ absorption. The resultant dehydration of the ASL in CF is thought to lead to compromised mucociliary clearance (MC) with mucus accumulation and inflammation. Osmotic agents such as hypertonic saline and mannitol deposited into the airway are postulated to reverse the ionic gradients produced by a failure of epithelial cells to secrete Cl^- and consequent increased Na^+ absorption. The upper palate of *Rana catesbeiana* has been used in our laboratory to study mucus characteristics and mucus clearance under normal and perturbed conditions. In previous studies when the effects of acute injury were evaluated in the epithelial tissue of the palate (as a model for airway cilia) the molecular biology techniques of zymography (for matrix metalloproteinases, MMPs 2 and 9) and Western blot for tissue inhibitors of MMPs (TIMP 1) yielded results consistent with mammalian and clinical studies. We showed that the epithelium of the frog palate can serve, not only as a physiological model for cilia function but also as a model to study inflammatory mediators. In the present study using blockers of Cl^- and HCO_3^- , we created a fluid-depleted frog palate for the study of osmotic agents on MC, surface liquid depth (SLD) and mucus rigidity with an eventual goal to improve available treatments to aid lung function and reduce adverse effects in CF. Employing hypertonic saline (HS) to test the model, we have shown marked improvement in MC from the FD condition that was corroborated with an increase in SLD compared to the FD condition and a decrease in mucus rigidity. These results are consistent with results in mammalian models and clinical studies that concluded the primary action of an osmolyte placed in the airway is to draw water out of the epithelial cells into the surface liquid and mucus improving MC. Using this model it is possible to create N, FD and restored conditions in the same ex-vivo experiment. Our results corroborated with those obtained in more elaborate mammalian model systems that showed it would be a valid model in which to initially test new osmotic agents. Two recent sets (n=5 each) of experiments using nebulized 2% saline + isotonic Frog Ringer's (2/3 R+ 1/3 distilled water) in one experiment and high molecular weight dextran in another, were more effective than 2% saline alone to restore MC. To show repeatability, the %coefficient of variation of MC was determined in each individual experiment and the sets compared using a t-test which showed no difference in two separate sets of studies of the MC times under N conditions. Thus we can conclude that experiments under relatively standard conditions with subjects drawn from the same population offer a very stable

estimate of error variance that can only add confidence to these results. Therefore further studies on these agents or combination of agents are recommended in this model to benefit CF treatment. Study supported by the CIHR.

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UP-REGULATION AND ACTIVATION OF CLC-2 MEDIATED CHLORIDE TRANSPORT IN MURINE NOSE AND LUNG SUGGESTS AN ALTERNATIVE THERAPEUTIC TARGET FOR CYSTIC FIBROSIS

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Background: Multiple sodium and chloride channels on the apical surface of nasal epithelial cells contribute to periciliary fluid homeostasis. The nasal potential difference test (NPD) can be used in vivo to quantify the activity of these channels during perfusion with ion channel agonists and inhibitors. The objective of this study was to utilize the NPD to quantify CIC-2 function in mice that overexpress CIC-2 using the putative CIC-2 agonist lubiprostone and the potent and specific peptide CIC-2 inhibitor GaTx2. For this purpose, we have generated a novel, epithelial-directed TetOn CIC-2 mouse model (k18rtTA-hCIC2). CFTR knockout and CIC-2 knockout mice were also studied.

Methods: Nasal and lung tissue was harvested from doxycycline treated and untreated k18rtTA-hCIC2 mice, and visualized by Western blot to verify increased CIC-2 expression after doxycycline treatment (IP injection 40mg/kg 24 hours prior to harvest). k18rtTA-hCIC2, CIC2KO, and CFTRtm1Unc-Tg(FABPCFTR)1Jaw/J (CFTRKO, gut corrected with wtCFTR) mice were sedated and intubated prior to NPD measurement. Baseline NPDs were performed on k18rtTA-hCIC2 mice prior to receiving doxycycline, then performed again following doxycycline treatment, and the results compared. NPD solutions included "Ringers": a baseline Ringer's saline solution, "Amiloride": Ringer's + amiloride, "Zero Chloride": a chloride-free gluconate-substituted Ringer's solution + amiloride, "Lubiprostone": Zero Chloride + 20μM lubiprostone, and "GaTx2": Lubiprostone + the CIC2 inhibitor GaTx2 at 200nM. For NPDs on k18rtTA-hCIC2 and CIC2KO mice, all solutions also included 30μM CFTRinh 172 to reduce CFTR contribution. The solutions for CFTRKO and CIC2KO mice did not include CFTRinh 172 and GaTx2, respectively.

Results: Treatment with doxycycline significantly increased the CIC-2 expression level and the lubiprostone response in eighteen (18) k18rtTA-hCIC2 mice, with an average response in the untreated mice of -1.58 ± 0.41 mV (mean $\pm$ SEM, n=18), and -3.99 ± 0.57 mV (p=0.0006) in the CIC-2 up-regulated mice. This lubiprostone response was partially inhibited by GaTx2; untreated mice recovered 1.16 ± 0.53 mV upon addition of GaTx2, while doxycycline treated mice recovered 1.59 ± 0.42 mV. No significant change was observed in CIC-2 up-regulated mice in response to the amiloride or zero chloride solutions as compared to the untreated control. Six (6) CFTRKO mice showed a large hyperpolarization indicative of chloride efflux upon addition of the lubiprostone solution (-6.19 ± 3.05 mV), which was partially inhibited by GaTx2 ($+2.1 \pm 1.76$ mV). Five (5) CIC2KO mice showed a reduced response to the lubiprostone solution (-1.11 ± 0.53 mV), suggesting some level of lubiprostone activation of CFTR or other chloride channels.

Conclusions: Overexpression and activation of CIC-2 leads to improved NPD readings in vivo, suggesting that it is available as an alternative pathway for epithelial chloride secretion in murine nose and lung. The utilization of CIC2 as an alternative chloride efflux channel could provide clinical benefit to patients with CF.

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GENISTEIN: EFFECTS ON THE R117H MOUSE JEJUNUM

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We have previously shown that female wild-type mice (Wt, C57BL/6J) fed a genistein-containing diet (600 mg/kg food, 600G) for 1-month have

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significantly elevated basal levels of jejunal chloride (Cl) secretion (measured as transepithelial short circuit current, I_{sc}) compared to mice fed a genistein-free diet (OG). In Wt mice, this was a gender-specific response to 600G diet; there was no response in males. In this study, we fed male and female R117H cystic fibrosis (CF) mice, either 600G or OG diet for 1-month. Basal I_{sc} was unchanged in 600G male mice (39.2±11.7 μA/cm², n=9) compared to OG controls (34.9±7.7 μA/cm², n=9). Basal I_{sc} was unchanged in 600G female mice (36.7±8.6 μA/cm², n=10) compared to OG controls (29.3±6.5 μA/cm², n=7). Comparable steady-state increases in I_{sc} in response to forskolin (10 μM, bilateral) were observed in 600G and OG male and female mice. There was no effect of the inhibitor of the Na/K/2Cl co-transporter, bumetanide (100 μM, basolateral), on the forskolin-stimulated I_{sc} in either 600G or OG fed male and female mice. There was no difference in the % inhibition following the addition of acetazolamide (100 μM, bilateral) to block the bicarbonate secretory component, in 600G fed males (10.3±2.6 %, n=9) and 600G fed females (3.5±4.2 %, n=10), compared to their respective controls. In the male (n=8) and female (n=3-4) R117H mice, there were no measurable changes in jejunum morphology with the diets (villi length, number of goblet cells per villi, crypt depth, number of goblet cells per crypt). These data suggest that in both male and female R117H mice, a 4-week diet treatment with genistein, at this dose of 600G, does not provide any improvements in the overall magnitude of basal or forskolin-mediated secretion. These results are in stark contrast to the gender-dependent effects of the same dose and duration of dietary genistein on Wt mice. *S. Polito was supported by the Midwestern University Summer DO Fellowship Program. L. Al-Nakkash was supported by the Soy Health Research Fund. R117H mice were generously provided from Dr. M. L. Drumm (Case Western Reserve University).*

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ADVANCING A YEAST PHENOMICS MODEL FOR GENE INTERACTION NETWORK ANALYSIS OF F508DEL-CFTR BIOGENESIS

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We have developed a genome-wide phenotyping approach for *S. cerevisiae* designed to understand regulators of F670del-Yor1 biogenesis as a molecular model for F508del-CFTR. This strategy employs the genomic set of *S. cerevisiae* loss of function alleles for assessment of gene interactions, defined as a functional dependency of the F670del-Yor1 phenotype on other genes. The *S. cerevisiae* genome does not contain CFTR, but does include members of the broader ABC transporter family among which the phenylalanine corresponding to F508 is highly conserved. F670del-Yor1 is a mutation that has been characterized for its similarity to F508del-CFTR in terms of protein misfolding and defective cellular trafficking. Yor1 functions as a drug pump, and is the primary cellular resistance factor for the mitochondrial poison, oligomycin. When cultured on media containing oligomycin, cell proliferation is dramatically influenced by alterations in the biogenesis of F670del-Yor1. By combining the F670del-Yor1 allele into the collection of 5000 *S. cerevisiae* gene deletion strains, we have shown by quantitative phenotyping that there are hundreds of interacting genes modulating F670del-Yor1 biogenesis. We observe that the network influencing F670del-Yor1 bears strong resemblance to the corresponding network that underlies trafficking of F508del-CFTR, based on homology with human genes reported in the literature. Similarly, we found that the F670del-Yor1 gene network is predictive of novel gene interactions that influence F508del-CFTR processing. In light of these initial results, we recently advanced the predictive power of the model by use of secondary assays designed to prioritize gene interactions most relevant to cystic fibrosis, including: 1) delineating F670del-Yor1 gene interactions that are promoter independent (which we expect to be of highest translational relevance). To address this question, we expressed F670del-Yor1 from distinct promoters and repeated the genome-wide phenotypic survey; 2) establishing F670del-Yor1-dependence of each gene interaction. This is important to exclude genes that directly influence oligomycin sensitivity, independent of F670del-Yor1. We addressed this by quantifying oligomycin resistance when F670del-Yor1 is not expressed in the yeast deletion strain library. In addition to confirming the primary screen, these secondary analyses provide a highly accurate, robust, and detailed view of the F670del-Yor1 interaction network with regard to genes most likely to have human homologs and modulate F508del-

CFTR trafficking. The overall approach defines an unbiased screen for CF relevant therapeutic targets and a model for higher order network analysis.

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LOSS OF CFTR ALTERS GLIAL CELL FUNCTION AND MYELINATION IN NEWBORN PIGS WITH CYSTIC FIBROSIS

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene cause cystic fibrosis (CF). For decades, nervous system abnormalities have been reported in patients with CF. However, it has remained possible that such defects were secondary consequences of the disease. To explore this issue, we studied CFTR^{-/-} pigs, which develop disease that closely matches that in people with CF, and assessed the nervous system in newborns. Consistent with earlier reports, we found CFTR transcripts in the central and peripheral nervous systems. We found glial cells to be an important site of CFTR expression and activity, and loss of CFTR affected glial cell Ca²⁺ signaling. Because glial cells support axons, we examined axon structure and function in nerve bundles and found reduced axon density in CFTR^{-/-} pigs. Ultrastructural examination revealed myelin sheath abnormalities with characteristics similar to, but less severe than those in known neuropathies. Consistent with this, genes associated with demyelinating and dysmyelinating diseases, including myelin basic protein, proteolipid protein and myelin protein zero were increased in CFTR^{-/-} pigs. To test for functional consequences, we measured the conduction velocity of trigeminal and sciatic nerves and found it to be reduced. In addition, auditory brainstem evoked potentials revealed defects in auditory nerve function. Interestingly, intermediate phenotypes were often observed in CFTR^{+/-} pigs, suggesting a gene dosage effect. These results indicate that loss of CFTR directly affects nervous system function and may explain the longstanding, but previously unexplained findings of altered neural function in people with CF. They also raise the questions of whether nervous system abnormalities contribute to the pathogenesis of CF or produce a phenotype in people heterozygous for CFTR mutations.

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CREATION OF A CFBE410- CELL LINE WITH A SINGLE INTEGRATION SITE FOR THE EXPRESSION OF RARE CFTR MUTANTS

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There are currently over 1,900 different mutations that have been identified in CFTR. For a vast majority of these mutations, the effect on protein expression, processing, and function remains unknown. Cell-based systems can be useful in the evaluation of mutation effect upon function. However, uniform levels of CFTR expression are desirable so that the effect of mutations is correctly interpreted. Transient transfection of cell lines leads to a high level of expression in only a minor subset of cells while stable expression using an expression plasmid under antibiotic selection leads to highly variable expression. One approach to alleviate this variability is to integrate a recombinase target site into the genome so that only one heterologous cDNA is expressed per cell as previously demonstrated for MDCK cells (1) and fetal rat thyroid cells (2). However, neither cell line is human in origin or derived from tissues affected in CF (e.g. airways). To address this issue, we selected a human bronchial epithelial cell line derived from a CF patient (CFBE410-; F508del/F508del) for the introduction of recombinase-mediated single cDNA integration. The expression of endogenous CFTR RNA was found to be negligible in several CFBE clonal lines when analyzed by RT-

PCR. CFTR protein was also not detected by Western blot indicating that endogenous CFTR will not interfere with functional interpretation of exogenous CFTR mutants. CFBE cells were transfected with pFRT/lacZeo, a plasmid containing an FRT site for targeted recombination, as well as a Zeocin resistance gene for clonal selection. Four Zeocin-resistant cells lines were obtained and integration was checked by Southern blot using a probe specific for the Zeocin resistance gene. After digestion of the genomic DNA with three different restriction enzymes, all four clones revealed bands of the expected plasmid size of 8.1kb, indicating the presence of episomal plasmid. Further inspection of the pFRT vector revealed that it contains the SV40 origin of replication (SV40 ori). CFBEs were immortalized using a plasmid containing the SV40 virus and express the large T antigen, which binds the SV40 ori and enables episomal replication of the pFRT plasmid. To obtain genomic integration of the plasmid, three nucleotides of the SV40 ori were mutated that had previously been shown to prevent large T antigen mediated replication (3). After transfection and selection, single colonies were isolated to create six clones. Integration events were evaluated by Southern blot using EcoRV digested genomic DNA. The blot revealed no bands at the expected plasmid size and a genomic band of approximately 3kb for three of the six clones. These results confirm that a single genomic integration event has occurred for three of the clones, and that the plasmid is no longer able to replicate episomally via its SV40 ori. The CFBE cell line with the FRT recombinase target site provides a new human epithelial cell-based tool to study the effect of disease-associated mutations upon CFTR function.

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CFTR IS REQUIRED DURING DEVELOPMENT OF THE ZEBRAFISH LIVER AND PANCREAS

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Cystic fibrosis is a disease characterized by mucosal buildup in several organs due to defective fluid secretion caused by mutations in a chloride channel, CFTR. In addition to decreased lung function, many patients also develop pancreatic insufficiency, cystic fibrosis related diabetes, and liver fibrosis. Although CFTR has been well characterized *in vitro*, the pathophysiology of the CF remains poorly understood. With the development of new animal models of CF, symptoms of liver and pancreas disease have been identified early in life, indicating that the defects in the function of these organs may arise during their morphogenesis. To identify the role of CFTR during organogenesis of the liver and pancreas we turned to the zebrafish, a well characterized developmental model. We developed several new reagents which have allowed us to investigate the developmental functions of *cftr*. Using immunofluorescence, *in situ* hybridization, and live imaging of a GFP-tagged *cftr* transgenic line, we have found that *cftr* is expressed in the exocrine pancreas and liver. To determine whether *cftr* is required for the development of these organs we generated *cftr* mutant zebrafish using TAL Effector Nucleases and utilized an antisense morpholino against Cftr. In addition to zebrafish-specific defects in fluid secretion early in development, analysis of mutants revealed that *cftr* is required for the morphogenesis of the zebrafish liver and pancreas. In mutant zebrafish, the size of the liver and exocrine pancreas are reduced. In addition, the β -cells of the pancreatic islet are reduced similar to the reduction observed in human patients. Importantly, these phenotypes are observed before the liver and pancreas begin to function, indicating that these morphogenesis defects precede tissue destruction due to blockage of the organ ducts. Together, these studies reveal that *cftr* is a critical component of zebrafish organogenesis and indicate that the symptoms observed in human patients may arise during development.

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PRIMARY RABBIT NASAL SEPTAL EPITHELIAL CULTURES FOR STUDIES OF CYSTIC FIBROSIS SINUS DISEASE

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Transgenic cystic fibrosis (CF) murine models have greatly facilitated studies of CF pathogenesis and treatment. We recently described and characterized primary cell culture of murine nasal septal epithelium (MNSE) grown at an air-liquid interface that demonstrates excellent differentiation and robust, physiologic ciliary beat frequency stimulated by ATP and other agonists. Small rodents, however, do not reproduce key aspects of human airway physiology. For example, molecules developed with the intent of restoring CFTR function may exhibit species specificity – a fact underscored by lack of CFTR activation in MNSE by the potentiator VX-770. As experimental epithelia, rabbit tissue provides an excellent model for studies of human sinus disease. Rabbit sinonasal epithelium consists of 80 to 90% ciliated cells – similar to the human sinus and nasal cavities – and *in vivo* methods for investigating rabbit sinusitis are well established and suited for studies of therapeutic intervention. The objectives of the current experiments were to develop primary rabbit nasal septal epithelial (RNSE) cultures and evaluate their usefulness as a model of transepithelial transport and CFTR function in particular. RNSE was cultured at an air-liquid interface on filter supports to confluence and full differentiation. One rabbit septum yielded an average of 180 cultures (20-fold higher than a murine septum). Optimization of culture conditions was conducted to achieve approximately 80-90% ciliated cells as judged by scanning electron microscopy. Differentiation occurred within 14 days and all inserts attained resistance > 500 Ω/cm^2 . Monolayers were mounted in Ussing chambers to investigate pharmacologic manipulation of ion transport. RNSE exhibited a change in forskolin-stimulated current (ΔI_{sc} in $\mu\text{A}/\text{cm}^2$) compared to MNSE (6.3 $\pm$ 0.2 vs. 16.8 $\pm$ 2.1, respectively; $p < 0.0001$). However, VX-770 stimulated CFTR-mediated Cl^- secretion in RNSE (1.4 $\pm$ 0.1) whereas no measurable response was detected in MNSE. All cultures demonstrated inhibition of forskolin and VX-770 stimulated current by the CFTR inhibitor INH-172 (5 μM). In summary, standardized, well characterized RNSE cultures represent a useful model for studying CFTR activity, and provide advantages over murine nasal epithelial tissues. Culture yield from the rabbit septum is 20-fold greater than the murine septum. Although forskolin-dependent Cl^- secretion was modest in comparison to MNSE, activation with VX-770 indicates important molecular similarities between rabbit and human CFTR, including usefulness of rabbit epithelial cultures for testing CFTR potentiation in the future.

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DIRECT CARDIOVASCULAR EFFECTS OF THE CFTR ΔF508 MUTATION

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Background: The CFTR- ΔF508 mutation has a high carrier frequency, and female carriers have decreased blood pressures. CF patient bronchial smooth muscle cells have decreased calcium flux, but CFTR-null mouse aortic smooth muscle cells have a paradoxical increase in vasoconstriction. We speculated that intracellular retention of the dysfunctional CFTR protein produced by the ΔF508 mutation interferes with calcium mobilization and decreases myogenic tone.

Objective: To test the hypothesis that ΔF508 homozygous pigs have decreased aortic contractile responses to calcium channel activation.

Methods: CFTR-null and ΔF508 heterozygous pigs were generated by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. Animals were crossed, and thoracic aortas were harvested from homozygous progeny within 6h of birth. Aortic tone was assessed by wire

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myography. Data were compared by ANOVA. To assess translational implications, umbilical arteries were harvested from one infant diagnosed with presumed CF (meconium ileus with both parents $\Delta F508$ carriers, genotype pending) and 3 control infants.

Results: Compared to aorta from null or wild-type pigs, aorta from $\Delta F508$ pigs had decreased KCl-induced constriction (null 5.0 ± 0.4 g, wild-type 5.1 ± 0.4 g, dF/dF 3.8 ± 0.3 g, $N=11-21$, $P < 0.01$). When aorta from additional litters were incubated for 5 hours in a physiologic saline solution, those from $\Delta F508$ piglets generated decreased myogenic tone (null 6.2 ± 0.5 g, wild-type 5.8 ± 0.4 g, dF/dF 3.9 ± 0.6 g, $N=11-37$, $P < 0.001$), with a greater impairment seen in dF/dF females (3.1 ± 0.6 g) than dF/dF males (4.4 ± 0.9 g). Combined inhibition of intracellular calcium channels with 2-APB (IP3R antagonist) and ryanodine (RyR antagonist) diminished aortic tone, recapitulating the phenotype seen in dF/dF piglets (null 3.3 ± 0.8 g, wild-type 3.5 ± 0.5 g, dF/dF 2.6 ± 0.4 g, $N=4-9$, $P=0.32$). While umbilical arteries obtained from all 3 control humans developed significant myogenic tone 90 minutes after passive stretch and incubation in physiologic buffer (2.4 ± 0.6 g), umbilical arteries obtained from the infant with presumed CF did not (-0.8 g). Responses of control umbilical arteries were similarly diminished by preincubation in the IP3R and RyR antagonists (0.1 ± 1.1 g).

Conclusions: Aorta from pigs homozygous for the common $\Delta F508$ mutation have decreased tone. We speculate sarcoplasmic reticulum retention of CFTR- $\Delta F508$ interferes with calcium channel activation, decreases vascular resistance and contributes to the lower arterial pressures seen in those carrying the $\Delta F508$ mutation.

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STRUCTURAL ABNORMALITIES IN THE NEWBORN CF PIG LUNG

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In newborn CF pigs we have observed tracheal cartilage ring defects, abnormal appearing airway smooth muscle bundles, and reduced tracheal and mainstem bronchi caliber. Additional studies using micro-CT based imaging of the newborn pig tracheal lobe, the porcine equivalent of the human right upper lobe, have found that the percentage of CF airway size reduction was greatest for the large airways and tapered as they became smaller and more peripheral. In this study, we asked if the CF pig tracheal lobe volume is normal at birth and if distal airspace development is dependent upon CFTR. Tracheal lobes from non-CF and CF newborn pigs were instillation fixed at a pressure of 20 cmH₂O and CT scanned. The lobes were manually segmented from the scans to acquire an estimate of lobar volume. The average tracheal lobe volume in non-CF was 8.45 ± 0.48 cm³ and in CF 6.95 ± 0.45 cm³ ($p < 0.05$), a reduction of approximately twenty percent. A similar trend was observed when we measured total lung volume with CT in spontaneously breathing newborn pigs (81.2 ± 5.3 cm³ vs. 63.8 ± 7.1 cm³ for non-CF and CF, respectively). Following CT scanning, the tracheal lobes were processed for morphological assessment. Mean linear intercept, an estimate of the volume to surface ratio of acinar airspaces, was determined based upon tracheal lobe histology. No statistically significant differences were found between genotypic groups. The mean linear intercept length was 65 ± 2.5 μ m for non-CF and 60 ± 2.1 μ m for CF. In summary, in the newborn CF pig lung we have observed a reduction in caliber of the large airways and in lung size. Additional morphometric studies of alveolar size, number, and surface area will be required to fully understand the extent of these congenital abnormalities and their importance in the pathogenesis of early CF lung disease.

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A FUNCTIONAL CFTR ASSAY USING PRIMARY CYSTIC FIBROSIS ORGANOID

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We have recently established conditions allowing long-term expansion of epithelial organoids from human intestine, recapitulating essential features of the in vivo tissue architecture. Here, we apply this technology to study primary intestinal organoids of patients that suffer from cystic fibrosis (CF). As expected, we found CFTR located at the apical membrane of cells in the organoids. Forskolin induces a rapid swelling of wild-type organoids, but not of organoids derived from CFTR-deficient mice or CF patients. This phenomenon is phenocopied by CFTR-specific inhibitors. Function of the common, temperature-sensitive CFTR-F508del mutant is restored upon incubation at low temperature, as well as by the corrector compounds VRT-325, Corr-4a, VX-809 and the potentiator VX-770. In organoids derived from different CFTR F508del homozygous patients, we observed differential capacity of (combinations of) drugs to restore CFTR function suggesting patient-specific drug efficacy. We have also established methods to measure drug efficacy in CF organoids grown from rectal biopsies after their use in intestinal current measurements (ICM), and found correlation between ICM and our novel forskolin-induced swelling assay. This relatively simple and robust assay will facilitate diagnosis, functional studies, drug development as well as personalized medicine approaches in CF.

NEW THERAPIES, BIOMARKERS & OUTCOME MEASURES

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PREDICTING SUSTAINED RESPONSE TO BRONCHITOL™ TREATMENT IN PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Cystic fibrosis (CF) causes significant progressive pulmonary disease; identification of predictors of response to long term therapy is important given that CF treatment is life-long, onerous, expensive and complex. The efficacy and safety of Bronchitol™ (inhaled dry powder mannitol) has been established in two large phase III trials (CF-310, CF-302). We sought to determine the independent contribution of baseline factors to treatment response from this population, and establish whether early FEV1 response might be useful in predicting longer term impact on lung function.

Methods: A retrospective analysis in patients, completing 26 weeks blinded therapy. Responders were defined as patients with a sustained response during the 26-week double-blind treatment period, response was defined as $\geq 5\%$ relative improvement in FEV1 (% pred) over the treatment period. Stepwise logistic regression was used to determine independently contributing baseline and on-treatment factors. Results from the week 6 on-treatment time-point were explored to assess the sensitivity and specificity of response thresholds to predict a sustained response over 26 weeks.

Results: Baseline Predictors: Two baseline factors were significantly associated with response; treatment with Bronchitol™ vs control ($p=0.0038$) and the baseline rate of %/yr decline in FEV1 ($p=0.0068$). Sensitivity and specificity tests showed moderate utility but not high enough to be clinically useful. At a cut off of 50%, the sensitivity and specificity for predicting a sustained response were 35% and 83% respectively.

On-treatment Predictors: All thresholds explored provided clinical utility as changes from baseline after 6 weeks were similarly predictive of

response over the whole 26 weeks eg, 41% of patients had $\geq 6\%$ response; sensitivity was 87% and specificity 83% [Table]. Improvement in % pred FEV₁ $>0\%$ after 6 weeks of BronchitolTM was significantly correlated with sustained response over 26 weeks ($p<0.0001$).

Conclusion: Although baseline rate of decline in FEV₁ independently predicts sustained response to BronchitolTM, its inadequate sensitivity excludes too many responders to be clinically useful. While basing treatment on patient phenotype does not appear to be useful, the utility of a range of response thresholds predicting long term response after 6-weeks of treatment with BronchitolTM provides clinicians with an approach that improves the certainty of response, minimises unnecessary exposure and risk, and is consistent with an individualised treatment strategy for patients with CF.

Threshold - FEV ₁ relative change % predicted	% of patients meeting/ exceeding threshold	Utility of week 6 data to predict a treatment effect over Weeks 6, 14, 26	
		Sensitivity (%)	Specificity (%)
> 0%	61.2	86.8	78.2
$\geq 2\%$	54.8	87.9	78.8
$\geq 4\%$	49.0	85.8	76.5
$\geq 6\%$	41.3	86.5	83.0
$\geq 8\%$	34.9	83.5	84.5
$\geq 10\%$	29.1	76.2	85.2

Table 1. Sensitivity and specificity of different response thresholds at Week 6 predicting overall response in the Bronchitol ITT population

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RESULTS OF THE PHASE 3 STUDY OF ATALUREN IN NONSENSE MUTATION CYSTIC FIBROSIS (NMCF)
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Background: Ataluren enables readthrough of premature termination codons to produce full length, functional CFTR in patients with nonsense mutation CF (nmCF), who comprise ~10% of all patients with CF.

Methods: A Phase 3, randomized, double blind, placebo controlled clinical trial evaluated the efficacy and safety of ataluren in patients with nmCF. Eligible patients ≥ 6 yrs of age with nmCF and %-pred FEV₁ between 40-90% were randomized to receive ataluren 10, 10, 20 mg/kg orally TID or placebo for 48 wks. Stratifications included chronic inhaled antibiotic use, age, and %-pred FEV₁. Primary and secondary endpoints were %-pred FEV₁ change from baseline at 48 wks and pulmonary exacerbations (PEX), respectively. Tertiary endpoints included nasal potential difference.

Results: In all, 238 patients were randomized; the intent-to-treat population comprised 232 patients (116 ataluren, 116 placebo). The primary endpoint compared mean relative changes in % pred FEV₁ at Wk 48, and demonstrated a treatment difference of 3% favoring ataluren (-2.5% change on ataluren vs. -5.5% change on placebo; $p=0.124$, primary analysis); the average treatment effect across all post-baseline visits demonstrated a treatment difference of 2.5% favoring ataluren (-1.8% change on ataluren vs. -4.3% for placebo, $p=0.048$). Over 48 wks, the PEX rate was 23% lower for ataluren versus placebo ($p=0.099$). Among the *a priori* stratifications, only the interaction of treatment with chronic inhaled antibiotic use was statistically significant (nominal $p=0.007$). In patients not being treated with inhaled antibiotics, a 6.7% difference in the relative change in % pred FEV₁ at Wk 48 favoring the ataluren treatment group was seen (nominal $p=0.015$); the average treatment effect was 5.5% in favor of ataluren across all post baseline visits (nominal $p=0.009$). Similarly, a 43% lower PEX rate over 48 wks (nominal $p=0.012$) favoring ataluren was observed in this subgroup. These effects appeared to be driven by patients receiving chronic inhaled tobramycin and concur with *in vitro* data which shows that aminoglycosides antagonize ataluren-induced readthrough, suggesting an explanation for the observed subgroup effect. The tertiary endpoint of nasal potential difference

did not demonstrate a treatment difference in this study. Safety profiles were similar for ataluren and placebo, other than cases of reversible Grade 3-4 creatinine elevations, which were associated with the combination of nephrotoxic antibiotics with ataluren. Preliminary data from the ongoing open-label extension study are encouraging.

Conclusion: Ataluren treatment over 48 wks resulted in trends towards improved lung function and pulmonary exacerbation rate compared to placebo in patients with nmCF; a larger treatment benefit was observed in the subgroup of patients not receiving chronic inhaled aminoglycoside therapy, commensurate with *in vitro* data.

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THE SYNTHETIC AMINOGLYCOSIDE NB124 SUPPRESSES CFTR PREMATURE TERMINATION CODONS MORE EFFECTIVELY THAN GENTAMICIN AND PRIOR SYNTHETIC DERIVATIVES
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Certain aminoglycosides induce translational readthrough of premature termination codons (PTCs). However, toxicity and lack of efficacy deter treatment with clinically available aminoglycosides for genetic diseases caused by PTCs, including CF. Previously, our laboratories showed that using a structure-based approach, the novel aminoglycoside derivative NB54 exhibited reduced cellular toxicity while also demonstrating efficacy that equaled that observed with gentamicin *in vitro* and *in vivo*. In this study, we provide new evidence that a second generation of aminoglycoside derivatives, NB74, NB84, and NB124, further enhance efficacy and reduce toxicity, resulting in translational readthrough that exceeds gentamicin and other prior aminoglycoside derivatives.

NB derivatives were first screened in a dual luciferase reporter system transiently transfected into CFBE41o- cells encoding several relevant CFTR PTC contexts. In R1162X, NB124 caused dose dependent readthrough of nonsense transcripts, achieving 2.5-fold over control at 100 μ M, and exceeding gentamicin (1.4-fold, $P<0.0001$); other NB derivatives were intermediate. Similar efficacy was observed in W1282X (1.9-fold NB124 over control vs. 1.3-fold gentamicin, $P<0.0001$) and G542X (1.5-fold NB124 over control vs. 1.1-fold gentamicin, $P<0.0001$) constructs.

CFBE41o- cells stably transduced with the R1162X transgene demonstrated partial restoration of CFTR-dependent cAMP stimulated current following 48 hr incubation with aminoglycosides at 250 μ g/mL. Stimulated Isc was 2.8 μ A/cm² in NB124 treated monolayers ($P<0.0005$) vs. 0.5 and 0.3 in gentamicin and vehicle control, respectively. Western blotting confirmed translation of full-length CFTR C-band that was superior with NB124 treatment (5.5-fold, $P<0.005$) vs. vehicle or gentamicin (each 2-fold over control). Similar results were observed in CFBE41o- transduced with W1282X CFTR, with NB124 providing greater CFTR dependent Isc (15.9 μ A/cm² NB124 vs. 5.2 and 3.9 following vehicle and gentamicin treatment, respectively, $P<0.0005$). Dose-dependent rescue of CFTR currents were observed in both CFBE41o- lines with NB124 treatment, with peak effects at 250 μ g/mL. In primary HBE cells expressing G542X/F508del, NB124 treatment rescued forskolin-dependent (20 μ M) Isc to 3.1 μ A/cm² compared to 1.2 μ A/cm² in control treated cells ($P<0.01$); NB124 treatment improved CFTR dependent Isc activity to ~12% of WT-CFTR and was greater than that observed with gentamicin, NB54, and other derivatives. In toxicity studies using cochlea cell monolayers, NB124 exhibited 4.3 fold lower LD50 than gentamicin, indicating an efficacy:toxicity ratio at least 15X greater than gentamicin. The synthetic aminoglycoside NB124 significantly enhances readthrough of PTCs and rescues CFTR activity as shown by several complementary assays. The current results support further research efforts to optimize synthetic aminoglycosides for translational readthrough, and could result in agents suitable for long-term treatment of the basic CF defect found in ~10% of CF patients and many other genetic diseases.

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NANOPARTICLES FOR SITE-SPECIFIC GENOME EDITING IN CYSTIC FIBROSIS

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Triplex-forming peptide nucleic acids (PNAs) are synthetic molecules that are capable of binding to targeted sites in genomic DNA, enhancing the homologous recombination of short 50-60 base-pair “donor DNA” fragments. This results in specific genome modification. Unlike plasmid delivery or whole-gene insertion, this type of gene modification is both heritable and constitutes true site-specific gene editing. We have created polymer nanoparticles containing triplex-forming PNA and donor DNA capable of mediating the correction of the common $\Delta 508$ mutation in CFTR.

We designed three tail-clamp PNA molecules that bound near the $\Delta 508$ mutation (Fig. 1A). Gene correction can be detected by allele-specific PCR (AS-PCR) using an allele-specific forward primer. These molecules were incorporated into poly(lactide-co-glycolide) (PLGA) nanoparticles, which were 100-200 nm in diameter (Fig. 1B). Nanoparticles containing donor DNA and PNA-2 were able to mediate correction of the $\Delta 508$ mutation in CFTR (Fig. 1C). This correction was confirmed both by direct sequencing of clones derived from corrected populations, with about 1% cells corrected after a single treatment. Modification was confirmed using a chloride flux assay (Fig. 1D). In addition, after two treatments at 2 mg/mL high dose, up to 42% of treated CFBE cells showed significant chloride efflux (average slope 139.9 Δ AFU/ Δ min) with forskolin. In a preliminary study in a CF mouse model, we treated two mice for one week with intranasal nanoparticles targeting murine CFTR and one mouse received blank nanoparticles. Nasal potential differences were measured, showing that one of the mice treated with CFTR nanoparticles had a -4.1 mV change in response to a 0Cl + amiloride + forskolin compared to baseline response prior to treatment. Further studies are underway at this time. This system represents a new technology for specific editing of the CFTR gene.

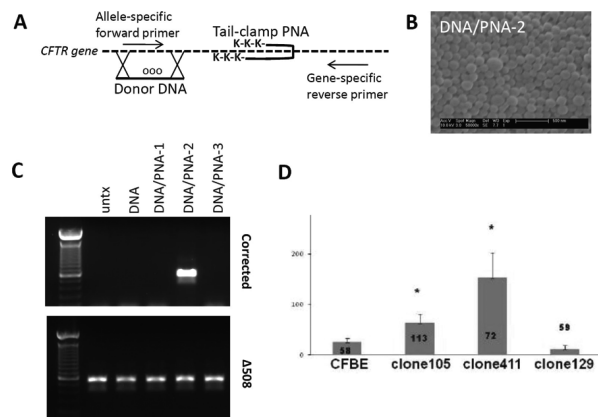


Figure 1. (A) Schematic of PNA and donor DNA. (B) Scanning electron micrograph of PLGA nanoparticles containing DNA targeting CFTR and PNA-2. Scale bar is 500 nm. (C) Allele-specific PCR shows that nanoparticles containing donor DNA and PNA-2 mediate correction of CFTR. (D) Chloride efflux was measured using N-[ethoxycarbonylmethyl]-6-methoxy-quinolinium bromide (MQAE), a fluorescent indicator dye. CFBE cells had significantly lower chloride efflux than clones 105 and 411, which were derived from cells treated with nanoparticles. Clone 129 is another un-modified control.

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HYPERPOLARIZED GAS MRI OF IVACAFTOR THERAPY IN PERSONS WITH CYSTIC FIBROSIS AND THE G551D-CFTR MUTATION

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Background: Hyperpolarized Gas MRI (HG-MRI) allows direct, high-resolution imaging of certain non-radioactive isotopes of noble gases, such as He-3. Images from patients with CF show numerous “ventilation defects” – areas of the lung into which inhaled gas does not flow due to airway obstruction. Ventilation defects correlate with disease severity. Ivacaftor improves FEV₁ in patients with CF who have the G551D-CFTR mutation.

Methods: This single-center, Phase 2, single-blind, placebo-controlled study evaluated the effects of ivacaftor on ventilation as revealed by HG-MRI in 8 subjects with CF who have the G551D-CFTR mutation and to assess the utility of HG-MRI as a biomarker of lung disease. Subjects were required to have FEV₁ >40% predicted to enroll. Subjects received ivacaftor 150 mg q12h for 4 weeks and placebo for a total of 4 weeks in a cross-over design. Helium-3 MRI imaging was conducted on Days 0, 14, 28, 42, and 56.

Results: HG-MRI revealed ventilation defects at baseline in all subjects, including those with FEV₁ >90% predicted. Treatment with ivacaftor for 28 days led to a substantial reduction in total ventilation defects [8.20% as determined by human reader (HR) ($P=0.0547$) and 12.81% by computer algorithm (CA) ($P=0.0078$)]. Reductions in total defect volume were 0.48 L as determined by HR ($P=0.0313$) and 0.68 L by CA ($P=0.0078$) as assessed by HG-MRI. These changes were associated with an increase in percent predicted FEV₁ of 12.8 percentage points ($P=0.0078$). Four participants reported one or more adverse events (abdominal distention, pulmonary exacerbation, pain, oropharyngeal pain, dry throat, and headache). All were mild and none resulted in study discontinuation.

Conclusions: HG-MRI could provide a novel endpoint in clinical trials of CF, one that both corroborates traditional pulmonary function testing and provides unique information, including absolute quantification of disease burden, information on burden in each lung or lobe, and assessment of burden in patients with relatively normal spirometry. Adverse events were consistent with previous studies of ivacaftor.

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NITROGEN MULTIPLE BREATH WASHOUT IS MORE SENSITIVE THAN SF₆ BASED LCI MEASUREMENTS AT DETECTING LUNG ABNORMALITIES IN CHILDREN WITH CF

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Background: Lung clearance index (LCI) measured by multiple breath washout has been shown to be a sensitive marker of early lung disease and an important outcome measure in clinical trials. In an effort to validate a nitrogen based multiple breath washout system against the gold standard mass spectrometry based technology, we found LCI measured by nitrogen washout, was higher compared with the gold standard, but that there was no systematic bias in the differences between these two systems. We further compared data collected from these two systems to explore whether the observed differences could be explained by patient demographic characteristics of disease severity, measured by traditional lung function tests.

Methods: In a cross-over design, healthy and CF children performed MBW by mass spectrometry (AMIS 2000; Innovision A/S, Odense, Denmark) using 4% SF₆ and by nitrogen washout (Exhalyzer D, Eco Medics AG, Switzerland). In most children (89%) we also measured traditional lung function tests (spirometry, body plethysmography). We used simple linear regression to determine whether the mean difference between LCI measured by MBW-SF₆ and MBW-N₂ was associated with demographic characteristics (e.g. age, height, weight) and other measures of lung function (FEV₁

from spirometry and FRC from body plethysmography). Both lung function outcomes were corrected for body size and interpreted as percent predicted.

Results: To date 50 healthy children (median age 11 years (range 3-17)) and 44 children with CF (median age 11 years (range 3-17)) completed MBW measurements using both the mass spectrometry and N₂ washout; 44 and 40 children, respectively, also had spirometry and/or plethysmography measured. The difference in LCI between the two MBW systems was not associated with weight-for-age or height-for-age centiles. However, when compared to FEV₁ (percent predicted), the difference between the two systems was greatest at lower values of FEV₁ ($\beta=0.02$, $p=0.004$), such that LCI measured by nitrogen washout was higher (thus more abnormal) when FEV₁ was more abnormal. After correcting for body size, the difference in LCI between the two systems was also greater for higher values of FRC by plethysmography ($\beta=-0.02$, $p=0.03$), demonstrating that for poor lung volumes LCI measured by N₂ is more abnormal. In addition, FRC measured by plethysmography was 0.32L lower compared with FRC measured by mass spectrometry; whereas the FRC by plethysmography was very similar when measured by nitrogen washout.

Conclusion: These results suggest that LCI measured by the nitrogen based multiple breath washout is more sensitive than SF₆ based measurements of LCI at detecting lung abnormalities, as determined by spirometry and plethysmography, in children with CF.

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DESIGN CHALLENGES AND EFFICIENCY MAXIMIZATION FOR PHASE 2 CLINICAL TRIALS IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) affects approximately 30,000 people in the US and 70,000 worldwide. This relatively small patient population poses challenges to researchers especially considering most studies do not allow participants to be enrolled in multiple clinical trials simultaneously and that specific inclusion/exclusion criteria make the eligible patient population smaller yet. Phase 2 proof of concept studies in CF can easily be underpowered simply due to feasibility issues. New and promising therapies or drugs may be prematurely abandoned in early phases as a result of a small, underpowered study. Given the orphan status of CF, maximizing the efficiency of Phase 2 studies is paramount.

We examine several common CF endpoints, including inflammatory markers, lung function and pulmonary exacerbations, and design considerations related to these endpoints that can impact the efficiency of a trial. Among these considerations are effect size and variability estimation, length of study and randomization schemes. Special consideration is given to the potential confounding impact of conducting clinical trials in the presence of CF standard of care (SOC), in particular, the use of cycling inhaled antibiotics. Biomarkers and clinical measures can oscillate with the use of cycling antibiotics, therefore, timing of baseline and end-of-study measurements impact trial efficiency and analysis methods, especially in studies with a shorter follow-up period or those with imbalance across treatment groups.

Through a review of published clinical trial data and simulation we conclude that a 1 month study can be conducted most effectively in an "off" SOC period to avoid a possible treatment effect washout or "ceiling" effect from SOC. The efficiency of a 2-4 month study design differs by endpoint and timing of SOC. For example, timing of enrollment can affect power by up to 7% when conducting a time to event analysis of pulmonary exacerbations. Likewise, allocating more subjects to receive treatment over placebo can reduce overall sample size by 8% when a response to treatment is highly variable in a biologic marker. Wading through the complexity and interrelatedness of these and other design considerations, while preserving clinical relevance, can be confusing. We give design recommendations for several scenarios commonly seen in CF research.

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IMPACT OF ACUTE ANTIBIOTIC USAGE ON THE PULMONARY EXACERBATION ENDPOINT IN A CYSTIC FIBROSIS RANDOMIZED TRIAL

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Rationale: Pulmonary exacerbations (PEs) are a key outcome in pivotal cystic fibrosis (CF) clinical trials. During a trial however, physicians prescribe treatment based on their own diagnoses rather than whether an a priori PE definition is met. Thus, it is common for antibiotics to be given during a clinical trial for events not considered a PE. The objectives of this study were to investigate the impact of this non-PE antibiotic usage on the risk of a subsequent protocol-defined PE. The hypothesis is that among younger CF patients with more mild disease, treating early with antibiotics could reduce the risk of a subsequent PE, and ultimately produce an attenuated treatment effect for the PE endpoint in a randomized clinical trial.

Methods: This retrospective study utilized data collected from the randomized, placebo-controlled trial of azithromycin in children with CF ages 6-18 uninfected with *Pseudomonas aeruginosa* (Saiman et. al. 2010 JAMA). The primary endpoint for the current study was time to PE utilizing an a priori definition. Antibiotic usage for reasons not meeting the PE definition was treated as a time-dependent covariate in Cox proportional hazards models for the PE endpoint. Sensitivity analyses were performed to re-estimate the treatment effect for the PE endpoint after accounting for non-PE antibiotic usage.

Results: Among 260 randomized patients in the trial, 61/129 (47%) placebo participants and 43/131 (33%) azithromycin participants received acute antibiotics prior to and not meeting the PE endpoint (15% difference, 95% CI:3%,26%). This antibiotic usage, predominantly for increased cough or sinus symptoms in the absence of other criteria needed to meet the PE endpoint, was not significantly associated with risk of a PE in the azithromycin group (hazard ratio [HR]:0.80, 95% CI:0.27,2.41). In comparison, a trend towards a reduced risk of subsequent PE in the placebo group was identified although non-significant (hazard ratio [HR]:0.68, 95% confidence interval [CI]: 0.36, 1.29). Despite the lack of significance of this association, non-PE antibiotic usage was a confounder for the comparison of the treatment groups in terms of the PE endpoint. Azithromycin usage was associated with a 50% reduction in PEs as compared to placebo not adjusting for non-PE antibiotic usage (HR:0.50, 95% CI:0.31,0.79) and a 56% reduction in PEs after accounting for the higher non-PE antibiotic usage in the placebo group (HR:0.44, 95% CI:0.30,0.65).

Conclusions: Antibiotic usage for reasons not considered a PE is common within a CF trial, and can confound the results of the PE endpoint when differentially prescribed between treatment groups. Understanding the clinical significance of these antibiotic events is necessary for continued optimization of the PE definition and in order to derive a more robust endpoint for future CF trials.

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HYPOFERREMIA REFLECTS INCREASED RISK OF ANTIBIOTIC TREATMENT IN CYSTIC FIBROSIS

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Background: Reduced blood iron content (hypoferremia) affects many patients with advanced cystic fibrosis (CF). We have shown that more severe hypoferremia is associated with lower percent-predicted FEV₁, body mass index, and hemoglobin, higher plasma interleukin-6 (IL-6) levels, and a

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greater burden of diabetes. Therefore, hypoferrremia reflects disease severity. We have also demonstrated that serum iron levels increase significantly following intravenous antibiotics for CF pulmonary exacerbation (CFPE). To investigate whether iron-related biomarkers could predict an increased risk of treatment with systemic antibiotics, we calculated from the medical records of 53 adult patients the elapsed time between these measurements and their next systemic antibiotic course (i.e., antibiotic-free days).

Methods: A range of clinical, hematologic, and microbiologic data were collected at a point of stable health. Clinic, telephone, and admission notes were reviewed along with medication and prescription records subsequent to data collection. Using antibiotic-free days as the dependent variable, dichotomous variables associated with differential treatment risk were identified by log-rank test. Cox proportional-hazards regression was used to assess the independent effect of risk factors on antibiotic-free days. No subject was considered more than once. $P < 0.05$ was deemed statistically-significant.

Results: The following features were associated with increased risk of antibiotic treatment: age < 24 years (HR 2.4, 95% CI 1.2-5.0), BMI < 20.5 kg/m² (HR 1.9, 95% CI 1.0-3.5), diabetes (HR 1.8, 95% CI 1.1-3.2), serum iron < 42 µg/dL (HR 1.9, 95% CI 1.1-3.2), and sputum *Aspergillus* colonization (HR 2.1, 95% CI 1.0-4.4). A Cox proportional-hazards model was generated in which younger age, lower serum iron, and *Aspergillus* colonization were independently associated with a greater likelihood of antibiotic treatment in this cohort. The hazard ratio fell by 6.7% (95% CI 3.1-10.2) for each additional year of life and by 1.9% (95% CI 0.8-3.0) for each 1 µg/dL increase in serum iron ($P < 0.001$ for both). Subjects with and without *Aspergillus* had similar serum iron levels and ages.

Conclusions: Lower serum iron concentration, younger age, and isolation of *Aspergillus* species from sputum independently predicted an increased risk of systemic antibiotic treatment. A single serum iron reading may be useful in assessing future need for antibiotics. The effect of age on antibiotic prescription risk could reflect barriers to treatment among our older patients but more likely signifies a bias toward longevity in patients with fewer CFPE events and milder disease. Our observation that *Aspergillus* colonization was associated with a higher risk of receiving antibiotics contributes to a mixed body of literature on this topic. The manner by which these factors impact antibiotic prescribing practices should be prospectively studied using validated symptom inventories and a specific definition for CF pulmonary exacerbation.

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SERUM IGF-1 DURING CYSTIC FIBROSIS PULMONARY EXACERBATION: TRENDS AND BIOMARKER CORRELATIONS

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Background: Insulin-like growth factor-1 (IGF-1) is an anabolic hormone that reduces protein turnover and gluconeogenesis, enhances glucose utilization by muscle, and promotes weight gain. Abundant clinical work, mostly in children, has shown that serum IGF-1 levels are abnormally low in cystic fibrosis (CF) and that low levels are associated with impaired growth. However, little is known about IGF-1 in the context of CF pulmonary exacerbation (CFPE). This study was done to evaluate changes in serum IGF-1 following standard therapy for CFPE and to assess whether serum IGF-1 correlated with other clinical metrics before and after these interventions.

Methods: Serum samples were obtained from 12 adult CF patients within 24 hours of hospital admission for CFPE and within 24 hours of completing their inpatient course. We defined CFPE as the onset of new or acutely worsened sinopulmonary and constitutional signs and symptoms that included the following: increased cough frequency, increased daily sputum expectoration, wheezing, dyspnea, fever, chills, sweating, anorexia, and weight loss. Biomarkers were measured during early and late CFPE. Therapy included intravenous antibiotics, nutritional support, airway clearance, and glycemic control. A subset of subjects also had serum samples obtained during a clinic visit when they were deemed to be at baseline. In addition, serum samples were obtained from 8 healthy control subjects. A commercially-available ELISA was used to quantify serum IGF-1. Biomarkers were

analyzed by Spearman correlation (ρ) and paired t-tests. $P < 0.05$ was significant.

Results: Data are presented as mean $\pm$ SD. Subject age was 32 ± 11 years. Percent-predicted FEV1 (FEV1%) and weight at early CFPE were $34 \pm 14\%$ and 54.3 ± 11.9 kg, attesting to severe disease. Serum IGF-1 levels at baseline in CF subjects were significantly lower than those in healthy controls ($p < 0.001$). In addition, serum IGF-1 was reduced in CF subjects during an exacerbation compared to baseline ($p < 0.01$). In CF subjects, serum IGF-1 rose by 114.7 ng/mL (95% CI 55.6-173.7 for mean difference) between early and late exacerbation. Serum IGF-1 correlated with the following parameters at early and late CFPE, respectively: FEV1% ($\rho = 0.67$ and 0.78), serum iron ($\rho = 0.82$ and 0.84), hemoglobin ($\rho = 0.75$ and 0.69), transferrin saturation ($\rho = 0.76$ and 0.91), and alpha-1-antitrypsin (A1AT) ($\rho = -0.66$ and -0.71). IGF-1 correlated with serum IL-6 only at the end of therapy ($\rho = -0.85$).

Conclusions: IGF-1 levels are reduced in adult subjects with CF and there is further reduction seen during exacerbation. Therapy for CFPE improves serum IGF-1 levels. A direct relationship between IGF-1 and FEV1% before and after treatment raises the possibility that IGF-1 could serve as a surrogate endpoint for pulmonary function in clinical trials. In the context of therapy for CFPE, IGF-1 is closely correlated with biomarkers of iron homeostasis, suggesting a possible role for IGF-1 in regulating iron bioavailability during inflammatory states. The novel observation of an indirect correlation between IGF1- and A1AT also warrants future investigation.

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REGULATION OF INTRACELLULAR Ca^{2+} BY SECRETORY LEUKOPROTEASE INHIBITOR (SLPI): A NOVEL THERAPEUTIC TARGETING NEUTROPHIL MIGRATION IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) is generally diagnosed early in childhood and is characterised by sustained neutrophil dominated inflammation from a very young age. Conversely, recruited neutrophils are ineffective in the clearance of bacteria and play a significant role in development of bronchiectasis. Identification of agents modulating neutrophil migration constitutes a relevant therapeutic goal for reducing inflammation and subsequent tissue destruction. Studies have shown that SLPI is anti-inflammatory in such disorders as atherosclerosis and lung emphysema. The aim of this study was to investigate SLPI as a novel therapeutic specifically targeting excessive neutrophil recruitment in CF.

Ethical approval from Beaumont Hospital Institutional Review Board was acquired and written informed consent obtained from all study participants. Patients with CF ($n=10$) were heterozygous for the $\Delta F508$ mutation and illustrated a median FEV1 of 39% (23-53). Neutrophil (2×10^5) migration in response to fMLP (10^{-6} M) and IL-8 (10ng) in the presence or absence of recombinant human SLPI (240-480nM) or oxidised SLPI was measured employing a Boyden chamber. The effect of SLPI on cytoplasmic levels of inositol 1,4,5- P_3 (Ins-1,4,5- P_3) and Ca^{2+} flux was assessed fluorometrically. Actin polymerisation, talin and vinculin cleavage was assessed by Western blot analysis. Statistics were analysed using GraphPad Prism 4.0 with significance determined at $p \leq 0.05$.

Here, we show that SLPI is a neutrophil cytoplasmic protein and that exogenous SLPI significantly inhibits the migration of neutrophils isolated from healthy control donors and individuals with CF ($p < 0.05$). The mechanism of inhibition involved regulation of Ca^{2+} release from intracellular calciosomes. We also provide evidence that the anti-migratory effect mediated by SLPI can be overcome by allowing influx of Ca^{2+} from the external environment, by inclusion of exogenous Ins-1,4,5- P_3 , or by inactivation of the antiprotease by oxidation. Notably, in addition to inhibiting IL-8-induced filamentous actin formation, SLPI potently blocked Ca^{2+} dependent calpain induced cleavage of the cytoskeletal membrane anchors talin and vinculin ($p < 0.05$).

In conclusion, the release of Ca^{2+} from intracellular stores is activated upon cell stimulation and migration via occupancy of the Ins-1,4,5- P_3 sensitive channel, a process now shown to be regulated by SLPI. The novel findings of this study highlight the therapeutic potential of SLPI for reducing the number of neutrophils migrating to the CF airways.

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THE TOPICAL ANTICHOLINERGIC IPRATROPIUM REDUCES CHLORIDE RESPONSES MEASURED BY NASAL POTENTIAL DIFFERENCE

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Introduction: Although the nasal potential difference (NPD) technique allows physiological assessment of airway epithelial ion transport in vivo, and provides measurement of CFTR function in vivo, little work has been undertaken to determine if any endogenous control mechanisms exist which can alter airway epithelial ion transport in vivo. The two main control mechanisms in the airways, namely adrenergic and cholinergic, are modulated by two classes of inhaled medications – albuterol stimulates beta adrenergic receptors and ipratropium blocks cholinergic receptors. Although beta-adrenergic bronchodilators are known to stimulate Cl⁻ secretion, the effect of anti-cholinergic medications is not known. With the recent interest in this class of agents following the Phase III trial in CF (J CF 2011 10 suppl 1 S21) we examined the effect of topical anti-cholinergic agents on NPD in human subjects in vivo.

Methods: Nasal PD was measured using our standard technique (ERJ 1994; 7: 2050). The effect of ipratropium (bromide) was assessed in 2 protocols – in six healthy non-CF volunteers:

- (i) baseline - baseline + ipratropium – baseline,
- (ii) baseline - amiloride - amiloride / low Cl⁻ - amiloride / low Cl⁻ + ipratropium - amiloride / low Cl⁻.

Results: Addition of ipratropium to the physiological buffer (Krebs solution) decreased absolute NPD (making it less negative) by approximately 1 mV. However, following pre-treatment with amiloride / low Cl⁻, the subsequent effect of ipratropium was a reduction in absolute NPD of 7.6 mV, which reversed almost completely with washout. Importantly, those nostrils with the largest Cl⁻ response demonstrated the largest response to ipratropium.

Conclusion: Ipratropium alters Cl⁻ secretory responses as measured by the NPD. Further studies in CF subjects are currently underway.

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CORNEAL ENDOTHELIAL CHANGES IN CYSTIC FIBROSIS

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Background: Corneal endothelium plays an important role in maintaining stromal hydration and transparency of the cornea. Cystic fibrosis transmembrane conductance regulator (CFTR) is present in corneal endothelial cells. Malfunctioning CFTR in cystic fibrosis (CF) may cause changes in corneal endothelial morphology. Physiologic stress and pathologic changes of the aqueous humor also cause changes. Examining the corneal endothelium for endothelial cell density, coefficient of variation, and the percentage of hexagonal cells can help identify early changes in corneal cells. The purpose of this study was to examine corneal endothelial changes in patients with CF and to evaluate possible mechanisms for any changes.

Methods: A specular microscope was used to record one central image of the right eye of each subject. Three groups of subjects were compared: CF patients, age-matched healthy subjects, and age and FEV1-matched asthma patients. Once obtained, the image was analyzed for cell density, coefficient of variation, and percentage of hexagonal cells.

Results: Study populations included 26 CF patients, with a mean age of 11.8 years and a mean FEV1 of 94%, and 20 asthma patients, with a mean age of 11 years and a mean FEV1 of 102%. The healthy control group contained 26 patients with a mean age of 11.5 years. Patients with CF had a significantly higher coefficient of variation (p=0.002) and lower percentage of exagonal cells (p=0.005) than the healthy control group. CF patients also had a significantly higher coefficient of variation (p=0.009) and a lower percentage of hexagonal cells (p=0.011) than the asthma group. The endothelial cell density was not significantly different between the CF group and the control group (p=0.0864) or the CF group and the asthma group (p=0.961).

Conclusions: Patients with CF have a higher coefficient of variation and a lower percentage of hexagonal cells than age-matched healthy and age and FEV1-matched asthma patients. As these changes are early indicators of pathologic change in the cornea, we believe they are reflective of abnormal CFTR function.

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UNRAVELING THE MECHANISM OF VX-770 (KALYDECO)

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VX-770 (Kalydeco) is the first CF drug that targets directly the root of CF pathogenesis, namely the malfunction of CFTR channels. Although proven effective in clinical trials, very little is known about its mechanism of action. VX-770 potentiates WT-CFTR as well as many disease-associated mutations by prolonging their open time. Since the traditional strict coupling model for CFTR gating demands that the formation and separation of the NBD dimer be obligatorily coupled to the opening and closing of the gate respectively, the only two ways to prolong the open time is to delay NBD dimer separation or to impede ATP hydrolysis. But none of these two possible mechanisms can offer a nice explanation for the effect of VX-770 on G551D-CFTR, a mutation that likely prevents NBD dimerization from happening due to its unique position in the signature sequence and for the same reason eliminates ATP hydrolysis. Recently, we identified a new pathway in CFTR gating that allows ATP to capture the post-hydrolytic channels before the gate closes and shuffle it back to “re-enter” the original pre-hydrolytic open state to initiate another hydrolysis cycle. Inspired by this finding, we proposed that VX-770 promotes such a “re-entry” pathway by stabilizing the post-hydrolytic open state. Such a “re-entry” pathway delays channel closure by recycling the about-to-close post-hydrolytic channel for another round of ATP hydrolysis. Therefore, the length of each opening burst is correlated to the number of ATP molecules hydrolyzed. This hypothesis is supported by several unpublished findings. First, we found that ATP-independent activity of WT-CFTR is increased by >6 fold in the presence of VX-770, a consequence of stabilizing the post-hydrolytic open state. This finding also provides a nice explanation for how VX-770 works on G551D-CFTR as G551D-CFTR channels lose all ATP-dependent activity yet retain intact ATP-independent opening events. Second, since the “re-entry” pathway requires binding of ATP to the post-hydrolytic open state, it is predicted that the frequency of the “re-entry” events depends on [ATP]. Consistent with this idea, we found that the open time of WT-CFTR is significantly prolonged at high [ATP] (882 ± 141 ms, $n = 25$ in 10 mM versus 438 ± 34 ms, $n = 17$ in 100 μ M) in the presence of VX-770. The most compelling evidence came from studying a mutant CFTR, R352C, that allows us to “see” ATP hydrolysis taking place within each opening burst. In the presence of VX-770, we found more opening events containing hydrolysis of more than 1 ATP molecule. VX-770 is the progenitor of medication targeting CFTR, but it benefits only a small portion of the CF population. Thus, understanding its mechanism and site of action is pivotal for future development of new drugs that could benefit more CF patients.

Results: The comparison of means between the ages of the two groups (control group vs cystic fibrosis group), revealed no statistically significant difference ($p=0.271$). The analysis of the control group vs CF group values for sodium showed: Cut-off: 13.5mmol/L; Sensitivity: 73.4%; Specificity: 70.6%; and for chloride: Cut-off: 20mmol/L; Sensitivity: 68.1%; Specificity: 72.9%. The sodium showed higher levels in the CF group (median: 18mmol/L; sd:12.65mmol/L) than in the control group (median: 11mmol/L; sd:4.78 [$p<0.001$]). We also observed an increase in the level of chlorine in the CF group (median: 26mmol/L; sd:13.48) over the control group (median: 16mmol/L; sd:8.42 [$p<0.001$]). When evaluating the group of patients with pancreatic insufficiency (PIG) vs control group (CG), a statistically significant difference was observed in the levels of sodium (median: 18mmol/L; sd:13.25 and control group median: 11mmol/L; sd:4.78 [$p<0.001$]) and chloride (median: 25.50mmol/L; sd:14.20 and control group median: 16mmol/L; sd:8.42 [$p<0.001$]).

Conclusion: The levels of sodium and chloride in the saliva of CF patients were higher compared with the control group. The results obtained thus far indicate that the saliva can be a potential diagnostic resource for CF, however more studies are needed to support this hypothesis.

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A RANDOMIZED DOUBLE BLINDED PLACEBO CONTROL TRIAL OF DOXYCYCLINE AS AN ADJUNCTIVE ANTI-INFLAMMATORY AGENT DURING CF EXACERBATION (DOXY)

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Unrelenting neutrophilic inflammation is a hallmark feature of CF airway disease and is largely responsible for extracellular matrix (ECM) remodeling, bronchiectasis, and mortality related to CF lung disease. Recently, our laboratory has described a novel neutrophil chemoattractant (proline-glycine-proline (PGP)) derived from ECM breakdown via a specific protease cascade. We have shown that one of the proteases involved in this pathway, matrix metalloproteinase-9 (MMP-9), is inhibited by doxycycline and that doxycycline can inhibit the generation of PGP from collagen via CF sputum *ex vivo*. However, it is unknown if this effect may be seen in patients with CF lung disease.

The hypothesis of this study is that doxycycline can reduce the MMP-9 activity in the sputum and blood of patients during hospital exacerbation, thereby reducing inflammatory indices and potentially impacting clinical endpoints. The primary outcome of this study is safety/tolerability of doxycycline in the CF patient population and change in MMP-9 activity in sputum and blood samples. The secondary outcomes of the study include measures of inflammatory biomarkers in sputum and blood, change in lung function, and sputum and blood doxycycline levels.

This study is a single center, randomized double-blinded placebo controlled study of oral doxycycline (100 mg) twice a day versus placebo as an adjunctive therapy during inpatient CF exacerbation. Inclusion criteria included patients undergoing uncomplicated inpatient CF exacerbation who were colonized with *Pseudomonas aeruginosa*. Randomization was conducted by UAB Research Pharmacy. Sputum and blood samples were collected at the beginning of hospitalization and prior to doxycycline; sputum and blood were also collected at end of hospitalization. Patients were followed up 1 month after study completion with phone call.

To date, both the doxycycline and placebo agent were well-tolerated with no serious adverse events noted and only one adverse event (rash) potentially related to the drug; patients had no sequelae noted at one month after therapy. We are currently in the process of completing the study and expect unblinding to occur shortly. The results related to biochemical and clinical endpoints will be made available shortly thereafter.

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CENTRALIZED INTESTINAL CURRENT MEASUREMENTS (ICM): CFTR ACTIVITY IN FRESH AND COLD-STORED RECTAL BIOPSIES, AND COMPARISON OF FORCEPS VS SUCTION BIOPSY

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Background: We developed common SOPs that quantify CFTR activity in human rectal biopsies, demonstrating outstanding sensitivity and specificity. To reduce barriers in the use of ICM as a CFTR biomarker for inclusion in clinical trials, we examined centralization of the electrophysiologic aspects and examination of the impact of biopsy methods on the CFTR signal (forceps vs suction).

Methods: Human rectal biopsy tissue was obtained at four study sites ($n = 27$ subjects, all non-CF), placed immediately in iced RPMI buffer. Three types of ICM studies were completed: 1) Comparison of fresh vs cold-stored specimens (18-24 post-biopsy, onsite); 2) Comparison of samples studied simultaneously at two sites after 18-24 hrs cold storage; and 3) Comparison of forceps and suction-based rectal biopsies (fresh samples). All studies were within subject comparisons. Biopsies were dissected and immediately studied under voltage clamp conditions (Physiologic Instruments, San Diego, CA). Tissues were warmed to 37°C by a circulating pump and continuously gassed with 95%O₂/5%CO₂ in RPMI 1640 media + 25 mM HCO₃. Tissue was treated with indomethacin (10 μ M, 20 min), amiloride (100 μ M, 10 min), forskolin + IBMX (10 and 100 μ M, 10 min), carbachol (100 μ M, serosal – 10 min), and bumetanide (100 μ M, 10 min). For (1), Least Square Means (LS-Means) of short circuit changes of CFTR currents (forskolin/IBMX + carbachol) were compared for Day 01 vs Day 02 ($n=17$ subjects). For (2), Intraclass Correlation Coefficients (ICC) for CFTR currents were compared for Site 01 vs Site 02 ($n=7$ subjects). For (3), descriptive statistics of CFTR currents for all suction vs forceps biopsies were compared ($n=3$ subjects, $n=22$ biopsies).

Results: Fresh vs cold-stored specimens (18-24 hrs): The LS-Means of CFTR currents for fresh biopsies were 155.85 μ A/cm² compared with 73.81 μ A/cm² for stored biopsies ($p=0.04$). Samples studied simultaneously at two sites after 18-24 hrs cold storage: ICC for CFTR currents was 0.49 ($p<0.001$). Forceps vs suction-based rectal biopsy: Mean CFTR currents for forceps biopsies were 234.29 μ A/cm² (SD=54.56) compared with 209.76 (SD=42.77) for suction biopsies.

Conclusions: The results indicate that CFTR currents in human rectal biopsy samples following cold storage (18-24 hrs) retain approximately 50% of the response observed in fresh tissue, and that CFTR currents from biopsies studied under parallel conditions at two sites following cold storage (18-24 hrs) demonstrate moderate correlation. CFTR currents from biopsies obtained by forceps or suction demonstrate similar CFTR responses within subjects. The results suggest that ICM assays can be centralized, and further support the development of this biomarker for clinical trials of CFTR modulators.

Performed through the CFF-TDN, and supported by CFFT.

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LONG-TERM SAFETY AND EFFICACY OF IVACAFTOR IN PERSONS WITH CYSTIC FIBROSIS WHO HAVE THE G551D-CFTR MUTATION

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Objectives: To assess the safety and efficacy of ivacaftor over 96 weeks in the open-label extension study (PERSIST) in adolescents/adults and chil-

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dren with CF who had completed the prior placebo-controlled trials (STRIVE and ENVISION).

Methods: The PERSIST study enrolled 144 adolescents/adults who completed STRIVE (age ≥ 12 years) and 48 children who completed ENVISION (age 6-11 years). All subjects received ivacaftor 150 mg q12h in addition to their prescribed CF therapies. Results through 48 weeks of PERSIST for STRIVE subjects and through 24 weeks of PERSIST for ENVISION subjects are presented.

Results: Subjects who were previously treated with ivacaftor for 48 weeks had sustained improvements in FEV₁, weight, and respiratory symptoms after an additional 48 (adolescents/adults) or 24 weeks (children) of ivacaftor treatment. Among those who had received placebo in the earlier studies, improvements in FEV₁, weight, and respiratory symptoms were evident and of similar magnitude to those observed in the prior studies among ivacaftor recipients. In adolescents/adults, time to first exacerbation as defined by a modified Fuchs' criteria was similar in the first 48 weeks of PERSIST to that seen in the 48 weeks of STRIVE; an increase in exacerbations in the first 48 weeks of PERSIST to that seen in the first 48 weeks of STRIVE was observed with no increase in the number of exacerbations per subject between the two trials. Respiratory AEs were the most common. SAEs were reported in 29 adolescents/adults (20.1%) and 4 children (8.4%). One adolescent/adult and 1 child discontinued from the current study due to an AE. No new clinically important safety concerns were identified.

Conclusions: Ivacaftor provided durable treatment effects for up to 96 weeks. In subjects who received placebo in the earlier studies, improvements were of similar magnitude to those observed in the prior studies among ivacaftor recipients. Ivacaftor treatment delayed the onset of exacerbations and the reduction in the number of exacerbations per subject observed with ivacaftor treatment in STRIVE was sustained in PERSIST. The safety profile of ivacaftor was generally consistent with that observed during the initial studies.

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Effect of ivacaftor on lung function and weight during the first 24 and 48 weeks of the PERSIST Study.

Trial Grouping	Weeks of PERSIST Study	Total weeks on ivacaftor	Absolute change from baseline (SD) in % predicted FEV ₁	Absolute change from baseline (SD) in weight (kg)
STRIVE	0 [‡]	48	9.4 (8.3)*	3.4 (4.9)*
Ivacaftor Group	48	96	9.5 (10.1)*	3.9 (6.3)*
STRIVE Placebo group	0 [‡]	0	-1.2 (7.8)*	0.25 (2.7)*
	48	48	8.0 (8.1) [†]	3.5 (4.4) [†]
ENVISION	0 [‡]	48	10.2 (15.7)*	6.1 (2.9)*
Ivacaftor Group	24	72	10.1 (14.2)*	8.5 (3.8)*
ENVISION	0 [‡]	0	-0.6 (10.1)*	2.9 (1.8)*
Placebo group	24	24	7.5 (10.9) [†]	6.5 (3.5) [†]

*Change from previous study baseline

[†] Change from PERSIST baseline

[‡]Last time point in previous study

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PATIENT-REPORTED OUTCOMES IN PHASE 3 TRIALS OF IVACAFTOR IN SUBJECTS WITH CF WHO HAVE THE G551D-CFTR MUTATION

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Objectives: Two Phase 3 studies were conducted to evaluate the effect of ivacaftor in subjects with CF who have a G551D-CFTR mutation. Changes on the Cystic Fibrosis Questionnaire-Revised (CFQ-R) scales were evaluated in both studies.

Methods: Both trials were randomized, double-blind, and placebo-controlled. Subjects received placebo or ivacaftor 150 mg q12h for 48 weeks in addition to their prescribed CF therapies. In STRIVE, 161 subjects ≥ 12 years of age were dosed. In ENVISION, 52 subjects 6-11 years were dosed. The CFQ-R is a disease-specific, health-related quality of life (HRQOL) measure for CF (Quittner et al., 2005). It consists of generic and CF-specific scales that measure respiratory symptoms, health perceptions and daily functioning in several domains (i.e., physical, social, treatment burden) over the past 2 weeks. It has developmentally appropriate versions for children,

ages 6 to 11 (interviewer administered), and self-report versions for ages 12-13, and 14 through adulthood. In STRIVE, results from the child and adolescent/adult versions were pooled. In ENVISION, both the child interview and parent/caregiver versions were used. Each domain is scored on a 100-point scale, with higher scores indicating better HRQOL. For the CFQ-R Respiratory Symptoms Scale, a difference of at least 4 points is considered the minimal important difference (MID). MIDs for other domains are being established. As previously reported, cough, pulmonary exacerbations, oropharyngeal pain, headache, pyrexia, upper abdominal pain, nasopharyngitis and upper respiratory tract infection were the most common adverse events.

Results: The treatment difference in the mean, pooled CFQ-R Respiratory Symptoms scores for ivacaftor vs. placebo through Week 48 was 8.6 points ($P<0.0001$) in STRIVE and 5.1 points ($P=0.1354$) in ENVISION. Differences between groups were detected at the first on-treatment visit (day 15) in both studies and were maintained at subsequent visits in STRIVE. In STRIVE, the treatment difference for ivacaftor was 4.4 points ($P=0.0055$) for Physical Functioning, 4.3 points ($P=0.0026$) for Social Functioning, 3.3 points ($P=0.0021$) for Eating Disturbances, and 3.3 points ($P=0.0419$) for Treatment Burden. The treatment differences for the Body, Emotion, and Digestive scales (2.7, 2.1, and 0.5 points, respectively) were better in the ivacaftor vs. placebo groups, although these differences were not statistically significant ($P=0.0857$, $P=0.0956$, $P=0.7317$, respectively). No other domains showed statistically significant treatment effects in ENVISION, except for Weight and Body Image scores through Week 48 on the CFQ-R Parent version.

Conclusions: Both studies demonstrated clinically meaningful improvements in respiratory symptoms that were rapid, durable, and of comparable magnitude. There were statistically significant improvements in other domains, although the MID for those domains has not been yet established.

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LOWER ADHERENCE TO CHRONIC PULMONARY MEDICATIONS IS ASSOCIATED WITH INCREASED HEALTH SERVICE UTILIZATION IN PATIENTS WITH CYSTIC FIBROSIS

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Objective: To assess the association between adherence to pulmonary medications and health service utilization among patients with cystic fibrosis (CF).

Methods: Patients ages 6+ years with a CF diagnosis and a prescription fill for a pulmonary medication (oral azithromycin, inhaled aztreonam, colistin, dornase alfa, hypertonic saline and tobramycin) were identified from the MarketScan® Commercial Claims and Encounters database (1/2005 to 6/2011). Medication possession ratios (MPRs) were computed for each pulmonary drug during a 12-month study period and then averaged to create a Composite MPR (CMPR) for each patient. Patients were categorized as having low (<0.50), moderate (0.50-0.80) or high (≥ 0.80) adherence based on their CMPR. All-cause and CF-related health service utilization during the study period were compared across CMPR categories using negative binomial regression models adjusted for gender, age, region, employment status, insurance, comorbidities, index year, number of unique CF medications and healthcare utilization in the 6 months prior to the study period.

Results: The sample consisted of 3,287 CF patients. After adjusting for patient characteristics, low versus high CMPR was associated with significantly higher rates of all-cause and CF-related inpatient/emergency room (ER) visits and with significantly lower rates of all-cause and CF-related outpatient visits during the study period. Medium versus high CMPR was associated with significantly higher rates of CF-related inpatient/ER visits (Table1).

Conclusions: In comparison to high adherence, low and moderate adherence to chronic CF pulmonary medications was associated with increased risks of all-cause and CF-related inpatient and ER visits. Supported by Novartis Pharmaceuticals Corporation.

Table 1: Comparison of Overall and CF-related Health Service Utilization among CF Patients with High, Moderate and Low Adherence

	Incidence Rate			Adjusted Incidence Rate Ratios (IRR)			
	Low MPR [A] N=1675	Moderate MPR [B] N=949	High MPR [C] N=663	IRR(95% CI) [A] vs. [C]	P-value	IRR(95% CI) [B] vs. [C]	P-value
Outpatient visits (N)							
All-cause	22.84	24.35	22.99	0.91 (0.86 - 0.96)	0.0012*	0.97 (0.91 - 1.03)	0.2985
CF-related	12.29	14.85	12.71	0.90 (0.83 - 0.97)	0.0038*	1.01 (0.93 - 1.09)	0.8410
Inpatient + ER visits (N)							
All-cause	1.44	1.22	0.99	1.18 (1.04 - 1.33)	0.0096*	1.10 (0.96 - 1.25)	0.1852
CF-related	1.00	0.94	0.70	1.17 (1.01 - 1.34)	0.0313*	1.19 (1.02 - 1.38)	0.0246*

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NUTRITIONAL STATUS MEASURES AMONG PERSONS WITH CF CARRYING THE G551D-CFTR MUTATION WHO RECEIVED IVACAFTOR OR PLACEBO IN PHASE 3 CLINICAL TRIALS

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Background: Patients with CF often have difficulty gaining and maintaining weight; therefore, weight and BMI are monitored in clinical trials. Malnutrition is associated with worsening lung function in children with CF and is also an independent predictor of mortality in this population.

Methods: Statistical analyses of weight and BMI were conducted in subjects enrolled in one of two randomized, double-blind studies in children with CF 6 to 11 years of age (ENVISION) or adolescents/adults ≥12 years of age (STRIVE). Screening % predicted FEV₁ was required to be 40-90% for adolescents/adults and 40-105% for children. Subjects received placebo or ivacaftor 150 mg q12h for 48 weeks plus prescribed CF therapies. In those ≤20 years, BMI and weight were adjusted for age and gender and analyzed as BMI- z-scores and weight- z-scores.

Results: The safety profile of ivacaftor and placebo were comparable. In STRIVE, 161 subjects were randomized, dosed, and included in the analysis of weight and BMI while 47 were included in the z-score analyses. In ENVISION, 52 subjects were randomized and dosed; all were included in weight, BMI, and z-score analyses. In STRIVE, 3 placebo and 9 ivacaftor subjects were pancreatic sufficient (PS). In ENVISION, 1 subject in each group was PS. At STRIVE baseline, mean (SD) weight was 61.5 (14.1) kg and mean BMI was 21.8 (3.6) kg/m². At ENVISION baseline, mean (SD) weight was 30.9 (8.6) kg and mean BMI was 17.0 (2.2) kg/m². The differences between the ivacaftor and placebo groups in mean weight change were 2.7 kg (P=0.0001) in adolescents/adults and 2.8 kg (P=0.0002) in children through 48 weeks. The differences between the ivacaftor and placebo groups in weight z-scores were 0.33 (P=0.0260) in adolescents/adults and 0.39 (P<0.0001) in children. Between-group differences for BMI were 0.93 kg/m² (P<0.0001) among adolescents/adults and 1.09 kg/m² (P=0.0003) among children. In the cohort of 12- to 20-year-olds, BMI z-score in the ivacaftor group improved to above the 50th percentile; those of the placebo group worsened (difference: 0.33; P=0.0490). In the children, mean BMI z-scores increased in the ivacaftor group and fell below the 50th percentile in the placebo group (difference: 0.45; P<0.0001). In subjects 12- to 20 years, the difference in height z-score between treatment and placebo was +0.05 at 24 weeks and +0.06 at 48 weeks. In children, the difference was +0.06 at 24 weeks and +0.12 at 48 weeks; none reached statistical significance.

Conclusions: Treatment with ivacaftor led to improvement in nutritional status as measured by increases in mean weight gain and BMI in both studies.

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VX-809 FACILITATES ΔF508-CFTR TARGETING TO NEUTROPHIL PHAGOSOMES

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Phagocytic host defense is compromised in CF patients due to deficient chloride supply to the neutrophil phagosomes, which leads to deficit in microbicidal oxidant production. Thus, pharmaceutical enhancement of ΔF508-CFTR targeting to neutrophil phagosomes may alleviate or rescue the phagocytic defect. To test the hypothesis, we established three promyelocytic cell lines which expressed EGFP alone (PLB-985-EGFP), EGFP-wt-CFTR (PLB-985-EGFP-wt-CFTR) or EGFP-ΔF508-CFTR (PLB-985-EGFP-ΔF508-CFTR), respectively. These cells were differentiated into neutrophil-like cells after DMSO induction for 5 days. Phagosomal isolation and flow cytometry analysis demonstrated that both wild-type CFTR and ΔF508-CFTR were capable of trafficking to the phagosomes, but the wild-type CFTR targeted the organelle more efficiently and ~4 fold higher than the mutant one. This finding indicates that there exists a molecular mechanism for ΔF508-CFTR to be recruited to phagosomes, albeit less efficiently. Corrector drug VX809 was applied to the DMSO-differentiating cells at various doses (0, 0.3, 3 μM). The expression of EGFP-wt-CFTR and EGFP-ΔF508-CFTR in the isolated phagosomes was compared. The data showed that VX809 significantly affected EGFP-ΔF508-CFTR phagosomal targeting in a dose-dependent manner. The high-dose VX809 (3 μM) increased the mutant CFTR targeting by ~1.5-2 fold. In contrast, the compound had little effect on the wild-type CFTR targeting. This preliminary study suggests the feasibility of pharmaceutical modulation of ΔF508-CFTR in CF neutrophils to rescue their microbicidal defect. Moreover, the established system will be useful for compound screening to search for effective CFTR correctors in neutrophils. This work is supported by a research grant to GW from Vertex Pharmaceuticals Incorporated.

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THE RISK OF FEMALE GENDER ON SURVIVAL IN CYSTIC FIBROSIS

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Background: Previous studies have suggested a female disadvantage with respect to survival in cystic fibrosis (CF). However, as advancements in therapies have improved life expectancy, this gender disparity has been challenged. This study was undertaken to examine whether or not there is still a gender inequality for life expectancy in CF since the advent of improved pulmonary therapies.

Methods: We conducted a retrospective cohort analysis of survival in 32,766 CF patients entered into the United States Cystic Fibrosis Foundation Patient Registry (CFFPR) between January 1, 1995 and December 31, 2007. Subjects contributed person years to the analysis corresponding to age at entry into the cohort through age of death. Subjects not recorded as dying during the study period were classified as censored at the time of last observation for the purposes of analysis. Subjects who underwent lung transplantation or had no age of diagnosis for CF were excluded. Kaplan-Meier survival estimates and Cox regression models with relevant covariates were used to compare mortality rates between men and women with CF.

Results: Kaplan-Meier analysis showed a significant difference between men and women (p < 0.001) with a median life expectancy of 36.0 years (95% confidence interval [CI] 35.0 - 37.3) in women and 38.7 years

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(95% CI 37.8 – 39.6) in men. Cox regression analysis demonstrated the risk of death to be higher for women than men (hazard ratio (HR) = 1.25; 95% CI 1.180-1.328). Multiple variables were analyzed and incorporated into the multivariate model if found to have a statistically significant impact on death ($p < 0.05$). These included race, genotype, body mass index (BMI), height, age of diagnosis, year of birth, pancreatic insufficiency, percent predicted FEV1 (FEV1%), pulmonary exacerbations, co-morbidities such as CF related diabetes mellitus (CFRD) and asthma, and sputum colonization with organisms including mucoid and nonmucoid *Pseudomonas aeruginosa*, methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*, *Haemophilus influenzae*, *Achromobacter species*, *Burkholderia cepacia complex*, *Aspergillus species*, and nontuberculous mycobacteria infections. In the multivariate model, female gender was still found to be a significant risk factor for death. Interestingly, there were no interactions between microbial data or other variables on gender-specific mortality.

Conclusion: Using recent data from the CFFPR, this study shows that CF women have a decreased life expectancy relative to men despite incorporating influences of morphometric factors, sputum microbiology results, and CF related co-morbidities. The explanation for this gender disparity is largely unknown and supports the need for further investigation into the mechanism, including evaluation of sex hormone mediated effects.

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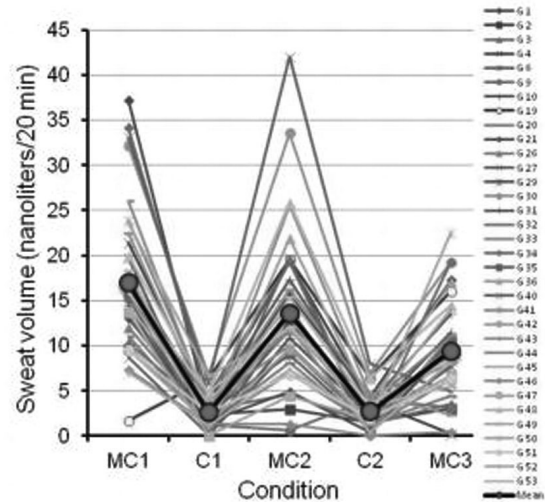
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CHOLINERGIC POTENTIATION OF CFTR-DEPENDENT SECRETION BY INDIVIDUAL, IDENTIFIABLE SWEAT GLANDS

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To evaluate systemic, CFTR-directed therapeutics, we developed an optical bioassay that monitors CFTR-dependent and -independent sweat secretion in parallel for ~50 individual, identified glands in a subject; each gland is assessed before and after treatment. In this assay, CFTR-dependent secretion is stimulated with an intradermal injection of a "beta-cocktail" consisting of isoproterenol and aminophylline (to activate CFTR) and atropine (to block cholinergically-stimulated sweating). Sweat gland size and cholinergically-stimulated secretion rates vary markedly. Therefore, CFTR-dependent, beta-sweat rates are expressed by their ratio to CFTR-independent, cholinergically-stimulated sweat rates for each identified gland. We initially treated the cholinergic and adrenergically stimulated sweat sessions as independent. However, Sato, (AJP, 1983, 245, C189) determined that cholinergic stimulation strongly potentiates beta-adrenergically stimulated production of cAMP, but did not determine if this influenced secretory rates. We found that prior stimulation with 1 μ M methacholine (15 min, just prior to beta test) exerted a profound potentiating effect on the subsequent secretory response to cocktail. The plot shows final volumes of 34 identified glands that were followed across 2 cocktail-only conditions (C1, C2) and 3 MCh-cocktail conditions (MC1-3), plus mean volume for all glands in each condition. The average across condition C1, C2 = 2.8 $\pm$ 1.6 and MC1-3 = 13.7 $\pm$ 6.1 nL/gland/20 min. The average potentiation factor (MC/C) was 4.89. With identified glands as the units of analysis a paired t test gave $P = 1 \times 10^{-13}$ and analysis using lmer from R (R_Development_Core_Team. 2011, R Foundation for Statistical Computing: Vienna, Austria) on log transformed data gave $t = 14.57$. MCh Potentiation Factors (PFs) were estimated for 4 other subjects: 3 had a mean PF of 2.2 and one of 27.6. No response in MC condition was observed in 11/12 pancreatic insufficient CF subjects, or if the beta agonists were omitted from the cocktail (atropine only). Potentiation, because it is CFTR-dependent, increases assay sensitivity for partially functional CFTR.

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DETECTION OF CFTR-DEPENDENT SECRETION IN INDIVIDUALLY IDENTIFIABLE SWEAT GLANDS OF A PATIENT HOMOZYGOUS FOR $\Delta F508$ CFTR

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Objective: Sweating stimulated by beta-adrenergic agonists provides a near-linear readout of CFTR function. We have developed an in vivo assay that quantifies such secretion in individual, identified glands. In addition to quantifying the effects of CFTR-directed therapies, the assay can also be used to identify subjects who, for unknown reasons, retain residual CFTR function even though they harbor two "severe" mutations. This was first discovered by Veeze et al. (JCI, 1994, 93, 461), who used Ussing chamber measurements of intestinal suction biopsies to demonstrate residual, CFTR-dependent secretion in 4/28 (14%) of subjects homozygous for F508del. Such subjects might benefit from CFTR potentiator therapies.

Methods: During the development of our single gland, ratiometric sweat assay, we tested 12 pancreatic insufficient CF subjects at least 4 of whom were homozygous for F508del. Sweating was stimulated with intradermal methacholine and secretion rates for ~50 identified glands per subject were determined. The same glands were then stimulated with a beta cocktail consisting of isoproterenol, aminophylline, and atropine (to block CFTR-independent sweating). Control subjects always secrete to the beta cocktail as do CF carriers, who on average secrete about half as much (Behm, Pediatric Res, 1987, 22, 271). CF subjects do not respond (Sato, J Clin Invest, 1984, 73, 1763).

Results: We measured methacholine-stimulated secretion from >630 identified glands from 12 CF-pancreatic insufficient subjects. In 536 glands from 11 subjects (25-125 glands per subject) none responded to cocktail over a 30 min period. However, in one F508del/F508del subject, 29/94 glands (31%) identified at 2 different sites secreted to cocktail 3/33 at one site and 26/61 at a second site. The responses were very small; the largest response was a 1.1 nL bubble; the average for responding glands was 0.35 nL. The total response, expressed as a ratio of total cocktail/methacholine volumes, was 0.004; this is 1.5% of a mean ratio of 0.26 obtained for 6 WT subjects. This same subject also responded to a cocktail that was not preceded by the potentiating methacholine stimulus, but that had been augmented with an additional phosphodiesterase inhibitor; no response was observed to this condition in a different subject homozygous for F508del. Combining our samples with those of Veeze et al. gives 5/40 or ~12% of F508del/F508del CF patients who might have residual CFTR function.

Conclusions: The standard paradigm in clinical testing compares average responses across groups of subjects, but evidence is now compelling that therapies and dosing strategies can be optimized by taking account of relevant individual differences. Because systemic CFTR-directed therapeutics act directly on CFTR within sweat gland cells, individual glands become the

units of analysis for within-gland, paired statistical treatments that provide sufficient statistical power to quantify treatment effects for that individual. This could help identify individuals who are likely to benefit from CFTR potentiators. Supported by Cystic Fibrosis Foundation Therapeutics-WINE08A0.

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COSTIMULATORY MOLECULES AS BIOMARKERS IN CF

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Rationale: Cystic fibrosis (CF) is characterized by chronic airway and systemic inflammation complicated by intermittent episodes of acute exacerbations. To identify exacerbations, clinicians rely on changes in clinical symptoms or impaired lung function. The latter is often a later marker of disease; therefore, specific biomarkers of the inflammatory and immune response are needed to improve the care of CF patients. We previously demonstrated that costimulatory dyads, including CD40-CD40L, CD 80/86-CD28/CTLA4, and OX40L-OX40 are capable of regulating inflammation in multiple inflammatory disorders and expression levels of cell bound and soluble isoforms serve as biomarkers for disease activity in sepsis, SLE, and MI. Therefore, our goal is to investigate the predictive value of costimulatory pathways in CF exacerbations.

Methods: We began a 2-year longitudinal study of adult CF patients in July 2011. Subjects will have blood for flow cytometry, serum and plasma collected at each clinic visit and the beginning, middle (day 7) and end of each exacerbation requiring IV antibiotics. All clinical data including FEV1, presence of *Pseudomonas* or MRSA, use of inhaled antibiotics and clinical symptoms are recorded.

Results: We have currently enrolled 32 subjects. Subjects have an average age of 26 (44% male) with an average FEV1 of 50%; 65% have at least one copy of DF508. In all, 85% were colonized with *Pseudomonas* and 25% with MRSA. Monocyte OX40L is upregulated in CF patients compared to healthy controls (10.76% vs. 2.7%; $p=0.01$). While there was no significant change with exacerbation, the absolute degree of OX40L expression correlated with the degree of FEV1 decline during exacerbation ($p=0.05$). In concert, OX40 was expressed on CD4+ cells and levels decreased with treatment of exacerbation. CD86 was constitutively expressed on circulating monocytes and levels decreased with the onset of exacerbation. Levels of CD86 correlated inversely with circulating levels of IL-6 ($p=0.01$). Further, levels of CD86 correlated inversely with the degree of FEV1 drop during exacerbation ($p=0.02$). Interestingly, at baseline, patients colonized with MRSA had higher levels of monocyte CD86 expression ($p=0.06$). CD80 expression was not altered. While expression of CD28 on CD4 and CD8 cells was not altered, expression of the soluble isoform of CD28 was upregulated in CF patients compared to healthy controls. During exacerbation, levels of sCD28 dropped significantly with therapy ($p=0.07$). Finally, similar to prior reports, sCD40L was upregulated in CF patients compared to healthy controls ($p=0.01$). However, there was no association with presence or treatment of exacerbation and there was no alteration in monocyte expressed CD40 in CF patients.

Conclusion: These data establish that CF patients exhibit changes in circulating costimulatory molecule expression, and changes in expression levels may predict the severity of exacerbation. With additional data points we hope to establish a biological baseline for each subject that will allow us to determine whether intrasubject changes can serve as robust biomarkers in CF.

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RELEVANCE OF HLA-G IN CF

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HLA-G is a non-classical HLA class I antigen expressed as membrane bound and soluble (sHLA-G) isoforms with a restricted tissue distribution and anti-inflammatory functions. The level of HLA-G expression is partly controlled by a polymorphism of insertion/deletion (INS/DEL) of 14bp (rs16375) in the 3'-untranslated region of the gene (Rizzo et al. Immunogenetics 2005). sHLA-G levels in biological fluids correlates with inflammatory diseases and treatment response (Rizzo Pharmacog Genom, 2006), suggesting a possible role both in immunopathology and as biomarker in CF.

This study aimed to identify possible relevance of HLA-G in CF.

We evaluated sHLA-G levels by ELISA before and after at least 12 days IV antibiotic therapy for pulmonary exacerbation in plasma samples from 50 CF patients and in exhaled breath condensate (EBC) from 29 of them; from healthy volunteers we analyzed 71 plasma and 20 EBC samples. Distribution of 14bp INS/DEL in 236 CF patients was analyzed by Real Time PCR. In the presence and absence of *Pseudomonas aeruginosa* (Pa) lipopolysaccharide (LPS) HLA-G expression was analyzed by ELISA and Western blot in CF and non-CF peripheral human monocytes, CF (IB3-1) and isogenic non-CF (C38) airways cell lines, while in wild-type and CF mice Qa2 (murine HLA-G homolog) was quantified (Comiskey Human Immunol 2003).

We observed lower sHLA-G plasma levels in CF patients in comparison with non-CF ($p<0.001$). After therapy plasma levels increased versus non-CF ($p=0.014$). EBC sHLA-G levels were higher in CF patients before treatment ($p=0.05$), decreasing after therapy versus healthy subjects ($p=0.83$).

CF patients with chronic Pa lung infection presented higher EBC sHLA-G levels ($p=0.0285$), negatively correlated with FEV1% ($p=0.039$, $r=-0.68$). Bacterial LPS is known to induce HLA-G expression. We observed higher HLA-G expression in CF monocytes (23.9 ± 4.4 versus 10.2 ± 2.3 ng/mL; $p=0.02$), IB3-1 cells (31.9 ± 1.4 versus 13.5 ± 1.3 ng/mL; $p=0.01$) and CF mice BALFs in comparison with non-CF. In all these three models LPS induced significantly higher increase of HLA-G than in non-CF.

Genetic analysis reported a correlation between chronic infection and DEL allele which is associated with higher HLA-G production than INS allele ($p=0.027$, $p=0.001$ respectively). DEL allele by up-modulation of HLA-G production could influence the risk of infection persistence, acting as immune-escape molecule.

The lower sHLA-G plasma levels in CF patients than non-CF and the increase after therapy sustain a role in systemic inflammation control. On the contrary, the enhanced EBCs levels in patients with chronic lung infection and the highest LPS-responsiveness of CF monocytes, airway cells and mice suggest an implication of infection in local HLA-G expression.

These results propose a role of HLA-G molecules to control and monitor both inflammation and therapeutic efficacy in CF patients.

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CORRELATIONS BETWEEN CLINICAL DISEASE ACTIVITY, SEVERITY AND QUALITY OF LIFE IN STABLE ADULT CF PATIENTS: CONTRIBUTIONS OF C-REACTIVE PROTEIN AND CALPROTECTIN AS BIOMARKERS

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Background: Many parameters describe the disease severity of CF patients (FEV1%pred., BMI and CXR), but do not entirely encompass clinical disease activity. We have previously validated the discriminating and predictive power of a clinical scoring system (1). In this study, we are investigating the potential role of serum CRP and calprotectin as biomarkers of disease severity and activity.

Methods: We recruited clinically stable adult CF subjects from our centre. We prospectively determined the combined clinical-complications sub-scores of the clinical scoring system, ranging between 0 (worst) and 50 points (best), as well as the CF specific QOL score (CFQ-R) (2). CRP and calprotectin were measured in plasma samples by ELISA.

Results: Baseline data were obtained from 24 females and 27 males (n=51). Mean age 33 ± 13.6, mean FEV1% pred. 62.6 ± 28.2, mean BMI 21.9 ± 4.1, mean clinical-complications score 33.7 ± 7.5, mean CRP (mg/L) 7.20 ± 6.71, mean calprotectin (ng/mL) 146 ± 157. The combined clinical-complications score significantly correlated with a wide spectrum of QOL domains, particularly Health perception (r = 0.57, p < .0001) and Physical domains (r = 0.56, p < .0001). It also significantly correlated with parameters of disease severity (FEV1% pred. (r = 0.7, p < .0001), CXR scores (r = 0.68, p < .0001), and BMI (r = 0.47, p = 0.0004)) as well as biomarkers CRP (r = 0.59, p < .0001) and calprotectin (r = 0.44, p < .0016). Both CRP and calprotectin significantly discriminated between all 3 different levels of disease activity, (defined by the clinical-complications scores) (mild > 35, moderate 25-35, severe < 25). They also discriminated patients with severely reduced (FEV1%pred. < 40) versus moderately (40-70%pred.) and mildly reduced (>70% pred.), but not between the groups with moderate and mild reduction in FEV1% pred. As described in our previous study, there was a strong, and curvilinear relationship between FEV1% pred. and the clinical-complication scores (r² = 0.558, p = 0.0029). Patients with elevated CRP > 5 compared to those with < 5 (p = 0.01) or calprotectin > 70 compared to < 70 (p = 0.0029) at baseline, exhibited a greater change in clinical complications score in response to smaller decreases in FEV1%pred.

Conclusions: The results of this study suggested that the clinical-complications score significantly correlated with a wide spectrum of quality of life domains as well as with disease severity. Both CRP and calprotectin discriminated patients with different disease activity and severity. Our results suggest that patients with high levels of CRP and/or calprotectin have more clinically active disease, as their clinical activity score changes more significantly with small changes in FEV1.

1. Matouk E. et al. *Eur Respir J* 1997;10:2004-2013. *Eur Respir J* 1999;13:552-559.

2. Quittner AL. et al. *Chest* 2009;135:1610-8.

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VARDENAFIL CORRECTS F508DEL-CFTR LOCALIZATION AND CHLORIDE TRANSPORT ACROSS THE INTESTINAL MUCOSA

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Nasal potential difference measurements have previously shown that vardenafil, a phosphodiesterase type 5 inhibitor, improves CFTR-mediated chloride secretion across the nasal mucosa of mice homozygous for the F508del mutation (CF). The present work aimed at studying the potential of vardenafil to rescue CFTR function across the rectal mucosa, representative of the GI tract. Moreover, the work aimed at validating immunohistochemical assays to allow detection of (sub)cellular localization of CFTR in colon tissue preparations from mice and at evaluating their efficacy as a biomarker of correction of the localization of F508del-CFTR after vardenafil treatment.

Distinct rectal potential difference (RPD) profiles were obtained in CF and normal homozygous wild-type mice (WT). Sodium absorption, measured by the response of 10⁻⁴M amiloride (in the presence of 5x10⁻³ M barium to block potassium channels), was much higher in CF (40.2 ± 4.0 mV) than in WT mice (22.6 ± 1.9 mV; p < 0.001). Chloride secretion recorded in the presence of chloride-free solution and 10⁻⁵ M forskolin was twice as low in CF (-4.2 ± 0.5 mV) as in WT mice (-9.4 ± 0.9 mV, p = 0.002). Heterozygous mice showed preserved sodium transport (20 ± 1.8 mV) but reduced chloride secretion (-5.4 ± 1.5 mV). Chloride secretion was restored in CF mice treated with a single IP dose of 0.14 mg/kg vardenafil; values after treatment (-9.3 ± 1.2 mV) were similar to those obtained in untreated WT mice.

Immunohistochemical assays were evaluated by a normalized ratio of F508del-CFTR related fluorescence (RF) between the apical membrane and the cell. A discriminative (mean (interquartile range)) RF between CF (1.7 (1.5-1.7)) and non-CF labelings (2.1 (1.7-2.3); p-value = 0.02) was noted in untreated conditions. The RF was increased in vardenafil treated CF tissues (2.4 (1.9-2.7)) as compared to untreated CF tissues (p-value < 0.001).

As conclusions, our findings pointed out the rectal mucosa as an additional target tissue to study in vivo ion transport abnormalities in CF. The RPD test discriminates between CF and non-CF and the test can be used to investigate the efficacy of therapeutic strategies to rescue CFTR. As previously demonstrated at the airway level, vardenafil restores chloride secretion across the gastrointestinal epithelium. Immunolocalization of CFTR protein in colon tissues is a valuable tool to quantify CFTR localization at the apical membrane. Vardenafil seems to act as a corrector of the cell mislocalization of F508del-CFTR.

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EFFECT OF HYPERTONIC SALINE ON LUNG CLEARANCE INDEX IN INFANTS AND PRESCHOOL CHILDREN WITH CF: A PILOT STUDY

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Hypertonic saline has been shown to be effective in improving mucociliary clearance in cystic fibrosis patients. The ISIS study was designed to evaluate the effectiveness of hypertonic saline compared to isotonic saline in infants and young children with cystic fibrosis in a multi-center, randomized, parallel group trial. In the Toronto site only, an add-on pilot study was

designed to measure lung clearance index in those subjects participating in the ISIS study to evaluate its utility as an outcome measure.

Methods: Multiple breath washout (MBW) measurements were offered as an add-on study to all participants recruited into the ISIS study in Toronto at enrollment, and one year after treatment. Our MBW set up is identical to that described by Gustafsson et al. (ERJ, 2003) and uses 4% sulfur hexafluoride (SF₆) as a tracer gas and an AMIS respiratory mass spectrometer (Innovision A/S, Odense, Denmark).

Results: Parents of all 26 subjects enrolled at the Toronto site consented to the study and all 26 were able to perform baseline multiple breath washout assessment. Twenty-four of 26 (92%) of the subjects had paired baseline and follow-up measures at one year. There was a significant reduction in LCI from baseline to follow-up in the hypertonic saline arm ($\beta = -1.32$ (95% Confidence Interval -2.61 to -0.04), $n=11$, $p=0.04$); while the LCI remained stable in the isotonic saline group ($\beta = 0.226$ (95% Confidence Interval -0.913, 1.365) $n=13$, $p=0.698$). A trend was seen for an overall treatment effect compared to isotonic saline, with an effect size of -1.57 (95% Confidence Interval -0.07 to 3.2; $p=0.08$, $n=24$).

In a sub-group analysis of preschool children, this treatment effect was greater (-2.20 (95% Confidence Interval -0.15; 4.55), $p=0.07$, $n=14$), and driven by changes in the hypertonic saline arms of preschool children ($\beta = 2.66$ (95% Confidence Interval -4.38, -0.95), $n=6$ $p=0.02$). In infants treated with hypertonic saline, LCI did not change ($\beta = 0.58$ (95% Confidence Interval -0.62, 1.79), $n=5$, $p=0.345$), whereas LCI increased significantly in the isotonic saline group ($\beta = 1.03$ (95% Confidence Interval 0.08, 1.98), $n=6$, $p=0.03$).

Discussion: Lung clearance index is highly feasible in an interventional study in infants and preschool children with cystic fibrosis. Although the treatment effect was not significant in the overall group, the effect size was higher than that reported in a short term study in older children (Amin, Thorax 2010). This supports the concept that LCI may be a suitable endpoint for interventional studies in this age group.

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SYNCHROTRON PHASE CONTRAST X-RAY IMAGING CAN DETECT AIRWAY SURFACE LIQUID DEPTH CHANGES FOLLOWING AEROSOLISED HYPERTONIC SALINE TREATMENT

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Introduction: The fundamental role of adequate airway surface fluid (ASL) in the pathophysiology of CF airway disease is well understood, but the ASL depth has proven impractical and difficult to measure without invasive procedures. Our goal is to non-invasively assess ASL depth as an outcome measure for developing genetic CF airway treatments. We have developed high resolution synchrotron phase contrast X-ray imaging methods to enable monitoring of airway surface health and behaviour. Here we provide the first data of successful monitoring of hypertonic saline (HS) induced ASL depth change in *ex vivo* mouse trachea preparations.

Materials and Methods: Tracheas from $n=15$ C57Bl/6 mice were excised after lethal Nembutal anaesthesia, immediately ligated onto connecting glass tubing mounted horizontally, and extended to normal length in a custom-designed chamber that permitted thermostatic temperature control, and reciprocating tracheal airflow (flexiVent, SCIREQ) during X-ray imaging. HS aerosol (7% w/v) was delivered via an Aerogen nebuliser incorporated into the flexiVent (MMD 3.5 μ m) during the designated "inspiratory" phase of the airflow cycle.

Images were taken using 25 keV X-rays and a 0.18 μ m effective pixel size at the BL20XU beamline at SPring-8, Japan. A 1000 line pairs per inch

gold grid was placed immediately before the trachea, in order to reconstruct an image of the trachea surface from the sample-induced distortions of the grid observed 1 metre downstream. Ten images were captured before treatment, with 7 additional sets of 10 images taken at 3 minute intervals (totalling 18 minutes) after a 90 second delivery of HS aerosol.

Results: The grid X-ray imaging system allowed both the airspace-ASL and the ASL-cell surface interfaces to be identified, whereas only the airspace-ASL interface was detectable with standard phase contrast X-ray imaging. Delivery of HS for 90 seconds resulted in a transient increase of mean ASL depth in the trachea of 9 μ m, with the increased depth persisting for 9 mins before returning to baseline.

Discussion: The combination of high spatial resolution synchrotron phase-contrast X-ray imaging setup with the use of an interfering grid can reveal the depth of the ASL layer in live *ex vivo* tracheal airways. The duration of the ASL depth increase is consistent with *in vitro* data from air liquid interface studies. These findings represent proof of principle for potential non-invasive assessment of ASL depth in intact live animal models. Results of preliminary imaging studies in intact mice are currently being analysed. This method may be useful for rapid repeated and direct assessment of the effect of potential CF airway therapeutics *in vivo*.

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INFANT PFTS AS AN ENDPOINT IN THE INFANT STUDY OF INHALED SALINE RANDOMIZED CONTROLLED TRIAL

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Background: The lack of well-developed, validated clinical trial endpoints in infants with CF has hindered the conduct of interventional studies in this population. A recent observational study for the first time showed the feasibility of performing infant lung function tests in a multicenter fashion (Davis, Rosenfeld, AJRCCM; 2010). We therefore evaluated infant lung function tests as an exploratory endpoint in a substudy of the Infant Study of Inhaled Saline (ISIS).

Methods: The ISIS study was a 30 center randomized controlled trial of hypertonic saline vs. isotonic saline for 48 weeks in 321 children with CF <5 years of age. The infant PFT substudy was offered to families with infants 4 to 15 months of age at 15 selected sites. Infant PFTs (raised volume rapid thoracoabdominal compression technique and plethysmography) were performed at enrollment and Week 48. Four sites performed an additional infant PFT at Week 4.

Results: Seventy-three infants participated; 62 (85%), 45 (62%) and 36 (49%) had acceptable measures of functional residual capacity, forced flows/volumes and residual volume, respectively, at both visits. In a linear regression model adjusted for baseline lung function, gender, age, height and weight, the difference (95% CI) in mean change in lung function over the 48 week treatment period between the hypertonic saline and isotonic saline groups for FEV_{0.5}, FEF₂₅₋₇₅, FEF₇₅ and FRC respectively, were 38 (1,76) mL, 99 (-7, 204) mL/sec, 46 (-29, 121) mL/sec and -2 (-30, 26) mL. No serious adverse events related to Infant PFTs were reported. In 13 infants with acceptable measurements at randomization and Week 4, the mean change in FEV_{0.5} was 10 (SD 44) mL in the hypertonic saline group ($n=7$) and -3 (SD 38) mL in the isotonic saline group ($n=6$) (difference in mean change not significant between groups).

Conclusions: This is the first multicenter clinical trial using infant PFTs as an endpoint. Though we showed improved feasibility of infant PFTs in the experienced centers participating in the current study compared to inexperienced sites in our prior multicenter study, acceptable results for forced flows/volumes were obtained in only 62% of participants. Unlike the previ-

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ous multicenter observational trial demonstrating that FRC may be the most sensitive parameter to differentiate CF from health, $FEV_{0.5}$ was more sensitive to detecting a treatment effect of hypertonic saline. Results suggest that infant PFTs, particularly $FEV_{0.5}$, may be a potential endpoint for interventional trials in CF infants.

Sponsored by NHLBI and the CFF.

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PHASE 1 PHARMACOKINETIC AND SAFETY STUDY OF INTRAVENOUS GANITE™ (GALLIUM NITRATE) IN CF

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Background: Elemental gallium kills *P. aeruginosa* (PA), is active against PA biofilms in vitro, and treats three different animal models of chronic PA infections. Gallium nitrate (Ganite™) is FDA approved for intravenous (IV) administration.

Methods: This three site, open labeled, unblinded non-randomized study was a phase 1 safety and pharmacokinetic (PK) study of a 5 day IV infusion of Ganite™ at two different doses (cohort 1: 100 mg/m²/day and cohort 2: 200 mg/m²/day) in patients with CF infected with PA. Inclusion criteria included age 18 years and older, $FEV_1 > 30\%$ of predicted, no clinically significant renal or liver disease or osteoporosis. Safety was evaluated by laboratory assessments and collection of adverse events. Blood, urine and sputum samples were collected for PK assessment. Secondary endpoints included lung function and quantitative sputum microbiology.

Results: Twenty subjects completed the study (9 in cohort 1; 11 in cohort 2). Mean age was 32.8 yrs (± 9.5 SD), 50% female, with a mean FEV_1 of 2.25 liters (± 0.83 SD). Of 41 reported adverse events (AEs), 9 were deemed related to study treatment (5 in Cohort 1, 4 in Cohort 2). No serious AEs were noted. Creatinine did not change from the start of infusion to day 8: mean change -0.027, 95% CI (-0.076, 0.023). PK showed two phase elimination. Sputum concentrations reached levels that have biofilm inhibitory and bacteriocidal activity in vitro in 100% and in 78% respectively of patients at day 14. Mean change in FEV_1 was +118 cc (95% CI: 41 to 196) and +95 cc (95% CI: 31 to 160) at 14 and 28 days from start of dosing (Figure 1). The lung function effects were more prominent in cohort 2 [day 14: +169 cc (95% CI: 8 to 260); day 28: +85 cc (95% CI: -6 to +176)]. Mean sputum density of PA change at day 14 was -6.0 million/CFU per gram (95% CI: -117.3 to 105.7).

Conclusions: Ganite™ appears safe and with sputum levels in the anticipated therapeutic range. FEV_1 improved after a single dosing infusion with the more pronounced effect occurring in the higher dosing cohort. PA density change was in the appropriate direction but with high variability. Ganite™ offers a novel therapeutic agent for the treatment of chronic lower airway infection with PA in CF.

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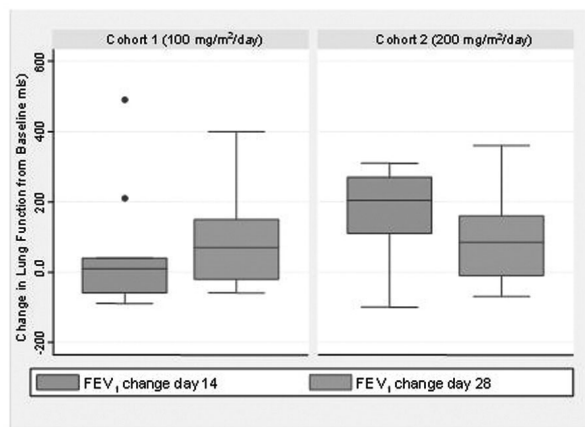


Figure 1. Change in lung function (FEV_1) by cohort

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A NOVEL SCORING SYSTEM IN CYSTIC FIBROSIS: EARLY PREDICTORS OF A 10% FALL IN FEV_1 - A PRELIMINARY REPORT

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Background: Scoring systems for assessment of cystic fibrosis disease severity have been published for 50 years without clinical utility. The aim of this scoring system is to utilize comprehensive clinical and laboratory data with specific statistical analyses to develop a disease predictive index.

Methods: CF patients treated at a large accredited pediatric and adult center during the five year period Jan 1, 2007 – Dec 13, 2011 were identified in the PortCF database. Baseline (start date) for each patient was determined by the first four month interval where $FEV_1\%$ -predicted was not suggestive of significant impairment ($FEV_1\%$ -pred $\geq 60\%$). During subsequent four month periods, minimum $FEV_1\%$ -predicted reported across health encounters was determined. Cox regression analyses were conducted to assess influence of patient characteristics on time to significant disease progression marked by a drop in $FEV_1\%$ predicted by ≥ 10 absolute percentage points (maximum of 3 year follow-up).

Results: In 81 patients aged 6-49 years at baseline (81% <18 years old) who met eligibility criteria, the cumulative probability of significant loss in pulmonary function was approximately 48% by end of year one (interval 3), 63% by end of year two (interval 6), and 70% by end of year three (interval 9). Patients positive for *Staphylococcus* (including methicillin resistant strains) at baseline were 2.4 times more likely to suffer a significant drop in any interval than patients who tested negative (HR=2.4, 95% CI (1.2-4.7), p=.013). Univariable survival analyses did not show significant differentials in hazard of event by gender, age, BMI, $FEV_1\%$ and FVC% predicted at baseline, *Pseudomonas aeruginosa* (+/-), nor by utilization of TOBI (p>.05). Further investigation of baseline BMI in children showed in females, BMI>20 corresponded to increased likelihood of a substantial drop in $FEV_1\%$ predicted (≥ 10 points) in any interval (HR=4.5, 95% CI (1.5-14.2), p=.009). In male children the hazard ratio was directionally similar although not significant (HR=2.2, 95% CI (0.6-8.7), p=.249). BMI interaction with age and gender was not evaluated in adult patients due to insufficient data.

Conclusions: Prevention of *Staphylococcus aureus* colonization may delay disease progression in CF. The cumulative probability of disease progression marked by a drop in $FEV_1\%$ predicted by ≥ 10 points was 70% by the end of three years of routine monitoring. In patients negative for *Staphylococcus*, the cumulative probability remained below 50% through the end of year three compared to 80% in those who tested positive (HR=2.4, p=.013). Further predictive markers will be delineated in this effort to create a CF disease predictive index scoring system with the eventual goal of studying such an index as part of a multicenter analysis of therapeutic efficacy.

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BETA-BLOCKER MANAGEMENT OF REFRACTORY HEMOPTYSIS IN CYSTIC FIBROSIS: A NOVEL TREATMENT APPROACH

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Background/Objective: Recurrent hemoptysis is a debilitating complication of cystic fibrosis (CF) and likely results from mucosal erosions into abnormal bronchial blood vessels due to chronic respiratory infection. The CF Pulmonary Guidelines (2010) advocate bronchial artery embolization in CF patients with massive hemoptysis who are clinically unstable. However, no consensus is reported on the management of massive and submassive hemoptysis in clinically stable patients. We hypothesize that the use of beta-blockade will decrease mean arterial pressure resulting in lower bronchial artery blood flow and subsequently, decrease the frequency and severity of hemoptysis, rate of hospitalizations, and usage of intravenous antibiotic.

Design: Retrospective chart review was performed on 12 CF patients with recurrent hemoptysis, ages 13 to 40 years old, along with a follow-up telephone survey to assess the effectiveness of beta-blockade for hemoptysis.

sis, tolerance of inhaled respiratory medications, activity tolerance, and potential adverse effects. Since the data was non-parametric, the Wilcoxon Signed Rank Sum Test (two-sided) was utilized with significance determined at the 0.05 or less level.

Intervention: Beta-blocker, specifically atenolol, was initiated in all subjects within 24 hours after experiencing recurrent hemoptysis episodes.

Results: The majority of patients (72.7%) had complete cessation of hemoptysis. There were significant decreases in the frequency of hemoptysis (p-value of 0.02) and the amount of hemoptysis (p-value 0.004). Rate of hospitalizations significantly decreased from 1.33 to 0.67 (p-value of 0.05) after initiation of atenolol. There was a trend toward statistical significance in the reduction of intravenous antibiotics use (p-value of 0.08). No statistical difference was found comparing the pre- and post-treatment means of forced expiratory volume in 1-second (p-value of 0.59). Very minimal adverse effects were observed with only 1 patient reporting intermittent facial flushing.

Conclusion: Beta-blockade, particularly with atenolol, appears to successfully treat, if not resolve, chronic, recurrent hemoptysis in CF. Beta-blocker therapy appears to maintain an effective safety profile in CF.

Average Rate of Hospitalizations, Intravenous Antibiotic Usage, FEV1, and Systolic Blood Pressure (SBP)

	Before Beta-Blocker (Mean, Standard Deviation)	After Beta-Blocker (Mean, Standard Deviation)	P-value
Hospitalizations	1.33 (0.49)	0.67 (0.78)	0.05
IV Antibiotics	1.33 (0.49)	0.67 (0.78)	0.08
FEV1 (% of predicted)	54 (20.02)	53.92 (17.68)	0.59
SBP (mmHg)	117.3 (13.83)	123.3 (16.51)	0.39

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EVALUATION OF NOVEL CXCR2 ANTAGONISTS TO COMBAT NEUTROPHILIC AIRWAY INFLAMMATION

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Background: Cystic fibrosis (CF) is characterized by a massive migration of neutrophils into the airways, release of reactive oxygen species and proteolytic enzymes, and ultimately deterioration in pulmonary function. Studies have shown that neutrophil count and activity are strong correlates of disease severity in CF. The chemokine receptor CXCR2 is expressed on the surface of neutrophils and bronchial epithelial cells and mediates neutrophil recruitment through binding of interleukin-8 (CXCL-8). We hypothesize that CXCR2 antagonists can reduce neutrophil migration and may have therapeutic use in patients with CF.

Methods: Using a pharmacophore model based on previously published CXCR2 antagonists, a novel set of candidate compounds was selected for CXCR2 inhibition screening. CXCR2 antagonism of candidate compounds was initially screened in a CXCR2-specific, cell-based, beta-lactamase reporter gene assay (CXCR2 TangoTM, Invitrogen). Sixteen antagonists showed potent antagonism of CXCR2 with IC₅₀ values less than 20 μ M. Cytotoxicity of these compounds was determined over a 4 log range of concentrations using MTT assays in the CuFi-1 and NuLi-1 human bronchial epithelial cell lines (with CF and non-CF phenotypes respectively). Compounds that exhibited less than 10% cytotoxicity at the highest concentration were selected for further experiments in a human neutrophil chemotaxis assay to investigate anti-inflammatory dose-response. In this assay, neutrophils were isolated from blood collected from healthy human volunteers, and were then incubated with CXCR2 antagonists over 4 logs of concentration. The neutrophils were added to the top plate of a membrane-based plate insert system, and CXCL-8 was added to the bottom plate. After four hours, the total number of migrated neutrophils was manually counted using Turk's solution, and the data was plotted using a sigmoidal Emax model.

Results: All compounds with the exception of CX4152, CX4338, CX312, and CX797 showed reduced cell viability of more than 10% at 100 μ M in MTT assays. CX4152 and CX4338 showed no cytotoxicity at concentrations up to 500 μ M, and were chosen for neutrophil chemotaxis assays. Both CX4152 (IC₅₀ 58.29 μ M, 95% CI 44.51 – 76.33 μ M, R² = 0.93) and CX4338 (IC₅₀ 57.76 μ M, 95% CI 47.81 – 69.77 μ M, R² = 0.94) showed inhibition of neutrophil migration when stimulated with CXCL-8. Cytotox-

icity was not significant even at ten times the concentrations that resulted in 50% maximal inhibition of neutrophil migration (IC₅₀).

Conclusion: CX4152 and CX4338 can reduce neutrophil migration *in vitro* and have a broad therapeutic index. Based on the positive *in vitro* results, we are currently conducting studies of CX4152 and CX4338 in a murine model of neutrophilic airway inflammation.

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CF PATIENTS' PREFERENCES AND PERFORMANCE CHARACTERIZATION OF A NOVEL AEROSOL DELIVERY PLATFORM TARGETING PULMONARY DEPOSITION OF AEROSOLS DELIVERED OVERNIGHT VIA NASAL CANNULA

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Nebulized chronic inhaled therapies contribute to daytime treatment burden despite the trend towards faster administration. Additionally, some therapies may not be efficacious or safe when delivered by inhalation at fast rates. A novel trans-nasal pulmonary aerosol delivery platform (TPD) has been developed to optimize the efficacy and safety of hyperosmolar, antibiotic, mucolytic and other inhaled therapies and to reduce the daytime treatment burden. TPD enables administration of aerosols via nasal cannula over extended periods of time, including overnight periods, with the intent to replace inhaled treatments administered during the day. TPD design incorporated CF patients' preferences identified in a human factor (HF) study. These preferences and the characterization of TPD performance over 8 hours of administration, without excessive accumulation of fluid in the nasal cannula or sputter, are reported here.

Methods: 31 US CF patients were recruited for a HF study conducted as one-on-one in-person interviews. The study assessed participants' preferences for overnight aerosol delivery via nasal cannula. TPD was designed to incorporate these preferences and to generate aerosol suitable for extended trans-nasal delivery via custom nasal cannula with optimized aerosol conductance, resembling in dimensions a supplemental oxygen nasal cannula. Particle size distribution and aerosol output were determined by laser diffraction and direct filter capture, respectively.

Results: ~90% of the patients indicated they would be willing to use the TPD device to replace their daily inhaled treatments, provided the TPD therapy resulted in an expected benefit. Multiple patients suggested to administer all of their daily inhaled treatments be administered in the TPD. The majority of the patients preferred the use of supplemental oxygen nasal cannula tubing compared to the CPAP tubing. Stand alone vibrating mesh produced aerosol with volume median diameter (VMD) of 5 to 11 μ m with >50% of the aerosol volume localized in large particles >4 μ m, leading to a declining aerosol output, excessive liquid accumulation and sputtering when administered via nasal cannula. In contrast, the aerosol produced by the TPD and emitted from the optimized nasal cannula had VMD of 1.5 μ m with 98% of the aerosol contained in particles below 4 μ m. Steady output of ~2.6 mL/hour of aerosol emitted from the nasal cannula was maintained for 8 hours without excessive rainout or sputter.

Conclusions: The TPD approach was well received by CF patients. TPD enables pulmonary aerosol administration via nasal cannula for extended periods of time and generates aerosol at a steady level of output for up to 8 hours without excessive fluid accumulation or sputter from the nasal cannula. Parion Sciences is developing the TPD for overnight administration of inhaled therapeutic agents with the intent of improving the efficacy and tolerability of these agents and reducing the daytime treatment burden in CF and other respiratory diseases.

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EVALUATION OF ANTIBODY TITER ANTI-*P. AERUGINOSA* IN PATIENTS UNDERGOING ERADICATION THERAPY

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Objectives: *Pseudomonas aeruginosa* (Pa) represents a major cause of lung inflammation and chronic infection. Early antibiotic therapy can eradicate Pa at the time of first infection delaying the onset of chronic infection. Eradication therapy is currently monitored only by microbiology cultures but some studies have evaluated the possibility of using additionally specific immunological markers. However the clinical relevance of the immunological response in the early stages of Pa is not clear and there is no conclusive evidence. The purpose of this study was to evaluate the immune response anti-Pa in the early stages of infection in patients undergoing eradication treatment.

Materials and Methods: We evaluated the sera of 62 patients with primary infection by Pa recruited into a multicenter study. An ELISA assay was used to test the presence of IgA and IgG anti-Pa. Lipopolysaccharide Pa (LPS) was used as the antigen. The cut-offs were 8 U/mL for IgA anti-LPS Pa and 6 U/mL for IgG anti-LPS Pa. Sera were collected at the first episode of colonization by Pa and after the eradication treatment (median 5 months, range 1-15 months). The treatment was considered effective if at least 3 culture tests were negative in a period of follow-up of 6 months (UK CF Trust); in the case of successful treatment we have a success in eradication therapy (S) if not, a failure (I).

Results: At the time of first infection the antibody titer of anti-Pa was below the cut-off value for IgA (mean 2.15 U/mL, ST 1.78) and for IgG (mean 4.43 U/mL, ST 3.09). There was no statistically significant difference noted between the two groups of patients with successful or unsuccessful eradication. After eradication therapy we noticed a rise in anti-PA antibody titer in patients still positive for Pa. This phenomenon has been found both for IgA (mean I=3.98 U/mL vs. mean S=1.85 U/mL, P=0.013) and IgG (mean I= 6 U/mL vs. mean S=3.64 U/mL, P= 0.039). Only for the IgG did the mean value reach the cut-off.

Conclusions: The study showed that the evaluation of specific anti-Pa may be useful to monitor the early stages of infection by Pa. The antibody titers at the beginning of the eradication treatment did not affect the outcome while an increase in antibody titer after or during the treatment should be carefully evaluated as a possible alert for treatment failure.

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IDENTIFICATION OF NEW TARGETS FOR ΔF508-CFTR RESCUE BY GENOME-WIDE SHORT INTERFERING RNA SCREENING

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The most frequent mutation in cystic fibrosis, ΔF508, causes the arrest of the CFTR protein in the ER and its degradation. Drug-like molecules called correctors are able to improve ΔF508-CFTR trafficking. However, correctors are characterized by a relatively low efficacy, particularly in primary airway epithelial cells. Indeed, the activity of ΔF508-CFTR correctors is largely influenced by cell background. In addition, the additivity/synergy observed between correctors, indicates that different treatments work by different mechanisms, and that cross-talk among different mechanisms may occur. These mechanisms may be universal or cell type specific. Therefore, novel strategies are required to develop rescue maneuvers that are more effective in the epithelial cells of CF patients.

To identify novel proteins involved in the processing of ΔF508-CFTR, we have adopted a functional genomics approach based on the screening of a Druggable-Genome siRNA library (6650 targets). Since the composition of the CFTR interactome and the responsiveness to rescue maneuvers may differ from one cell type to the other, we have utilized a human cell line (CFBE41o-) that is as close as possible to the original native epithelium, as the use of primary bronchial cells for screenings is not feasible.

The established conditions for high-throughput siRNA transfection in 96-well format were as follow: CFBE41o- cells were reverse transfected using 10 nM siRNAs and lipofectamine 2000. After 48 hours, the functional YFP-based assay was performed to determine the extent of ΔF508-CFTR activity in the plasma membrane.

The screening has identified 40 targets whose silencing causes a significant ΔF508-CFTR rescue in CFBE41o- cells. For 35 targets CFTR activity was increased by 35-50% relative to control-transfected cells, while for the remaining 5 targets CFTR activity was increased by 50-100% relative to control-transfected cells. The targets can be grouped into different functional classes, including ligases, kinases, cell receptors, and proteins involved in cell regulation.

Validation of results is ongoing. Silencing will be verified at the mRNA level, and the effect of silencing of each target will be confirmed using siRNA from other sources.

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IS MONITORING OF INTRAVENOUS TOBRAMYCIN IN CHILDREN WITH CF POSSIBLE WITH ONE BLOOD SAMPLE?

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Introduction: Intravenous tobramycin is often used for *Pseudomonas* infections in children with CF, but has a relatively small therapeutic index. Toxic levels can cause hearing loss and renal toxicity. There is limited data on the best strategy to monitor once-daily IV tobramycin in children. Peak serum levels correlate with antibacterial efficacy and trough levels with toxicity. A wide range in dosing and monitoring practices were found in a survey we distributed among international CF centers (n=25).

Aim: To design a pharmacokinetic model for tobramycin iv in which one blood sample (t=6) would be sufficient to assess effective as well as toxic concentrations.

Methods: Retrospective cohort of all children with CF treated with tobramycin IV between January 2007 and December 2010 and who had available tobramycin serum levels. In the current monitoring protocol, tobramycin serum levels are assessed at t=0, t=1 and t=6 hours after the start of infusion. NONMEM was used to assess pharmacokinetic parameters, to develop a population pharmacokinetic model and predict serum levels. We also collected data on hearing loss (high frequency audiometry) and renal function (serum creatinine).

Results: In all, 35 patients were included, who had a total of 128 courses of tobramycin and in which a total of 719 serum levels were assessed. A two-compartment model with zero-order intravenous infusion and allometric scaling for weight best fitted the observed data. NONMEM characteristics were found to be 6.1 L/h for clearance, 17.7 L for central volume of distribution, 1.1 L/h for intercompartmental clearance and 13.4 L for peripheral volume of distribution.

This model was found to structurally underestimate trough levels when using only t=6 as input. Thirty-six trough levels were higher than 0.5 mg/L in our study group. Only 36.1% was predicted accurately with the model. The model was also not able to predict peak levels accurately. There was a high variability in peak levels (t=1) in the study group. This was probably due to the fact that serum levels were not precisely taken on t=1 hour. No additional information was found with serum levels assessed in the consecutive treatment weeks, all serum levels remained stable. In only 11 patients audiograms were performed. Three out of 11 (27.3%) had hearing loss of >

15dB at 2 different frequencies. Creatinine was not measured on a routine basis. No signs of renal toxicity were found.

Conclusion: We failed to design a pharmacokinetic model which could accurately predict trough and peak levels based on $t=6$. The used blood samples were probably not taken at the exact documented times. Peak levels can vary greatly within a short period of time. Pharmacokinetic models may be of use, but must rely on accurately measured serum levels as input. For clinical practice we propose a monitoring protocol in which $t=0$ and $t=1$ is the minimum blood sampling for monitoring of tobramycin IV treatment. Monitoring of hearing loss and renal toxicity should be screened routinely as part of the clinical protocol.

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ASSESSMENT OF PULMONARY DISEASE BY LOW DOSE CT AND SPIROMETRY IN CF PATIENTS WITH MODERATE DISEASE: A COMPARISON

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Purpose: With improving treatment, classic spirometry may not be sensitive enough to assess and differentiate pulmonary involvement in CF subjects with minor to moderate disease. The aim of this study was to compare findings of spirometry to those of the low dose CT (LDCT), using the Bhalla CT scoring system.

Materials and Methods: Thirty-eight patients with CF, presenting for their yearly pulmonary assessment, underwent spirometry and LDCT in a stable condition. All CT scans were randomized and rated independently by 2 experienced staff radiologists, blinded to clinical information. Radiation dose was calculated. The Bhalla CT-scoring system was used to rate the images defining severity and extent of bronchiectasis, peribronchial thickening, generations of bronchial divisions involved, sacculations or abscesses, extent of mucous plugging, collapse or consolidation and number of bullae.

Results: The age of the patients (22 males and 16 females) ranged from 6 to 58 years (median age: 17 years); 42% being younger than 15 years. Mean FEV₁ was 83% $\pm$ 23.6, indicating that patients had a wide range of lung disease, but mostly with mild impairment and normal FEV₁. Mean effective radiation dose for LDCT was 0.51 mSv $\pm$ 0.56 mSv for patients younger than 15 years, and 1.54 mSv $\pm$ 1.32 mSv for patients older than 15 years, compared to 0.028 mSv $\pm$ 0.010 mSv for a PA chest radiograph. The median Bhalla score was 7 (range 1-18; highest possible score: 25). There was excellent interobserver agreement ($r=0.99$).

The LDCT scores correlated well with FEV₁ ($r_s=-0.77$, $p<0.001$), FEF₂₅₋₇₅ ($r_s=-0.73$, $p<0.001$), FVC ($r_s=-0.64$, $p<0.001$) and FEV₁/FVC ($r_s=-0.73$, $p<0.001$). The strongest linear association was found between LDCT score and FEV₁, and FEF₂₅₋₇₅ respectively.

Conclusion: Low dose CT scores correlate well with FEV₁ values, and seem to detect more lung abnormalities than suggested by mean normal FEV₁ values in this group of CF patients. More work is needed to further lower radiation dose, so that the latter is not a drawback for repetitive investigation.

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EXPOSURE-RESPONSE RELATIONSHIP FOR FEV₁ AND SWEAT CHLORIDE IN PATIENTS WITH CYSTIC FIBROSIS TREATED WITH IVACAFTOR, A CFTR POTENTIATOR

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Background: Ivacaftor (KalydecoTM) is a cystic fibrosis (CF) transmembrane conductance regulator (CFTR) potentiator that targets the under-

lying cause of disease, the defective CFTR protein, in patients with CF who have the G551D mutation. In clinical trials of these patients, treatment with ivacaftor led to substantial, statistically significant improvements in FEV₁, pulmonary exacerbations, respiratory symptoms and reductions in sweat chloride. The most common adverse drug reactions were headache, oropharyngeal pain, upper respiratory tract infection, nasal congestion, abdominal pain, nasopharyngitis, diarrhea, rash, nausea and dizziness.

Objectives: To establish the exposure-response relationship for FEV₁ and sweat chloride in patients with CF who were treated with ivacaftor.

Methods: Ivacaftor was studied in patients with a G551D mutation at 25 to 250 mg q12h for up to 28 days in a Phase 2a Study, and 150 mg q12h for 48 weeks in two Phase 3 Studies. It has also been studied in patients who were homozygous for the F508del mutation for 16 weeks in a Phase 2b Study. Population pharmacokinetic/pharmacodynamic (PK/PD) analyses were performed using pooled FEV₁ data and pooled sweat chloride data from these studies, respectively.

Results: The final PK/PD model for FEV₁ and sweat chloride is an E_{max} model with terms for baseline FEV₁ or sweat chloride, CFTR mutation, gender, age, and an effect compartment. In addition, a slope term was included for FEV₁ to account for natural changes expected with time. For an 18-year old male carrying the G551D mutation, the respective population mean (95% CI) E_{max} and EC₅₀ estimates were 0.322 (0.234, 0.403) L and 47.0 (5.03, 96.1) ng/mL for FEV₁. The E_{max} and EC₅₀ estimates were -50.6 (-52.9, -42.2) mmol/L and 100 (85.1, 112) ng/mL, respectively, for sweat chloride. Patients homozygous for the F508del mutation had E_{max} estimates that were not distinguishable from a null value of zero, indicating no treatment effect.

Conclusions: The population PK/PD models reasonably described the ivacaftor exposure-response relationship for FEV₁ and for sweat chloride in patients with a G551D mutation.

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CLINICAL PHARMACOLOGY PROFILE OF IVACAFTOR, A CFTR POTENTIATOR

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Background: Ivacaftor (KalydecoTM) is a cystic fibrosis (CF) transmembrane conductance regulator (CFTR) potentiator that targets the underlying cause of disease, the defective CFTR protein, in patients with CF who have the G551D mutation. In clinical trials of these patients, treatment with ivacaftor led to substantial, statistically significant improvements in FEV₁, pulmonary exacerbations, respiratory symptoms and reductions in sweat chloride. The most common adverse drug reactions were headache, oropharyngeal pain, upper respiratory tract infection, nasal congestion, abdominal pain, nasopharyngitis, diarrhea, rash, nausea and dizziness.

Methods: The drug interaction potential of ivacaftor with inhibitors and inducers of CYP3A, and substrates of CYP3A, CYP2C8 and CYP2D6 was investigated in several studies enrolling healthy volunteers. The effect of moderate hepatic impairment on the pharmacokinetics of ivacaftor was also evaluated.

Results: Ivacaftor exposure (AUC) was increased 8.5-fold by ketoconazole (a strong CYP3A inhibitor) and 3-fold by fluconazole (a moderate CYP3A inhibitor). Co-administration of ivacaftor with rifampin (a strong CYP3A inducer), decreased ivacaftor AUC by 89%. The co-administration of ivacaftor with midazolam (a sensitive CYP3A substrate) increased midazolam AUC by 1.5-fold, indicating that ivacaftor is a weak CYP3A inhibitor. Ivacaftor did not affect the AUC of rosiglitazone (a CYP2C8 substrate), desipramine (a CYP2D6 substrate), or the plasma exposures of an oral contraceptive (norethindrone/ethinyl estradiol).

Volunteers with moderately impaired hepatic function (Child-Pugh Class B, score 7 to 9) had an approximately 2-fold increase in ivacaftor AUC when compared with demographically-matched healthy volunteers.

Conclusions: Ivacaftor is a sensitive CYP3A substrate, a weak CYP3A inhibitor, but not an inhibitor of CYP2C8 or CYP2D6. Based on the degree of interactions observed in these studies, ivacaftor dose reduction is recommended when co-administering with strong or moderate CYP3A inhibitors and when administering to patients with moderate or severe hepatic impairment. Strong CYP inducers would be expected to substantially lower ivacaftor exposure and may affect efficacy. Caution and appropriate monitoring are recommended during co-administering of ivacaftor with some CYP3A

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substrates with a narrow therapeutic index. Health practitioners should consult the ivacaftor package insert prior to and during treatment for potential drug interactions and dosing recommendations.

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P38MAP KINASE INHIBITOR REDUCES CYTOKINE RELEASE BY *PSEUDOMONAS AERUGINOSA*-CHALLENGED BRONCHIAL EPITHELIAL CELLS

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Introduction: *Pseudomonas aeruginosa* (PA) is the leading pathogen in patients with cystic fibrosis, resulting in a strong inflammatory response and is a major contributing factor to the overall lung damage. It has been shown previously that P38 inhibitors can suppress the inflammatory response caused by PA, which could be an exaggerated response in CF. Specific kinase inhibitors are currently also being developed for a range of indications. The aim of this study is to evaluate the role of kinase signaling in, and the effectiveness of kinase inhibitors to suppress, PA-induced cytokine release in bronchial epithelial cells.

Methods: Human bronchial epithelial Beas-2B cells were pre-incubated for 2 hours with between 1 µg/mL - 0.00001 µg/mL of different commercially available kinase inhibitors targeting p38MAPK, MEK, JNK, Syk and C-src. After 2 hours the cells were stimulated with 2.5×10^7 CFU/mL of the *Pseudomonas* strain PAO1, which was washed out after 1 hour, and media containing 10 µg/mL gentamicin was added. Cell free supernatant was collected after 24 hours and the concentrations of interleukin-8 (IL-8) and IL-6 were determined using a sandwich ELISA. Each compound dose was triplicated and the data are presented as mean of the triplicates with standard deviation. IC₅₀ values were calculated using Excel Fit.

Results: IL-8 was significantly ($p < 0.05$) induced by PA in the Beas-2B cells (basal 365 ± 78 pg/mL, PA 2135 ± 160 pg/mL). The P38 alpha/gamma kinase inhibitor BIRB796 inhibited PA-induced IL-8 release in a concentration-dependent fashion; as the maximum inhibition was 44% at 1 µg/mL, no IC₅₀ was calculated. Other kinase inhibitors did not inhibit IL-8 release significantly. BIRB796 also showed concentration-dependent inhibition of IL-6 release with a maximum inhibition of 83%, and an IC₅₀ of 40 nM.

Conclusions: Only BIRB796, a P38MAPK inhibitor, compared to other kinase inhibitors, inhibited PA induced IL-8 and IL-6 release in Beas-2b cells suggesting that the P38MAP kinase is a dominant pathway in PA induced inflammation. We are exploring this further as a novel anti-inflammatory approach for bacterially-induced inflammation in CF.

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NOVEL BIOINFORMATICS APPROACH ACCURATELY IDENTIFIES CF MICE USING PLASMA OR TISSUE LIPIDOMICS

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Introduction: Patients with CF have abnormalities in blood fatty acid levels. Analysis of individual fatty acid levels or fatty acid ratios cannot reliably differentiate between CF and control samples because there are unresolved inconsistencies in their direction of change, even amongst arachidonic, linoleic, and docosahexanoic acids.⁽¹⁾ However, blood fatty acid profiles could be used as an outcome measure for CF therapies if there were a more comprehensive mode of analysis. We hypothesized that an algorithm to analyze multiple fatty acids could more accurately differentiate between CF and control groups. In this work, we validated the use of a novel algorithm to predict CF or WT genotype in fatty acid profiles from CF mouse models.

Methods: Fatty acid concentrations were measured in blood, liver, and/or adipose tissue samples from F508del/F508del (CF) and WT mice using mass spectrometry. Since age and diet affect fatty acid concentrations, we used mice of different ages (1 day, 3 weeks, and 6 weeks) and fed diets of varying lipid composition (low-fat and high-fat). Fatty acid concentra-

tions were input into the "Algorithm to Determine Expected Metabolic Alterations Using Mutual Information" (ADEMA) and the real values were discretized using B-spline curves. Related sets of fatty acids were determined by Elementary Mode Analysis on the de novo lipogenesis pathway. CF and WT specific fatty acid levels (signatures) were determined in order to train the ADEMA classifier. Accuracy, precision, and recall values were obtained using the leave-one-out cross-validation method.

Results: We used ADEMA on blood fatty acid profiles to predict CF or WT genotype with 100% and 79% accuracy in 3 and 6 week old mice, respectively, but could not accurately classify 1 day old mice. This suggests that CF mice have distinct blood fatty acid profiles at 3 and 6 weeks but not at 1 day old. When mice were fed different diets, ADEMA predictions of CF or WT genotype were most accurate in the adipose tissue samples from mice fed the high-fat diet. Genotype was least accurately predicted (<60%) in tissues of mice fed the animal-based low-fat diet. This suggests CF mice have a distinct blood fatty acid profile when fed the high-fat diet but not the animal-based low-fat diet.

Conclusions: Classification of CF status by analysis of fatty acid concentrations with ADEMA can be used in the well-controlled mouse model under different variables (age, diet) to determine which most affect fatty acid status. Ultimately, we need to test ADEMA's ability to classify CF status in human fatty acid profiles. If classification works, then "fatty acid correction" scored by ADEMA could be an outcome measure in clinical trials for the efficacy of CFTR potentiators, fatty acid supplementation, or other therapies for CF patients. Furthermore, ADEMA is generalizable to other classes of metabolites such as amino acids. We hope to apply ADEMA to other metabolites in the future.

Reference: 1. T.C. Coste et al. Clin Bioc 40, 511-20 (2007).

These studies were sponsored by the NIH.

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RESVERATROL FAILS TO INCREASE CHLORIDE SECRETION IN DF508 PRIMARY AIRWAY CELLS

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Resveratrol, a small organic compound found in red wine, has been reported to have various salutary effects. Indeed, resveratrol has been reported to increase CFTR expression and to increase cAMP stimulated chloride secretion across epithelial cells. However such effects have been reported using concentrations of resveratrol far in excess (>500 µM) of those achievable in human plasma (5-10 µM). We sought to evaluate the effects of resveratrol on CFTR expression and chloride secretion at clinically relevant concentrations. Resveratrol is a PDE inhibitor, increasing cAMP concentrations. Alone, resveratrol caused a dose-dependent increase in chloride secretion from T84 cells. In addition, resveratrol augmented the effects of sub-maximal levels of forskolin, consistent with actions mediated by the cAMP pathway. High concentrations of resveratrol were also able to cause a modest increase in CFTR levels when CFTR expressing 293 cells were incubated with compound overnight. Primary airway cells from a patient homozygous for the DF508 mutation were incubated overnight with 10 µM resveratrol. No increase in forskolin mediated currents was observed from resveratrol treated cells; though cells exposed to known CFTR correctors were responsive. We conclude that while high levels of resveratrol are effective in vitro, the low achievable levels of resveratrol in plasma suggest that resveratrol will have little beneficial effect in treating patients with cystic fibrosis.

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VALIDATION OF A NITROGEN (N₂) BASED MULTIPLE BREATH WASHOUT TECHNOLOGY IN HEALTHY CHILDREN AND CHILDREN WITH CYSTIC FIBROSIS

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Background: Multiple breath washout (MBW) derived measures of lung function such as the lung clearance index (LCI) have been shown to be abnormal in a high percentage of CF patients with normal spirometry. Recently, there has been a heightened interest in the utility of LCI as an outcome measure for clinical trials. Mass spectrometry based technology is considered the "gold standard" for measuring LCI, but is only available in a few centers around the world. In addition the technology is not commercially available, is expensive and uses sulphur hexafluoride (SF₆) as a tracer gas which is not readily available in the US. In this ongoing study we aim to validate a Nitrogen (N₂) washout system for use in the pediatric CF population.

Method: This is a cross-sectional study of healthy children and children with CF aged 6-18 years. CF children were recruited from the CF clinic at Sick Kids Hospital, Toronto, and healthy controls from siblings and community volunteers. Each participant performed MBW in triplicate by two methods: mass spectrometry (AMIS 2000; Innovision A/S, Odense, Denmark) using 4% SF₆ and 4% He as marker gases, and N₂ washout using 100% oxygen (O₂) (Exhalyzer D, EcoMedics AG, Durnten, Switzerland) using an indirect measurement of N₂.

Results: Fifty healthy children (median age 12 years (range 3-18)) and 37 children with CF (median age 11 years (range 6-17)) have completed MBW measurements using both mass spectrometry and N₂ washout. Reliable measurements could be obtained in all but one healthy and in 39 of 42 CF children. Measurements obtained and analyzed to date have shown good agreement between the two washout systems for the overall population studied (Limits of Agreement -2.5 to 1.2). In health, SF₆ LCI measured by mass spectrometry was on average 0.4 units lower than LCI measured by N₂ washout, with no systematic bias between the two techniques (Limits of Agreement -1.5 to 0.6). Differences between the two systems were greater in children with CF (on average 0.9 units lower for SF₆ (Limits of Agreement -3.4 to 1.5)). Inter-observer agreement of the offline analysis of the N₂ washout showed excellent agreement between the two operators (Limits of Agreement -0.5 to 0.4)).

Conclusion: These data suggest that, while there is no systematic bias between the two systems in healthy subjects, LCI measured by N₂ washout is significantly higher than SF₆ LCI in CF. The study also demonstrates the validity of the N₂ washout system for LCI measurement in school age children and forms the basis for subsequent validation studies in preschool children.

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SURFACE-MODIFICATION OF POLYMER NANOPARTICLES IMPROVES EFFECTIVENESS OF GENE DELIVERY IN CYSTIC FIBROSIS

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The underlying gene defect responsible for cystic fibrosis (CF) was identified in 1989 and there has been considerable interest in developing gene therapy based therapeutics for the disease ever since. Numerous studies and trials involving viral and liposomal vectors for plasmid DNA (pDNA) delivery have occurred, however due to safety concerns and lack of efficacy a successful therapy has yet to be developed. The use of biodegradable polymers provides a potentially safe and effective alternative to viral

and liposomal vectors for the delivery of pDNA to cells. In prior work, polymers have been found to be limited in different ways: nanoparticles of poly(lactic-co-glycolic acid) (PLGA) are non-toxic but low in efficiency whereas nanocomplexes of poly(beta-amino) esters (PBAE) are higher in efficiency but toxic. Here, we optimize the formulation of nanoparticles (NP) by blending PLGA with PBAE for use as gene delivery vehicles. Particles of different weight/weight (wt/wt) ratios of the two polymers were characterized for size, morphology, plasmid DNA (pDNA) loading and surface charge. NPs containing PBAE were more effective at cellular internalization and transfection of cells CF cells (CFBE41o-, F508del-CFTR) than NPs lacking the PBAE polymer. However, along with these delivery benefits, PBAE formulations still exhibited significant cytotoxicity. Surface coating of these blended particles with the cell-penetrating peptides (CPPs) mTAT, bPrPp and MPG via a PEGylated phospholipid linker (DSPE-PEG2000) resulted in NPs that reduced surface charge and cellular toxicity to levels comparable with NPs formulated with only PLGA. Additionally, these coated nanoparticles showed an improvement in pDNA loading, intracellular uptake and transfection efficiency, when compared to NPs lacking the surface coating. Although all particles with a CPP coating outperformed unmodified NPs, respectively, bPrPp and MPG coating resulted in 3 and 4.5X more pDNA loading than unmodified particles and approximately an order of magnitude improvement on transfection efficiency in CFBE41o-cells.

These particles can be administered safely via the intranasal, intratracheal and intravenous routes in a murine model. All routes of administration resulted in particle distribution throughout the lung in both proximal and distal airways. Studies of in vivo gene transfection efficacy are currently underway. Due to high transfection efficacy in vitro, this NP system has potential as a highly effective and minimally toxic, non-viral delivery vector for gene therapy treatment of CF.

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AN OBSERVER-REPORTED OUTCOME MEASURE FOR CHILDREN WITH CYSTIC FIBROSIS AGES 0-6 YEARS

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Study objective: To develop a validated CF-specific observer-reported respiratory, gastrointestinal, and systemic sign instrument for use as a clinical trial endpoint in children with CF ≤6 years of age. As a first step, in this paper we describe a Phase I study in which we developed a draft instrument suitable for field testing.

Methods: This was a qualitative study in which we conducted concept elicitation interviews with n=25 parents of children ages 0-6 years diagnosed with CF. A majority (76%) of parents were interviewed within 30 days of contact with the CF clinic for an acute change in their child's respiratory status. The data collected through these interviews were used to create a CF sign observational diary. The diary was subsequently revised based on cognitive interviewing with parents (n=8) and CF clinicians (n=16).

Results: The draft sign diary consists of 26 total items: n=11 respiratory, n=9 gastrointestinal, n=4 CF-related impacts, and n=2 reports of symptoms from child to parent (only ages 3-6). The respiratory signs include those related to breathing (n=5), cough (n=5), and runny or stuffy nose (n=1). The gastrointestinal signs include those related to bowel movement frequency and amount (n=3), stool appearance and consistency (n=2), gas and stomach bloating (n=2), and spitting up after eating (n=1) (for 3 years of age and younger). The impact signs include those related to activity, sleep, eating, and emotions (n=1 for each area). Finally, the symptom reports include those related to chest pain and stomach pain (n=1 each) (3 years and older).

Conclusions: Using a qualitative inductive methodology, we successfully obtained patient-centered data regarding observable pulmonary and gastrointestinal signs and impacts and have created a novel observer-reported outcome measure for young children with CF. Future studies will assess the measurement properties of the instrument.

Acknowledgment: This research was supported by a grant from the University of Washington - Institute of Translational Health Sciences and a research contract with Vertex Pharmaceuticals.

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CHANGES IN SPUTUM RHEOLOGY FOLLOWING *IN VITRO* TREATMENT WITH ALGINATE OLIGOMERS

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Progressive lung disease in cystic fibrosis (CF) patients represents a major cause of morbidity and mortality. Bacterial infections in the lungs of CF patients are typified by bacterial overproduction of alginate or extracellular polysaccharide. Current therapies include physiotherapy, antibiotic and mucolytic treatments. OligoG is a low molecular weight (ca. 2600 MW) oligosaccharide nanomedicine based on a defined alginate oligomer with 90-95% of the monomer residues as G-blocks. OligoG alginates possess many advantages, being biodegradable, effectively inert, non-toxic and safe for *in vivo* use (Clinical Phase 1 studies; EudraCT number: 2009-009330-33, www.clinicaltrials.gov identifier NCT00970346). The ability of OligoG to potentiate the activity of antimicrobial/antibiotic therapy and disrupt *in vitro* biofilms has previously been demonstrated in conventional microbiological assays, scanning electron- and confocal laser scanning- microscopy. In preliminary rheological studies, we demonstrated that OligoG is able to reduce CF sputum viscosity *in vitro*; potentially aiding the disruption of CF sputum and facilitating its clearance from the diseased lung.

We sought to definitively characterise the effect of OligoG on CF sputum in a series of individual adolescent and adult sputum samples (n=20) from patients attending clinics at the Cystic Fibrosis Unit in Cardiff with local ethical approval. In a separate series of experiments, to monitor potential changes in the efficacy of OligoG with disease state, we prospectively collected sputum from an individual patient over 16 weeks (n=10 samples). In these experiments, non-induced sputum samples were treated with OligoG ($\leq 2\%$) and/or Pulmozyme® (2.5 µg mL⁻¹) or hypertonic saline. A significant difference in elasticity (G') and viscosity (G'') between CF sputum in control vs 2% OligoG was confirmed (G' $p < 0.0017$; G'' $p < 0.0053$). In addition, the ability of OligoG to potentiate the changes in both viscosity and elasticity induced by Pulmozyme® alone was evident ($p < 0.0394$). Interestingly, the longitudinal studies over a 4-month period demonstrated consistency of the positive effect for OligoG, in contrast to Pulmozyme® treatment where changes in viscoelasticity were not uniformly observed throughout the study (a positive effect being evident in only 6/10 longitudinal sample analyses).

These studies demonstrated a consistent ability of OligoG to effectively modify the rheology of sputum from CF patients *in vitro*; significantly reducing the observed viscosity and elasticity. The ability of OligoG to reduce CF sputum viscoelasticity, potentiate the activity of Pulmozyme®, together with its previously described ability to potentiate the activity of antibiotics against multi-drug resistant bacteria, highlights its potential utility in the treatment of CF. In this respect, OligoG is now undergoing Phase IIa human trials (EudraCT number: 2010-023090-19, www.clinicaltrials.gov identifier NCT01465529).

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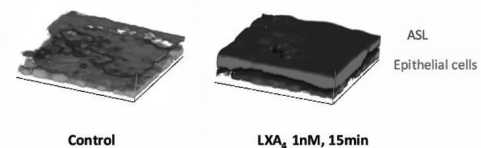
LIPOXIN A4 INCREASES AIRWAY SURFACE LIQUID HEIGHT, STIMULATES AIRWAY EPITHELIAL REPAIR AND DELAYS THE INVASION OF CYSTIC FIBROSIS BRONCHIAL EPITHELIAL CELLS BY *PSEUDOMONAS AERUGINOSA*

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In cystic fibrosis, the mutant CFTR gene results in decreased Cl⁻ secretion and hyperabsorption of Na⁺ in the airway leading to dehydration of the airway surface liquid (ASL) layer. The reduction of ASL height impairs mucociliary clearance and favours lung infection and inflammation which lead into a progressive lung destruction. The eicosanoid lipoxin A4 (LXA4) described as a signal for the resolution of inflammation is decreased in the

lungs of patients with CF (Karp et al, 2004). We previously reported that lipoxin A4 (LXA4) a pro-resolution anti-inflammatory lipid mediator stimulates Cl⁻ secretion (Urbach et al, 2003), ZO-1 expression and increases transepithelial electrical resistance in human airway epithelial cells (Urbach et al, 2008). Using confocal microscopy, luciferin/luciferase and gentamicin invasion assay we investigated the effect of LXA4 (1nM) on ASL height, on epithelial repair and on invasion of human bronchial epithelial (HBE) and cystic fibrosis bronchial epithelial (CFBE) cells by *P. aeruginosa*. Airway epithelial cells were cultured from bronchial brushing obtained through the SHIELD CF study (Study of Host Immunity and Early Lung Disease in CF). We found that LXA4 increased the ASL height in a dose and time dependent manner in both the HBE and CFBE cells. The effect of LXA4 on the ASL height was mediated by the Boc-2 sensitive FPR2 receptor, apical ATP release, P2Y11 purino-receptor activation and subsequent stimulation of Cl⁻ secretion. LXA4 also significantly stimulated epithelial repair in CF and non-CF airway epithelial cells. Cell proliferation and repair induced by LXA4 were found to be mediated by KATP channel activation. Finally, LXA4 delayed the invasion of HBE and CFBE cells by *P. aeruginosa*. In conclusion, we reported a novel role for LXA4 in increasing ASL height, stimulating epithelial repair and delaying the invasion of *P. aeruginosa*, which could lead to a new therapeutic route for CF patients.

Acknowledgements: Funded by the Children's Medical and Research Foundation in Ireland, the Health Research Board of Ireland and the French National Institute of Health, INSERM.

Lipoxin A₄ increases the airway surface liquid height in CF bronchial epithelium in primary culture

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UPDATE ON THE UK CF GENE THERAPY CONSORTIUM MULTIDOSE, NON-VIRAL, GENE THERAPY TRIAL

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The UK CF Gene Therapy Consortium has been working for several years to determine the clinical benefit of CFTR gene therapy. Our premise was that for such a therapy to achieve clinical benefit, repeated administration would be required, and that therefore a non-viral approach was needed. We demonstrated in laboratory and preclinical animal models that the cationic lipid, GL67A, originally developed by Genzyme Corp, was the optimal gene transfer agent, and designed a plasmid, pGM169, completely depleted of pro-inflammatory CpG motifs and driven by the EF1α promoter designed for prolonged expression (Hyde SC et al. Nat Biotech 2008). In a longitudinal observational study (the Run-in) we measured the variability of multiple outcome measures, both conventional and novel. These data have allowed us to perform power calculations and choose a) our primary

outcome (FEV₁), b) secondary efficacy outcomes (lung clearance index, various parameters on CT scan, quality of life questionnaire [CFQ-R], exercise capacity and activity, and selected sputum and serum inflammatory markers), and c) safety measures (clinical findings, exacerbation rate, gas transfer, sputum culture, serum inflammatory markers, renal and hepatic markers). The Run-in study also allowed us to identify the patients most suitable to progress to the trial. We excluded two subgroups a) too severe: those with such heterogeneity of pulmonary deposition on isotope scanning that successful delivery of the topical agent was considered unlikely or who had significant impairment of FEV₁ (<50% predicted) and b) too mild: FEV₁ > 90% predicted, in whom a primary outcome signal might not be achievable.

One hundred patients, aged 12 years, and above are being randomised in a 1:1 fashion to active treatment or placebo and will receive the nebulised agent at monthly intervals for 12 doses at a dose determined from our recently completed single dose (Pilot) study. The group size was determined on the basis of a 6% relative improvement in FEV₁. An adaptive design will be used for additional safety; the first 20 patients will receive 3 doses ahead of the rest of the cohort. Safety data will be examined by an independent committee following which, should there be no major concerns, the rest of the group will begin dosing. If safety is an issue, the administered dose will be halved. Patients will be invited to participate in either one, or both, sub-studies, being conducted to explore mechanisms; a) nasal administration followed by nasal potential difference (PD) and brushings for mRNA expression and b) pre- and post-treatment bronchoscopies for lower airway PD, gene expression and histology. The double-blinded nature of the trial means that final outcome data will only be available upon completion of the study. The trial was initiated in April 2012; here we will update on recruitment, projected time-lines and progress.

Funded by the Cystic Fibrosis Trust and the MRC and NIHR through the EME programme.

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THE CLINICAL UTILITY OF A HAND-HELD NASAL NITRIC OXIDE ELECTROCHEMICAL ANALYSER TO SCREEN PATIENTS WITH BRONCHIECTASIS FOR PRIMARY CILIARY DYSKINESIA (PCD) AND CYSTIC FIBROSIS (CF)

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Background: Measurement of nasal nitric oxide (nNO) levels has been validated as a useful screening tool for PCD. Reduced nNO levels are also seen in patients with cystic fibrosis (PWCF). Traditionally, nNO measurements have been performed using stationary, chemi-luminescence analysers during breath-holding or other volum-closure techniques (as per ATS/ERS Recommendations for Standardized Procedures for the Online Measurement of Nasal Nitric Oxide, 2005). As part of the development of a national diagnostic centre for PCD in the Republic of Ireland, we aim to assess the clinical utility of a hand-held nNO electrochemical analyser, the NIOX MINO®, to differentiate between PCD, CF, patients with non-CF/non-PCD bronchiectasis, COPD and normal healthy subjects.

Methods: Clinically stable patients were recruited from the hospital outpatient clinic over a 6-month period. Each subject completed a pro forma screening for a compatible PCD phenotype, nNO analysis using a NIOX MINO®, and one nasal brushing for electron microscopy (EM) analysis, obtained from below the inferior turbinate using an endocervical brush. nNO gas was aspirated via a nasal olive probe inserted into one nostril by use of passive sampling at a flow rate of 5mL/sec during normal relaxed tidal breathing. EM images will be reviewed internally by three clinicians blinded to clinical diagnosis, and externally at an international centre of excellence (UNC Chapel Hill). Independent T-tests were used to compare mean nNO values between groups.

Results: Twenty-six subjects were recruited (n=4 PCD, n=6 CF, n=7 non-CF/non-PCD bronchiectasis, n=4 COPD, n=5 healthy subjects). Mean nNO levels (ppb +/- SD) were 24+/-13(PCD), 40+/-39(CF), 251+/-273(non-CF/non-PCD bronchiectasis), 263+/-148(COPD) and 419+/-76(healthy control). nNO levels were significantly reduced in the PCD cohort when compared to patients with COPD (p-value 0.018) and normal healthy sub-

jects (p-value <0.0001). nNO levels were also significantly reduced in the CF cohort when compared to patients with COPD (p-value<0.04) and the normal healthy subjects (p-value<0.0001). However, there was no statistically significant difference between nNO levels in PCD and CF (p-value 0.49). Results of EM analysis are pending.

Conclusion: In this study, the hand-held NIOX MINO®

nNO analyser demonstrated ability to separate patients with PCD and CF from patients with COPD and normal healthy subjects, but was unable to distinguish between patients with PCD and CF. EM analysis may reveal subjects with previously undiagnosed PCD in the non-CF/non-PCD group, which may impact our results. However, based on our results to date in a single institution, tidal-breathing NO analysis using the NIOX MINO® cannot, on its own, differentiate CF and PCD, and further confirmatory testing such as ciliary EM analysis, sweat test and genetic studies are required.

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EVALUATION OF OXYGEN-ENHANCED MRI FOR DETECTION OF EARLY-STAGE CYSTIC FIBROSIS LUNG DISEASE

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Introduction: Evaluating therapies that target the basic defect in infants and young children with CF will require repeated assessments in order to monitor lung disease progression. MRI is a safe, non-invasive procedure capable of providing quantitative assessments of pulmonary function without radiation. An oxygen-enhanced (OE)-MRI technique has been shown to reliably assess regional lung dysfunction in CF patients with established lung disease. However, this non-invasive imaging technique has not been studied in CF patients with early-stage lung disease.

Methods: Cross sectional study of adult CF patients in comparison to a healthy non-CF adult control subject. OE-MRI data were obtained from a CF patient with early-stage (FEV₁% predicted = 100%) intermediate-stage (FEV₁% predicted = 60%), and a healthy control (FEV₁% predicted = 117%). Each subject was positioned in a clinical Siemens Espree 1.5T MRI scanner. While breathing room air rapid MRI acquisition was used to obtain T1 relaxation maps for multiple imaging slices to obtain 3D OE-MRI data. Each imaging slice was acquired in a single, <5 second breathhold to eliminate respiratory motion artifacts in the images. The procedure was then repeated under 100% oxygen. Mean T1air values were calculated from regions of interest in the upper and lower lungs for three contiguous imaging slices for each subject.

Results: Mean T1air values in the upper lungs of both the early-stage and intermediate-stage CF patients were significantly lower (more disease) when compared to the healthy subject (* p < 0.05, ** p < 0.01, respectively) [Figure 1]. OE-MRI was also able to discriminate between early and intermediate stage CF patients (p = 0.05). The mean T1air results were consistent between the right and left lungs for each subject.

Conclusion: These preliminary results suggest that OE-MRI may be able to sensitively detect early-stage CF lung disease. The next step is to test younger patients. Ongoing studies in CF patients will provide additional information to evaluate OE-MRI as a tool to safely monitor early-stage lung disease progression and response to therapy in infants and young children.

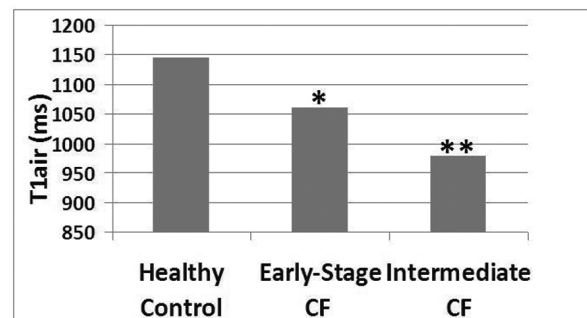


Figure 1. OE-MRI differentiates early CF lung disease from a

healthy volunteer.

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CHARACTERISATION OF THE EFFECT OF OLIGO G ON THE BACTERIAL CELL SURFACE OF PSEUDOMONAL BIOFILMS

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Pseudomonas aeruginosa is a common cause of morbidity in cystic fibrosis (CF) patients. Bacterial infections in the lungs of CF patients are typified by bacterial overproduction of extracellular polysaccharide such as alginate. We have previously shown that OligoG, derived and processed from alginate, an oligomer of the sodium salt of (1-4) linked α -L-guluronic acid (G), composed of 90-95% G (MW 2600 g mol⁻¹) potentiates the activity of conventional antibiotics (up to 500-fold) against a range of multi-drug resistant Gram-negative bacteria, and that OligoG has an ability to modify the rheology and structure of biofilms of *P. aeruginosa*. Whilst these effects have been extensively characterized, the mechanism and interaction with the bacterial surface is not well understood. We studied the interaction of *P. aeruginosa* (PAO1) and OligoG on cell surface structure (using atomic force microscopy; AFM), surface charge and cellular assembly (using sizing and zeta-potential analysis) and cell motility. In addition, the reversibility of the interaction was studied to determine the stability of OligoG/PAO1 binding on exposure to hydrodynamic shear. AFM analysis was performed on *P. aeruginosa* (PAO1) biofilms after washing and treatment with OligoG (0.5-10%, 20 mins) using a Dimension 3100 AFM (Bruker). Sizing and zeta-potential analyses were performed in electrolyte solutions of 0.1-0.001 M NaCl at pH 5, 7 and 9. Hydrodynamic shear experiments repeated the analyses following a rigorous 3 min washing regime (x2; 5,500 g). The ability of OligoG (at concentrations <10%) to affect bacterial motility was also studied by incorporating OligoG either into Isosensitest agar plates or into motility test agar (MTA) "stab" inoculations containing a redox indicator and observing bacterial spread of *P. aeruginosa* (PAO1) and *Proteus mirabilis* (NSM6) across/through the agar. AFM studies demonstrated the uniform distribution of OligoG on the bacterial surface following treatment. OligoG treatment increased the overall negative bacterial surface charge (at all observed pH values; $p > 0.05$). These changes were associated with 2-3 fold changes in cell-size as a result of "cell-clumping" and was not reversible even after exposure to hydrodynamic shear. OligoG inhibited bacterial motility in a clear, dose-dependent manner. OligoG concentrations >6% almost completely inhibited motility in *P. aeruginosa*, *P. mirabilis* and *Escherichia coli*. This study demonstrated that not only was irreversible binding of the OligoG to the bacterial cell surface of PAO1 in biofilms observed, but this binding was shown to modify the surface structure, surface charge as well as biofilm assembly and bacterial cell motility. These physical, surface-charge and structural effects may, in part, explain the observed action of OligoG on bacterial assembly, biofilm formation and antibiotic potentiation that has been previously described.

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LUNG CLEARANCE INDEX TO EVALUATE THE EFFECT OF IVACAFTOR ON LUNG FUNCTION IN SUBJECTS WITH CF WHO HAVE THE G551D-CFTR MUTATION AND MILD LUNG DISEASE

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Objectives: Ivacaftor has been shown to improve FEV₁ in subjects ≥ 6 years of age who have the G551D mutation. Drugs targeting the basic defect in CFTR may benefit patients with early stage disease, but demonstrating

this can be difficult. FEV₁ may be of limited value whereas a more sensitive test such as lung clearance index (LCI) may be more useful.

Methods: This Phase 2, randomized, double-blind, placebo-controlled, multicenter, crossover study evaluated the effect of ivacaftor, a CFTR potentiator, on LCI in addition to clinical and CFTR biomarker endpoints of CF lung disease. Multiple breath washout (MBW) measurements were performed using an open-circuit Innocor system and 0.2% sulphur hexafluoride (SF₆) as a tracer gas. Operators were qualified for the study by completing a pre-specified number of acceptable LCI tests assessed by the LCI Central Reader. Operators obtained ≥ 3 LCI tests for each subject at each visit and blinded data were analyzed centrally. Subjects with CF ≥ 6 years of age who have the G551D-CFTR mutation were screened. Eligibility criteria included percent predicted FEV₁ >90% and LCI above the upper limit of normal (7.4). Ivacaftor 150 mg or placebo was administered q12h for two 4-week periods separated by a 4-week washout period.

Results: Twenty-one subjects were randomized and 20 received at least one dose of study drug. Seventeen subjects completed both ivacaftor and placebo treatment periods. Mean (SD) age was 16.6 (10.9) years. Mean (SD) baseline LCI was 9.0 (1.5). The treatment effect of ivacaftor for adjusted mean change from baseline in LCI at Day 29 was -2.1 ($P=0.0004$). Mean (SD) baseline percent predicted FEV₁ was 97.2% predicted (10.6%). The treatment difference for the mean change from baseline in percent predicted FEV₁ at Day 29 was 7.0 percentage points ($P=0.0117$). The treatment difference for the mean change from baseline in sweat chloride at Day 29 was -45.9 mmol/L ($P<0.0001$). The treatment effect for the change from baseline in pooled CFQ-R Respiratory Domain score was 4.0 points versus placebo ($P=0.3796$). In the ivacaftor period, adverse events were reported in 13 subjects and serious adverse events were reported in 2 subjects. In the placebo period, AEs were reported in 15 subjects and SAEs in 1 subject. Cough, headache, vomiting, pyrexia, and nasal congestion were the most commonly reported. Post-hoc analysis demonstrated that LCI had superior power to detect change in this mild group compared with FEV₁, the latter requiring approximately 3 times as many patients as the former.

Conclusions: In subjects with CF who have mild lung disease, ivacaftor treatment improved ventilation inhomogeneity as measured by LCI and respiratory function as measured by FEV₁. LCI is more sensitive to change in this mildly affected group of CF subjects and may be a useful outcome measure for future interventional trials in such patients.

Sponsored by Vertex Pharmaceuticals Incorporated.

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COMBINATORIAL TRANSCRIPTOMIC, PROTEOMIC, AND METABOLOMIC ANALYSES REVEAL DYSREGULATION OF GLYCOLYSIS IN PRIMARY CF AIRWAY EPITHELIA AND NEUTROPHILS

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We conducted a multimodal analysis of primary CF and nonCF airway epithelia grown in normal glucose at an air-liquid interface (HBE), and neutrophils collected from the blood (PMN) of CF and nonCF subjects. To better understand the link between the dysfunction of CFTR and secondary defects like inflammation and the dysregulation of redox balance, we treated primary nonCF airway epithelia from 6 donors with the CFTR-inh-172 (20 μ M) for 72 hr to inhibit CFTR. We then used mass spectrometry to analyze differences in metabolites and proteins to determine which pathways were significantly impacted by CFTR inhibition. In this initial discovery phase we found a significant change in a number of pathways, but the most pronounced change was an increase in glycolysis and glucose metabolism. Given the link between altered glycolysis and abnormalities in redox balance and inflammatory signaling, we designed a study using gene array and proteomic analyses to examine differences in CF HBE and PMN. The purpose of this study was to evaluate whether CFTR dysfunction results in abnormalities in glycolysis in the two cell types most implicated in inflammatory signaling and lung deterioration in CF.

First, we studied the expression profile of PMN from CF subjects and from healthy controls aged 16 to 40 years. PMN were separated by Ficoll method and selected by CD16+ magnetic microbeads. RNA was immediately extracted and Illumina HT12 microarray platform used for analysis. We

studied 11 healthy controls and 26 CF patients. Genes, enriched for glucose and carbohydrate metabolism were differentially expressed in CF vs. nonCF, which is characteristic of a metabolic shift to a glycolytic phenotype. In addition, CF PMN exhibited higher levels of two glucose transporters (GLUT1 and GLUT3). These data indicate a previously undescribed abnormality in the metabolism of CF PMN.

Follow up proteomic studies in PMN and HBE cells also revealed the increased expression of glycolytic enzymes in CF vs. nonCF controls. The similarities in the regulation of glycolysis in CFTR inhibited (pharmacological model) and CF primary PMN and HBE cells suggest that the metabolic shift toward a glycolytic phenotype is due to CFTR dysfunction and may be a basic defect in CF cells. The data on a metabolic shift toward glycolysis in CF primary cells, using transcriptomic, proteomic, and metabolomic analyses were also confirmed by biochemical measures that indicated an upregulation in phosphoenolpyruvate and pyruvate. We conclude that the upregulation of glycolysis is related to CFTR dysfunction as evidenced by the impact of CFTR inhibition in nonCF primary cells, and independent studies conducted in 2 different primary cell types in 2 different labs. The implication of these results is that CFTR dysfunction is linked to a glycolytic metabolism, which in nonCF related studies has been extensively shown to promote redox imbalance and inflammatory signaling.

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ACTIVATION OF NRF2 BY TRITERPENOID IN CF EPITHELIA RESULTS IN INCREASED LEVELS OF GSH AND THE DECREASED OXIDATION OF PROTECTIVE PROTEINS

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To better understand secondary defects in CF like inflammation and the dysregulation of redox balance, we conducted an analysis of the effect of activating the transcription factor Nrf2 on GSH levels and differential protein oxidation in primary CF and nonCF airway epithelia (HBE) grown at an air liquid interface. The activation of Nrf2 decreases inflammatory signaling in CF epithelia to nonCF levels and improves redox balance. To examine the mechanism by which Nrf2 activation in CF cells is protective, we used LCMS to identify the change in GSH and protein oxidation following treatment with the triterpenoids CDDO and CDDO-Me. These triterpenoids serve as maximal activators of Nrf2 by stabilizing its dissociation from the inhibitor Keap1, and have been shown to be safe in animals and humans.

Cells were treated with 300 nM CDDO (in 0.5% DMSO), 300 nM CDDO-Me (in 0.5% DMSO), or DMSO (0.5%) for 24 hr. On the day of analysis cells were incubated with or without 1 mM H₂O₂ acutely for 5, 15, 30, 60, or 90 minutes. The purpose of this assay was to examine the ability of CF vs. nonCF cells to rebound from exposure to oxidants, which is a key indicator of the ability of cells to stave off harmful effects of oxidative stress. We found that whole cell GSH levels ranged between 2.4-3.2 mM in DMSO treated CF and nonCF cells. Rebound was diminished in DMSO treated CF cells versus DMSO treated nonCF cells, with a drop of ~63% in GSH levels at 5 min which returned to 86% of levels in CF cells untreated with H₂O₂ by 90 min. Conversely, nonCF cells exhibited an increase in GSH of 87% following a 5 min exposure to H₂O₂, which remained elevated for 90 min. The decrease in rebound observed in CF cells was corrected following treatment with CDDO, or CDDO-Me. CF cells treated with these triterpenoids exhibited an increase of ~55% in GSH levels following exposure to H₂O₂. While triterpenoids increased basal levels of GSH in nonCF cells, no significant effect on rebound was observed. Measurement of the rebound in thioredoxin (TRX) I oxidation mirrored our results with GSH, with CF cells exhibiting a significantly reduced rebound in TRX I oxidation. Parallel samples were incubated with BIAM, which modifies reduced proteins. We used the BIAM to purify reduced proteins and identified them by LC/MS. In the absence of H₂O₂, a number of protective proteins involved in GSH synthesis and anti-inflammatory signaling (e.g. PPAR γ) are significantly less labeled with BIAM in CF vs. nonCF cells indicating a significant basal oxidation of CF proteins. Following 5 min incubation with H₂O₂, a further loss of BIAM labeling of protective proteins is observed in CF cells, which was corrected by CDDO and CDDO-Me.

We conclude that CF cells exhibit a significant dysfunction in the response to oxidative stress which manifests in increased oxidation of protective proteins. Triterpenoid activation of Nrf2 is sufficient to effect a reversal of this phenotype resulting in an increase in GSH expression and reduction of the oxidation of anti-oxidant and anti-inflammatory proteins.

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NATURAL HISTORY OF CYSTIC FIBROSIS IN PATIENTS WITH NONSENSE-MUTATION-MEDIATED DISEASE

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Background: Nonsense mutations are Class I mutations associated with complete absence of CFTR at the epithelial cell membrane and occur in ~10% of the patients. The natural history of CF in this specific subpopulation is not well documented. An international Phase 3, randomized, placebo-controlled, 48-week clinical trial of the investigational new drug, ataluren, in nonsense mutation CF (nmCF) was recently completed; longitudinal data from the placebo arm in this study provides insight into the natural history of nmCF.

Methods: Eligibility criteria included age ≥ 6 years, sweat chloride >40 mEq/L, FEV₁ $\geq 40\%$ and $\leq 90\%$ of predicted, and presence of a nonsense mutation in at least 1 allele of the CFTR gene as documented by gene sequencing. Outcome measures included spirometry, pulmonary exacerbation rate, and health-related quality of life as measured by the Cystic Fibrosis Questionnaire-Revised (CFQ-R).

Results: The intent-to-treat population comprised 232 patients (116 ataluren, 116 placebo) from 36 centers in 11 countries. Most patients were heterozygous for nonsense mutations (85% ataluren, 87% placebo); the remainder were homozygous. The most common nonsense mutations were W1282X and G542X.

Of the 116 patients assigned to placebo, 103 completed 48 wks of blinded study treatment and had a valid Wk 48 spirometry measurement. Fifty-two were female and 51 were male. Median [range] age was 22 [8-53] yrs. The mean baseline %-pred FEV₁ was 60.3 [SD=15.1]; the mean baseline CFQ-R respiratory domain score was 67.5 [SD=17.4]. The mean relative change in %-pred FEV₁ at Wk 48 was -5.5% and was larger than the decline reported in the general CF population. This change in FEV₁ was not predicted by baseline age, baseline FEV₁, or baseline CFQ-R respiratory domain score. Chronic palliative treatment included inhaled antibiotics (57%), dornase alfa (76%), and hypertonic saline (45%). The mean [median, range] number of pulmonary exacerbations (PEX) for the placebo group, as defined by the modified Fuchs criteria, was 1.64 [1.0, 0-8.6]. The correlations of PEX rate with %-pred FEV₁ at baseline and with change in %-pred FEV₁ at Wk 48 were low ($r = -0.19$ and $r = -0.28$, respectively).

Conclusion: Baseline data from this study and longitudinal data from the placebo arm in this international multicenter study demonstrate that patients with nmCF have severe disease. Despite treatment with currently approved CF therapies, a substantial decline in %-pred FEV₁ was seen, which exceeded the decline previously reported for CF in general, suggesting that patients with nmCF have more severe disease. This decline was not predicted by age, %-pred FEV₁ or CFQ-R respiratory score at baseline. Treatment options aimed at improving the underlying cause of CF are clearly needed in this patient population.

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ALTERED LUNG WATER DENSITY IN CF ADULTS AS MEASURED BY QUANTITATIVE MRI

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Rationale: The long-term goal of our research is to establish a non-invasive quantitative MRI technique to monitor secretions in patients with CF. Translational investigations have a critical need for a quantitative measure of secretions that could be used repeatedly in a diverse population including young children and patients with very advanced lung disease. Such a measure does not currently exist of this immediate downstream consequence of altered CFTR function. A non-invasive imaging protocol that is capable of obtaining lung water density within a 9-sec breath-hold has been developed at UCSD for use with physiological studies. In healthy subjects, lung water density is a measure of water from blood and lung tissue. However, in subjects with CF, lung water density includes water from excess airways secretions. Quantitative measures of lung water density were obtained in adult CF subjects with mild to severe lung disease and results have shown locations of abnormal lung water density and the presence of air-cysts (e.g. absence of lung water).

Methods: Fifteen to eighteen sagittal slices encompassing the left and right lung were acquired on a 1.5T MRI with a fast gradient echo sequence that collects images in a single 9-sec breath-hold at FRC and TLC. Data were fit on a voxel-by-voxel basis to a single exponential to determine fractional lung density (FLD). FLD has a range of values from 0 (air) to 100 (100% fluid). Data were acquired in duplicate.

Results: Average FLD across repetitions for each lung at FRC were compared between 6 healthy subjects (5M/1F, average age=36.2, average BMI=25.9, FEV1[%pred]=88.3), 3 CF subjects with mild disease (2M/1F, average age=41.5, average BMI=21.0, FEV1[%pred]=71.7), 2 CF subjects with moderate disease (1M/1F, average age=23.5, FEV1[%pred]=58.5), and a single CF subject with severe disease (M, age=41, BMI=18.9, FEV1[%pred]=37). The average FLD for the left(L)/right(R) lung was found to be 18±4%/21±5%, 17±3%/20±2%, and 16%/17% for the mild, moderate and severe CF subject(s); and 20±3%/21±4% for the controls. FLD negatively correlated with age for both CF (L: r=-0.72/R: r=-0.57), and controls (L: r=-0.22/R: r=-0.35). The presence of air-filled cysts was determined by calculating the percentage of each lung (L/R) that contains voxels with an FLD<5% and was measured to be 4±5%/3±4%, 8±10%/6±7%, and 7%/5% for the mild, moderate and severe CF subject(s); and 1±1%/2±2% for the controls. The percentage of lung (L/R) with FLD<5% (air-filled cysts) correlated positively with age (r=0.96) for subjects with CF but showed no relationship with controls (r=0.35). Lastly, the percentage of lung with an FLD<5% correlated positively with FEV1[%pred] for controls but showed no relationship in subjects with CF (r=-0.35). To determine if the decrease in FLD at FRC observed in CF subject is due to air trapping, the average FLD for both lungs was evaluated at TLC. The average FLD was 13.5±1%, 15.5±1.5%, and 14.5% for the mild, moderate and severe CF subjects(s), respectively. The average FLD was ~12±2% for the controls.

Conclusions: These data suggest that FLD as measured by MRI may be useful in monitoring disease in CF.

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SERUM NEUROTROPHIN LEVELS CORRELATE WITH ACUTE PULMONARY EXACERBATIONS IN CYSTIC FIBROSIS

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Introduction: Pulmonary exacerbations in cystic fibrosis (CF) patients are a significant cause of morbidity and have a significant impact in quality of life. However, little is known about their pathophysiology. Despite the central role of pulmonary exacerbations in cystic fibrosis, there is no standard definition that is universally accepted. Therefore, the diagnosis of a cystic fibrosis pulmonary exacerbation (CFPE) relies on a clinician's decision to treat a group of symptoms and signs that differ from the patient's baseline status or his/her period of clinical stability.

While not validated, a frequently used definition of a CFPE is that from the Cystic Fibrosis Foundation Practice Guidelines (1). Another standard-

ized instrument that uses clinical criteria to define a CFPE, is the Akron's pulmonary exacerbation score (PES) (2).

Neurotrophins play a critical role in neural and immunologic mechanisms of airway inflammation and hyperreactivity, and their expression may correlate with severity of infection. Inflammation in the CF lung appears to be driven by local stimuli, mediators and chemoattractants, more than a local effect of a systemic inflammatory reaction. To this date, there is no standardized inflammatory mediator that has been validated as a biomarker of CFPE.

Objective: This study sought to determine whether serum levels of the prototypical neurotrophin factor, Nerve Growth Factor (NGF), correlate with the severity of CFPE and therefore can be used to assist in the diagnosis of a CFPE and guide treatment strategy.

Methods: Serum samples were obtained from patients followed in our Mountain State CF Center during routine or sick visits, and PES was calculated for each of those visits. Serum levels of NGF were measured on all samples collected. Spearman's correlation coefficient was calculated for NGF levels and the calculated PES.

Results: Serum NGF was positively correlated with the calculated PES (coefficient 0.555, p<0.01).

Conclusions: Serum NGF levels increase as the PES increases, suggesting that NGF levels may be useful as a biomarker of severity of CFPE; and therefore, assist the clinician to guide therapeutic decisions.

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MEASURING MUCUS VISCOSITY USING MICRO-OPTICAL COHERENCE TOMOGRAPHY DEVELOPED MICRORHEOLOGY TECHNIQUES

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It is well accepted that CFTR functions as an epithelial ion transporter of chloride and bicarbonate on the surface of human airways, and emerging evidence suggests these defects can also alter the physical and functional properties of airway mucus. Analysis of airway mucus from newborn CFTR (-/-) pigs in situ using fluorescence recovery after photobleaching shows that it is highly viscous compared to CFTR (+/+) littermate controls ($t_{1/2} = 12.9 \pm 1.0$ sec CFTR (-/-) vs. 9.2 ± 0.9 sec CFTR (+/+), $P < 0.05$, $N = 20$), demonstrating that viscosity may be an important abnormality of the CF defect. Our previous studies have shown that one micron resolution spectral domain optical coherence tomography (μ OCT) can quantitatively assess ASL and PCL depths, ciliary beat frequency (CBF) and mucociliary transport rates (MCT), all without the use of contrast dyes, exogenous microparticles, or other manipulations. In the present report, we have further advanced the methodology and developed a technique for native particle tracking microrheology to measure mean squared displacement (MSD) caused by Brownian motion to derive dynamic viscosity and validated the method by fluorescence microparticle tracking in preparation for in situ and in vivo measurements of mucus viscosity using μ OCT.

We first compared μ OCT-based endogenous particle tracking to traditional fluorescence particle tracking microrheology on the same expectorated sputum from non-CF donors. MSD results were very similar ($0.61 \mu\text{m}^2$ by μ OCT vs. $0.51 \pm 0.082 \mu\text{m}^2$ fluorescence particle tracking, $P = \text{NS}$), validating μ OCT-based measurements of the mechanical properties of mucus. We have also found that μ OCT-based native particle tracking analysis can readily distinguish CF from non-CF sputum ($0.03 \pm 0.01 \mu\text{m}^2$ vs. $0.51 \pm 0.15 \mu\text{m}^2$ Non-CF, $P < 0.05$, $N = 8-12/\text{group}$), recapitulating known differences in viscosity of induced CF sputum compared to sputum from healthy volunteers. Preliminary data has also been able to differentiate CF and Non-CF mucus using measured dynamic viscosity.

To test the feasibility of extracting MSDs from μ OCT images in vivo, we evaluated mucus specimens undergoing ciliary, axial ($100 \mu\text{m}$, 1Hz) and lateral (2 mm/min) motion, with magnitudes and rates designed to simulate physiologic motion expected in vivo. By subtracting the bulk motion vector caused by active MCT from the random motion trajectories, we were able to calculate MSDs under all motion conditions, including in situ ($0.51 \pm 0.14 \mu\text{m}^2$ no cilia vs. $0.53 \pm 0.22 \mu\text{m}^2$ with cilia, $P = \text{NS}$; $0.21 \pm 0.02 \mu\text{m}^2$ no motion, $0.27 \pm 0.03 \mu\text{m}^2$ axial motion, and $0.25 \pm 0.07 \mu\text{m}^2$ transverse motion, $P = \text{NS}$). This preliminary data supports the notion that microrheol-

ogy can be performed by μ OCT using native particle tracking in vivo while also providing two- and three-dimensional assessments of local viscoelastic forces present on the airway surface. μ OCT provides unique capabilities allowing us to monitor the rheology of mucus in situ while also simultaneously measuring the functional airway microanatomy of the epithelial surface.

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SELF-MODELING STRUCTURE OF INTESTINAL CURRENT MEASUREMENTS IN PATIENTS WITH CYSTIC FIBROSIS

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Objective: Clinical and translational studies in cystic fibrosis (CF) have provided novel therapeutic advancements for disease treatment. These studies often involve vast collections of biomedical data on each subject. Such extensive data are often collapsed into summary statistics obtained at specific points in the experimental protocol. We examined the efficiency of self-modeling regressions (SEMOD) to characterize intestinal current measurements (ICM) collected on CF and non-CF subjects as from a multi-site clinical study.

Methods: We implemented a computational statistics algorithm for fitting the SEMODs using Bayesian adaptive regression splines. The shape-invariant structure allows regression methods based on splines to be used to estimate smoothed versions of the ICM traces, resulting in noise reduction for subsequent analyses. Linear correlations were examined between the calculations implemented after each protocol-specific stimulus and the SEMOD-specific coefficients. Information from multiple curves were combined to show "fine structure" aspects of the curves that are visually obscured by noise in the individual ICM curves.

Results: We observed excellent agreement between AUC (area under the curve) and the SEMOD coefficients. The self-modeling structure predicted that, except for noise, these two quantities should be equal. Correlations ranged from 0.74-0.96, indicating that the prediction of the self-modeling structure was accurate.

Conclusions: The spline smoothing that we performed decreased the noise involved in computing individual quantities in the ICM studies, such as peak amplitude and AUC, which provide efficient, methodological improvements to all analyses of these ICM curve-specific calculations.

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VALIDATION OF HAND-TO-FOOT BIOELECTRICAL IMPEDANCE ANALYSIS WITH AIR-DISPLACEMENT PLETHYSMOGRAPHY IN THE ASSESSMENT OF BODY COMPOSITION IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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Background: Malnutrition is an indicator of a poor prognosis in patients with cystic fibrosis (CF). Traditionally, the growth of children with CF is monitored by using height, weight, and body mass index (BMI) measurements, but these techniques do not provide any insight into the nature of the child's body composition. The investigation of body composition in the population would be beneficial due to it expanding our understanding of the disease process, enabling the assessment of the effectiveness of medical and nutritional interventions, and may identify those children at most risk of deterioration. Hand-to-foot bioelectrical impedance analysis (BIA) is simple and non-invasive, making it particularly suitable for use in children. There is insufficient evidence of the validity of hand-to-foot BIA compared with air-displacement plethysmography (ADP) as the criterion method in pediatric CF patients.

Objective: Assess the validity of hand-to-foot BIA against ADP through measures of Body Fat percentage (BF%), Fat Mass (FM) and Fat Free Mass (FFM) in a sample cohort of CF patients attending treatment at the CF Center at the Goryeb Children's Hospital.

Design: Body composition was measured by hand-to-foot BIA and ADP in 15 patients (60% males) on the same day.

Statistical Analysis: Paired comparisons were made between hand-to-foot BIA and ADP in terms of body fat percentage, fat mass (lb) and fat free mass (lb) using the paired t-test.

Results: Average age was 13.9 years (SD = 2.9) with 60% males. Average BMI was 20.7 kg/m² (SD = 5.0). Results from paired comparisons showed a marginally lower body fat percentage for BIA [mean body fat percentage for BIA was 18.0% vs. 20.4% for ADP; p = 0.064]. Significantly lower fat mass was observed for BIA [mean fat mass for BIA was 23.0 lbs vs. 25.4 lbs for ADP; p = 0.047]. Fat free mass for BIA was, however, significantly higher for BIA [mean fat free mass for BIA was 93.5 lbs vs. 90.6 lbs for ADP; p = 0.032].

Conclusions: Significant differences were highlighted between the two methods in terms of fat mass and fat free mass. This indicates that based on the data, we could not validate the use of BIA for those two measures in the assessment of body composition assessment in pediatric CF patients. Future analyses will focus on examining differences/agreement between the two methods by gender and age group. Future research should look to; expand the sample size and increase to participation of females due to their worse prognosis; use a control group of healthy pediatric subjects for comparison and classification of body composition; perform longitudinal repeat assessments to provide insight into the changes in body composition in CF pediatric patients.

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IMPROVEMENTS IN LUNG FUNCTION IN PSEUDOMONAS COLONISED PATIENTS TREATED WITH INHALED DRY POWDER MANNITOL (BRONCHITOL™)

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Introduction: Patients with cystic fibrosis (CF) have lungs infected early in life causing a cycle of inflammation and damage. Approximately 60% of patients with CF have chronic respiratory infection caused by *Pseudomonas aeruginosa* (PA). Infection with this organism is a leading cause of morbidity and mortality in CF patients. Two large Phase III studies with inhaled mannitol have demonstrated improvement in lung function over 6 months. Data from these studies has been pooled to optimise analysis of subgroups, including those with pre-existing PA.

Methods: Data was pooled from two near identical multicentre, double-blind, randomised (3:2), controlled, parallel group phase III studies comparing mannitol (400 mg) and control (mannitol 50 mg) bid for 26 weeks. Of the total 600 patients, 283 (mannitol n=164, control n=119) comprised a PA infected sub-group in which lung function was compared between treatment arms.

Results: In the PA positive sub-group 78.1% were adults, and the baseline demographics for each treatment group were similar for age (mannitol 25.1 yrs, control 24.8 yrs), gender male (mannitol 54.9%, control 52.1%) and FEV1 % predicted (mannitol 61.3%, control 60.1%). In the PA positive group, 88% were on antibiotics, including nearly two thirds on either neb/inhaled tobramycin or colistin (mannitol 64.0%, control 65.5%). Baseline PA infection rates in the mannitol and control groups were 45.4% and 49.8% respectively.

In the PA positive patient group, the difference in mean change in FEV1 (mL) from baseline for mannitol 400mg bid (125.98 mL) vs mannitol 50mg bid (15.76 mL) was statistically significant (110.22 mL, p<0.001) over 26 weeks treatment.

Conclusion: Mannitol has demonstrated a significantly improved lung function sustained over six months, on top of existing best standard of care in PA positive CF patients, a group at particularly high risk.

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THE INVESTIGATIONAL CFTR CORRECTOR, VX-809 (LUMACAFTOR) CO-ADMINISTERED WITH THE ORAL POTENTIATOR IVACAFTOR IMPROVED CFTR AND LUNG FUNCTION IN F508DEL HOMOZYGOUS PATIENTS: PHASE II STUDY RESULTS

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Background: F508del, the most common mutation in CF affects folding and trafficking of the CFTR protein, resulting in little or no CFTR at the epithelial surface. Lumacaftor, an investigational CFTR corrector, was designed to enhance F508del-CFTR protein trafficking. Addition of ivacaftor to lumacaftor increased chloride transport in model cell systems in vitro. In cohort 1 of a clinical trial of lumacaftor and ivacaftor, data suggested that co-administration significantly increased CFTR function compared to lumacaftor alone in patients homozygous for F508del. Cohort 2 was designed to evaluate the effects of lumacaftor monotherapy (days 0-28, period 1) as well as co-administration of lumacaftor and ivacaftor therapy (days 28-56, period 2) on sweat chloride, lung function, and safety in patients homozygous and heterozygous for F508del.

Methods: 82 patients homozygous for F508del were randomized and received either lumacaftor (200, 400, or 600 mg qd) for days 0-28 (period 1) followed by lumacaftor (200, 400, or 600 mg qd) co-administered with ivacaftor 250 mg q12h for days 28-56 (period 2), or placebo (periods 1 and 2). In addition, 27 patients heterozygous for F508del received either lumacaftor 600 mg qd for 28 days followed by lumacaftor with ivacaftor 250mg q12h for 28 days, or placebo for 56 days. Primary endpoints were safety and pharmacodynamic effect during period 2 as assessed by change in sweat chloride, a biomarker of CFTR function. The key clinical efficacy endpoint was absolute change in percent predicted FEV1 (FEV1).

Results: Treatment of homozygous patients with lumacaftor alone (600 mg, period 1) resulted in a within-group -5.91 mmol/L reduction in sweat chloride (p=0.001) and a -6.41 mmol/L reduction compared with placebo (p=0.012). An additional -2.82 mmol/L reduction in sweat chloride was observed during period 2 (p=0.243). During period 1 there was a decrease in mean absolute FEV1 in patients homozygous for F508del in the 600 mg lumacaftor monotherapy (-2.87%) and placebo groups (-0.89%) (p=0.364). During period 2 statistically significant increases in FEV1 were observed in all co-administered dose groups versus placebo. The greatest increase was observed in the 600 mg lumacaftor plus ivacaftor group (n=20): a 6.1% within-group improvement (p<0.001) and an 8.6% treatment effect versus placebo (p<0.001). Most adverse events were mild to moderate in severity, and were similar between treatment and placebo groups. Additional safety and efficacy data including individual patient results, results for each dose group, and FEV1 data for delF508 heterozygotes will be presented.

Conclusions: In CF patients homozygous for F508del, 28 days of combination therapy with lumacaftor and ivacaftor produced clinically meaningful improvements in lung function, both within group and compared with placebo. Lumacaftor was well tolerated alone or with ivacaftor co-administration in these patients.

INFECTION/MICROBIOLOGY

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THE INCIDENCE OF RESPIRATORY VIRUS INFECTION IN ADULTS WITH CYSTIC FIBROSIS

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Introduction: Respiratory virus infections are common in children with cystic fibrosis (CF) and are associated with increased respiratory symptoms and lung function decline. To date, there has been no large prospective study of respiratory virus infection in adults with CF. We performed a single-centre observational study to determine the incidence and impact of common respiratory viruses in a cohort of adults with CF.

Methods: Patients were recruited from December 2010 to March 2011 and followed for 12 months or until death/lung transplantation. Sputum, nose- and throat-swabs were collected every 2 months and additionally at the onset of new respiratory symptoms. Samples were analysed by in-house polymerase chain reaction (PCR) assays to test for adenovirus, influenza A and B, metapneumovirus, parainfluenza 1-3, respiratory syncytial virus (RSV) and rhinovirus. Pulmonary exacerbation scores (Fuchs et al. 1994) and upper respiratory tract infection scores (Johnston et al. 1993) were recorded. Patients with a follow-up duration of <6 months were excluded from incidence calculations. Where patients remained positive for the same virus at ≥2 consecutive visits it was assumed to be a single episode of persistent infection. Data are presented as mean (SD) or median (IQR) as appropriate unless stated.

Results: One hundred adults with CF were recruited. Median age was 28 (23-36) years. Mean FEV1 at baseline was 59 (22) %predicted; 48% were male, 60% F508del homozygous and 73% chronically infected with *P. aeruginosa*. Two patients withdrew after the baseline visit, 3 died during follow-up and 1 underwent lung transplantation. Excluding withdrawals, 98 patients completed a mean of 6.6 (1.7) study visits over 11.5 (1.95) months. Virology results were available from 625 visits.

A total of 197 (31.5%) visits were positive for ≥1 respiratory virus. There were 9 instances of dual viral infection. Rhinovirus accounted for 72% of identified viruses, metapneumovirus 13.2%, adenovirus 4.1%, influenza A 3.6%, influenza B 2.5%, parainfluenza 2.5% and RSV 2%. Overall incidence of respiratory virus infection was 1.58 (95%CI 1.37-1.78) cases/patient-year. Incidence (95%CI) of individual viruses was: rhinovirus 1.1 (0.92-1.27), metapneumovirus 0.28 (0.17-0.38), influenza A 0.08 (0.02-0.13), adenovirus 0.07 (0.02-0.13), parainfluenza 0.06 (0.01-0.1), influenza B 0.05 (0.01 - 0.1) and RSV 0.04 (0.001 - 0.08) cases/patient-year respectively.

Patients experienced a mean of 2.6 (1.8) pulmonary exacerbations per year. Of virus-positive visits, 51% met pre-defined criteria for pulmonary exacerbation compared with 32% of virus-negative visits (OR 2.17, 95%CI 1.52-3.09; p<0.0001). Data on the long-term effects of respiratory viral infection on lung function will be available by October 2012.

Conclusions: Respiratory viruses are very common in adults with CF and are strongly associated with pulmonary exacerbation. Rhinovirus is the most frequent viral respiratory pathogen in adults with CF. Respiratory viruses represent an under-exploited therapeutic target for the prevention of pulmonary exacerbations in CF.

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CO-INFECTION WITH RESPIRATORY VIRUSES ENHANCES *PSEUDOMONAS AERUGINOSA* BIOFILM GROWTH

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In cystic fibrosis (CF), mutations in CFTR reduce or eliminate chloride secretion in the lung and thereby reduce the volume of airway surface liquid (ASL), which in turn suppresses mucociliary clearance of *Pseudomonas aeruginosa* and other pathogens trapped in the mucus. Accordingly, individuals with CF are susceptible to chronic infections, primarily by the opportunistic pathogen *P. aeruginosa*, which is particularly difficult to eradicate due to the high basal levels of antibiotic resistance and the ability of *P. aeruginosa* to form antimicrobial resistant biofilms. Over time the CF lung evolves into a highly inflamed and purulent environment that is the proximate cause of morbidity and mortality in these patients. Little is known about the mechanism by which *P. aeruginosa* establishes biofilms in the host, but it has been reported that CF patients show a reduced ability to clear *P. aeruginosa* acquired during respiratory viral infections and 85% of new pseudomonal colonization in CF patients followed a respiratory viral infection within 3 weeks. We hypothesized that virus co-infection promotes biofilm growth by *P. aeruginosa*. Using a live-cell imaging approach to grow *P. aeruginosa* biofilms on polarized human airway epithelial cells, we demonstrated a dramatic increase in *P. aeruginosa* biofilm growth on airway cells in the presence of a viral co-infection with respiratory syncytial virus (RSV). Similar increases in *P. aeruginosa* biofilm growth were demonstrated when airway cells were co-infected with the respiratory viruses human rhinovirus-14 and adenovirus-5. RSV co-infection with CF non-mucoid and mucoid *P. aeruginosa* clinical isolates produced increased biofilm growth on polarized airway epithelial cells. Notably, RSV dramatically augmented *P. aeruginosa* biofilm growth on polarized CF primary airway epithelial cells. Virus co-infection did not induce cytotoxicity or enhance the attachment of *P. aeruginosa* to the airway epithelium, but did promote a more rapid maturation of the biofilm, as demonstrated by earlier expression of the biofilm marker gene *tolA* and earlier loss of the planktonic marker gene, *fliC*. Finally, in vivo co-infection studies demonstrated a profoundly increased bacterial burden in mice co-infected with influenza A virus, as compared to *P. aeruginosa* alone, at 24 hours post-inoculation of *P. aeruginosa*. The current study provides evidence that respiratory viral infections can enhance the development of *P. aeruginosa* biofilms and suggests a potential role for respiratory viral infections in the transition of *P. aeruginosa* to chronic colonization in CF patients. While it has been well documented that viral infections are often accompanied by a secondary bacterial infection, this is the first study linking viral co-infection to the establishment of chronic bacterial colonization and biofilm formation, with important implications in the progression of CF lung disease.

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RSV STIMULATES MMP-9 RELEASE FROM CF EPITHELIAL CELLS AND INCREASES MMP ACTIVITY IN HUMAN LUNGS

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Respiratory syncytial virus (RSV) infection is a common trigger that promotes acute pulmonary exacerbation in children with cystic fibrosis (CF). We recently demonstrated that CF airway epithelial cells exhibit greater sensitivity to RSV infection, including enhanced monolayer disruption and markedly elevated viral replication. Previous studies have shown that matrix metalloproteinase -9 (MMP-9) activity is increased in CF airway secretions, due in part to elastase-driven conversion of MMP-9 zymogen to

active enzyme, and degradation of the MMP-9 inhibitor, TIMP (tissue inhibitor of metalloproteinase)-1 by elastase. In the current study, we tested the hypothesis that RSV is a specific stimulus for MMP-9 release from airway epithelial cells, and that children with RSV-induced respiratory failure have elevated MMP-9 activity relative to normal and disease controls.

Methods: For in vitro studies, CFBE41o- bronchial epithelial cells stably transduced with wt or F508del CFTR were infected with the RSV A2 strain. Cell lysates and media were monitored for MMP-9 expression post-infection using real time RT-PCR, Western blotting and activity measurements. Endotracheal aspirates were collected within 48 hours from children with RSV-induced respiratory failure. ALI (acute lung injury) subjects served as disease controls and intubated patients without lung disease served as "normals." MMP-9 levels were measured using activity assays and correlated with disease severity.

Results: In vitro experiments demonstrated up-regulation of MMP-9 mRNA, elevated MMP-9 protein release, and increased MMP-9 activity following RSV infection in CF epithelial cells compared to wt controls. TIMP-1 expression was also blunted following RSV infection (an effect expected to further augment MMP-9 activation). In patient samples following 48-hour intubation, active MMP-9 was approximately 8-fold higher in children with RSV bronchiolitis (n=28), compared to ALI subjects without RSV (n=23). Low levels of MMP-9 were observed in normal controls (n=10), whereas the highest levels of MMP-9 were noted in subjects with RSV bronchiolitis who later developed ALI, suggesting the importance of an interaction between airway epithelial damage, matrix remodeling and MMP-9 dysregulation following RSV infection. In subjects who developed ALI from RSV, higher active MMP-9 levels also correlated with a longer course of mechanical ventilation.

Conclusion: Our studies identify a direct link between RSV infection and MMP-9 release from CF airway epithelial cells. MMP-9 derived from epithelia is a likely contributor to overall MMP-9 levels observed following RSV-induced lung injury. Since the CF airway is characterized by high elastase activity, our data support the notion that RSV infection in CF may lead to increased (and dysregulated) MMP-9 in vivo. Together, the findings support investigation of RSV and MMP-9 as mediators of RSV dependent tissue injury in CF lung disease.

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VITAMIN D DEFICIENCY IS ASSOCIATED WITH INCREASED RISK OF LOWER RESPIRATORY INFECTION WITH *S. AUREUS* AMONG INFANTS & PRESCHOOL CHILDREN WITH CF

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Background: Vitamin D has a central role in the innate defense of the lung and in regulation of inflammation. Early childhood studies link vitamin D deficiency to increased prevalence and severity of respiratory infection and asthma. The relationship between vitamin D status and pulmonary infection and inflammation in cystic fibrosis (CF) has not been characterized in early childhood.

Aims: To examine cross sectional and longitudinal relationships between serum levels of vitamin D and lower respiratory infection (LRI) and inflammation in young children and infants with CF diagnosed through newborn screening.

Methods: Bronchoalveolar lavage (BAL) samples obtained prospectively at diagnosis of CF (~3 months) and annually until 5-6 years of age were analysed from children participating in the ARESTCF early surveillance program at Perth and Melbourne CF clinics. BAL fluid was analysed for inflammation and cultured for respiratory pathogens. Serum levels of 25-OH-vitamin D were measured at diagnosis, annually, and when clinically

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indicated. Vitamin D levels were seasonally adjusted prior to analysis and results controlled for gender, age, season of infection and pancreatic status.

Results: Paired serum 25-OH-vitamin D/BAL fluid data on 195 subjects, median age 2.48 (0.06-6.46) years, 52% male, were analysed. Vitamin D deficiency <50nmol/L (20ng/mL) was observed in 31(22%) subjects in first 2 years of life, which persisted to age 6 years in 29%, despite supplementation. Vitamin D deficiency produced 3.7-times increased odds of LRI, specifically with *S. aureus* (OR: 3.66 [95% CI 1.15-11.64], $p=0.014$), but not with other lower respiratory pathogens, and odds of *S. aureus* LRI were increased 8-fold in severe deficiency <30nmol/L (12ng/mL) (OR: 8.08[0.97-67.25], $p=0.053$). There were no statistically significant relationships between vitamin D and pulmonary inflammation in the analyses. There were consistent observations in longitudinal analyses of increased pulmonary inflammation at age 5-6 years among subjects who were vitamin D deficient in infancy, and among those who did not achieve optimal levels (or developed suboptimal levels) during the subsequent preschool period. However, these results did not reach statistical significance and require exploration in larger populations before definitive conclusions are drawn.

Conclusions: Vitamin D deficiency was common despite supplementation, and persisted throughout preschool years in one third of subjects. The significantly increased odds of *S. aureus* LRI in young children with suboptimal Vitamin D levels underscore the importance of sufficient supplementation in early life for current, and potentially, future lung health.

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GENOME SEQUENCE AND FUNCTIONAL COMPARATIVE GENOMICS OF *P. AERUGINOSA* RP73: DEFINING HOST-BACTERIAL INTERACTIONS IN A PERSISTENT LIFESTYLE

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P. aeruginosa can establish life-long chronic airway infections in CF patients. As the disease progresses, strain variants can be distinguished from the strain initially acquired. However, the genetic basis and the biology of host-bacteria interactions leading to a persistent "lung-lifestyle" of *P. aeruginosa* are not understood. As a model system in studying long term and persistent infections, we used a multi-drug resistant non-mucoid *P. aeruginosa* strain RP73 isolated 16.9 years after the onset of infection in a CF patient. To confirm the capability of RP73 in long term persistence, we estimated *in vivo* maintenance using a murine model of chronic lung infection. After two weeks postinfection, pathology analysis confirmed advanced chronic pulmonary disease including inflammation and mucous secretory cells. To link a persistent lifestyle with the potential of a genetic basis, we report the complete genome sequence of RP73 and comparative genomics analysis.

The *P. aeruginosa* RP73 chromosome is 6,342,034 bps in length and has an average 66.5%, G+C. These values and the pangenome structure show similarity and differences to the reference genomes of PAO1, PA14 and LESB58. RP73 contains 4 genomic islands, PAGI-1, -5, -10 and -9, the latter of which is not present in most reference genomes. Unique features of RP73 also include 2 insertions that correspond to possible prophages. Overall, large genomic regions (> 5000 bps) that differ between RP73 and in the genomes of other strains fall into documented regions of genome plasticity. The RP73 genome encodes 5975 ORFs but lacks an entire cluster of 32 genes from PA1694 to PA1725 encoding the type III secretion system. The genome sequence of RP73 indicated the deletion of genes involved in twitching motility, *pilA*, *pilV*, *pilW*, *pilY2* and *fimT*, a deletion in *pilC* and a stop codon after 138 amino acid in *pilO*. Phenotypic analysis of RP73 showed no twitching motility. The 46 genes involved in swarming motility are present; although RP73 showed motility defects. We identified genetic variations in both the *lpxO2* and *oprH* genes involved in LPS biosynthesis. Analysis of the chemical structure of lipid A by mass spectrometry confirmed specific structural modifications. RP73 is a non-mucoid strain having genes for alginate biosynthesis and regulation including *mucA*, except for a deletion of *algP*. The transcriptional regulator *lasR* has a frameshift mutation giving a change in ORF of 26 amino acids at the C-terminus. The ability to acquire iron for *in vivo* survival is impaired in RP73; the genes *pchD*

and *pvdD* responsible for siderophore synthesis have stop codons in their ORFs. Finally *pldA* coding for phospholipase D is not present in RP73.

The genome plasticity of *P. aeruginosa* particularly in RP73 strongly indicates that these alterations may form the genetic basis defining host-bacteria interactions leading to a persistent lifestyle in human lungs.

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PSEUDOMONAS AERUGINOSA (PA) PHENOTYPES ASSOCIATED WITH PERSISTENT EARLY INFECTION IN CF PATIENTS IN THE EPIC CLINICAL TRIAL

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Background: CF *Pa* isolates have diverse *in vitro* growth phenotypes that change over time during infection. Some phenotypes, including mucoidy and those conferred by inactivating mutations in the quorum sensing regulator LasR, have been associated with worse clinical outcomes. The objective of this study was to evaluate the association between phenotypes of early CF *Pa* isolates with failure to eradicate *Pa* after antibiotic treatment and with other microbiologic and clinical outcomes, focusing on those phenotypes conferred by LasR inactivation.

Methods: We analyzed *Pa* isolates collected during the 18-month EPIC Clinical Trial, which compared 4 *Pa* eradication regimens among children ages 1-12 years with newly acquired *Pa*. All isolates from the 112 participants who had baseline cultures positive for *Pa* were analyzed for 21 well-characterized phenotypes, including mucoidy, motility, and (among those previously associated with LasR inactivation) colony sheen, autolysis, wrinkliness, protease and pigment production, and growth characteristics. Because colony sheen and autolysis were previously correlated with LasR inactivation among clinical *Pa* isolates, these phenotypes were used as *lasR* surrogate phenotypes. Associations between all phenotypes and subsequent microbiologic and clinical outcomes were estimated, including time to next positive *Pa* culture, emergence of mucoidy, and time to pulmonary exacerbation.

Results: Isolates with *lasR* surrogate phenotypes were present among 22/112 (20%) children at baseline and were associated with subsequent mucoidy: 24% of children with *lasR* surrogates at baseline, and 4% of children without, had an emergent mucoid isolate ($p=0.017$). Wrinkly initial colony morphology, which has been linked to LasR inactivation and ability to form biofilms *in vitro*, was found in 13/112 (12%) of children at baseline. It was significantly associated with recurrence of *Pa* during the trial (Hazard Ratio [HR]: 1.99, $p=0.043$) and trended towards association with emergence of *lasR* surrogates: 20% and 2.5% of children with and without baseline wrinkly isolates, respectively, had subsequent isolates with *lasR* surrogates ($p=0.059$). No phenotype was significantly associated with risk of exacerbation.

Conclusions: *Pa* phenotypes usually considered to be adaptive are common, even among isolates from early CF infection. While both LasR inactivation and mucoidy have been associated with persistence and/or resistance to eradication, only a wrinkly colony phenotype was strongly associated in this early infection cohort with recurrent culture positivity. These results suggest that a wrinkly colony phenotype, associated both with LasR inactivation and biofilm formation, may be a biomarker for more aggressive and/or persistent infection, preceding identification of sheen, autolysis, and mucoidy.

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PSEUDOMONAS AERUGINOSA ANTIBIOTIC RESISTANCE IN BIOFILMS IS ENHANCED BY RESPIRATORY VIRUS CO-INFECTION

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The transition of bacterial infections from acute to chronic infections often involves the development of bacterial biofilms. Biofilms are sessile communities of bacteria that are characterized by an inherent resistance to antimicrobial agents. The combination of an upregulation of antibiotic resistance genes and production of a polymeric matrix surrounding the biofilm serves to protect the biofilm community of bacteria from the hostile environment in the host. *Pseudomonas aeruginosa* is a biofilm-producing, opportunistic pathogen critical to the pathogenesis of chronic lung diseases, including cystic fibrosis (CF). Little is known about the mechanism by which *P. aeruginosa* establishes biofilms in the host, but it has been reported that CF patients show a reduced ability to clear *P. aeruginosa* acquired during respiratory viral infections and 85% of new pseudomonal colonization in CF patients followed a respiratory viral infection within 3 weeks. Furthermore, seasonal trends in influenza A and respiratory syncytial virus (RSV) infections significantly correlate with the acquisition of the first *P. aeruginosa* culture, as well as chronic infection with *P. aeruginosa*, in CF patients. We hypothesized that virus co-infection stimulates biofilm growth and antibiotic resistance in *P. aeruginosa*. Using live-cell imaging, as well as plate-based approaches to grow *P. aeruginosa* biofilms on human airway epithelial cells, we demonstrated a dramatic increase in *P. aeruginosa* biofilm growth on polarized human airway cells in the presence of respiratory virus co-infection. The common CF viral pathogens, RSV, human rhinovirus-14 (hRV) and adenovirus (AdV) dramatically increased *P. aeruginosa* biofilm growth on airway cells, 4.6+/-0.65, 3.4+/-1.2 and 4.4+/-1.1 fold over control, respectively. To explore the mechanism and potential clinical consequences of this enhanced bacterial biofilm growth in the presence of respiratory virus co-infection, we examined the antibiotic resistance of the virus-stimulated bacterial biofilms. Notably, in the presence of RSV, hRV or AdV co-infection, *P. aeruginosa* more rapidly acquired antibiotic resistance to tobramycin and ciprofloxacin, two frontline antibiotics used to treat *P. aeruginosa* infections in CF patients. The enhanced antibiotic resistance of *P. aeruginosa* in the presence of virus co-infection corresponded to increased expression of the antibiotic resistance genes *mexA*, *mexX* and *ampC*, thus providing a potential mechanism for the enhanced antibiotic resistance to the aminoglycoside and quinolone classes of antibiotics. In summary, this study reports that respiratory virus co-infection promotes the biofilm growth and antibiotic resistance of *P. aeruginosa* and provides important implications in the acquisition of chronic *P. aeruginosa* infection in CF patients, a critical window in the pathogenesis of CF lung disease.

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ALX-009 POTENTIATES THE EFFECT OF TOBRAMYCIN AT KILLING PSEUDOMONAS AERUGINOSA BIOFILMS ON HUMAN AIRWAY CELLS

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Background: In CF airways, infections are typically dominated by *Pseudomonas aeruginosa*. Permanent eradication of *P. aeruginosa* in CF airways is impossible because, as current evidence suggests, *P. aeruginosa* forms antibiotic-resistant biofilms in the CF lung. Iron has been shown to promote *P. aeruginosa* biofilm formation on airway cells and, for reasons that are still largely unknown, the iron concentration in the airway surface liquid (ASL) of CF lung is 400-times higher than in a non-CF lung. Chelating iron may be a promising new therapy to eliminate *P. aeruginosa* biofilm formation on CF airway epithelial cells. Here, we investigate whether *P. aeruginosa* biofilms would become more susceptible to the action of inhaled tobramycin in the presence of ALX-009, a newly designated orphan drug containing lactoferrin, an iron-binding glycoprotein with bactericidal activity and hypothiocyanite (OSCN⁻).

Methods: *P. aeruginosa* biofilms were grown at the apical surface of human airway epithelial cells overexpressing ΔF508-CFTR, using a co-culture model we previously described. ALX-009 was prepared according to Alaxia's recommendation. At the end of each treatment, viable bacteria remaining attached to airway cells were determined by serial dilutions and spot titer to determine the colony-forming units (CFU) per mL. Data were log transformed and analyzed by one-way ANOVA and Bonferroni test. A p value of 0.05 or less was considered significant.

Results: ALX-009 alone had no significant effect on established biofilms formed by PA01 whereas tobramycin alone reduced established PA01 biofilms by 4 log units. ALX-009 and tobramycin together decreased established PA01 biofilms by 7 log units, an effect significantly larger than tobramycin alone (p<0.05). The efficacy of ALX-009 and tobramycin together was tested on clinical *P. aeruginosa* strains isolated from the sputum of CF patients. Whereas the effects of tobramycin and ALX-009 were not additive in disrupting biofilms formed by nonmucoid isolates (n=3), the combination of tobramycin and ALX-009 was additive for mucoid clinical isolates (n=3), reducing the CFUs of established biofilms by 2.5 to 3 log units, compared with ~1 log unit for each compound alone (p<0.05).

Conclusion: Inhalation therapy combining tobramycin with ALX-009, newly designated an orphan drug by the FDA and the European Medicines Agency, may be beneficial to a subset of CF patients by decreasing the airway bacterial burden in CF patients infected with *P. aeruginosa*. Phase I clinical trials are planned.

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TREATMENT WITH ALX-009 IN COMBINATION WITH AZTREONAM POTENTIATES THE KILLING OF PSEUDOMONAS AERUGINOSA BIOFILMS ON HUMAN CF AIRWAY CELLS

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Background: CF patients are plagued by recurrent airway infections dominated by *Pseudomonas aeruginosa* that result in a decline in respiratory function. In the CF airway, *P. aeruginosa* forms biofilms that are resistant to current antibiotic therapy. Inhaled aztreonam, an antipseudomonal antibiotic approved in 2010, reduces *P. aeruginosa* density in the sputum of CF patients, but does not eliminate infections. Recently, others and we have shown that biofilm formation by *P. aeruginosa* is enhanced by excess iron in the CF airway, and that a combination of tobramycin and an iron chelator dramatically reduces *P. aeruginosa* biofilms on human CF airway cells. Thus, in the present study we tested the ability of a newly designated orphan drug, ALX-009, to eliminate *P. aeruginosa* infections, either alone or in combination with aztreonam. ALX-009 is composed of lactoferrin, an iron-binding glycoprotein with bactericidal activity, and of hypothiocyanite (OSCN⁻). Both lactoferrin and OSCN⁻ are part of the normal innate immune response but their secretion by airway cells is reduced in CF.

Methods: *P. aeruginosa* (PA01) planktonic growth was assessed by measuring OD₆₀₀. *P. aeruginosa* biofilms were grown in vitro at the apical surface of human CF airway epithelial cells. Studies were conducted to determine if ALX-009 and aztreonam, alone or in combination, prevented biofilm production or disrupted biofilms on CF airway cells. The colony forming units (CFU) per mL were determined by serial dilutions and spot titer. Data were log transformed and analyzed by one-way ANOVA and Bonferroni test. A p value of 0.05 or less was considered significant. ALX-009 was prepared according to the recommendation of Alaxia Biotechnologies (Lyon, France).

Results: ALX-009 alone extended the lag phase of planktonic *P. aeruginosa* by ~4 h, delaying entry into stationary phase by 3-4 h compared with untreated control. Aztreonam alone did not affect the initial growth of planktonic *P. aeruginosa* for the first 3 h, after which a steady growth decline was observed. Interestingly, combining aztreonam and ALX-009 completely blocked planktonic growth of *P. aeruginosa*. ALX-009 alone and aztreonam alone impaired *P. aeruginosa* biofilm formation on CF airway cells by 1 and 3 log units, respectively. Aztreonam and ALX-009 together reduced *P. aeruginosa* biofilm formation on human airway cells by an additional 1 log unit compared with aztreonam alone. The combination of ALX-009 and aztreonam reduced mature biofilms established on airway cells by 4 log units, compared with 0 and 3 log units for each compound alone.

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Conclusion: Because a combination of aztreonam and ALX-009, newly designated as an orphan drug by the FDA and the European Medicines Agency, completely inhibits the growth of planktonic *P. aeruginosa*, reduces *P. aeruginosa* biofilm formation and disrupts established *P. aeruginosa* biofilms on CF airway cells, this combination of drugs may be an effective treatment to significantly reduce the burden of *P. aeruginosa* in CF patients.

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RATIONAL DESIGN OF A *PSEUDOMONAS AERUGINOSA* BACTERIOPHAGE THERAPEUTIC AND ITS EFFICACY IN A MURINE LUNG INFECTION MODEL

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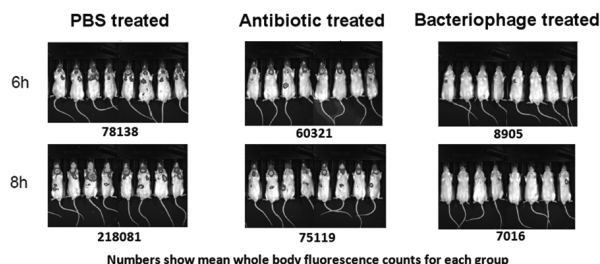
Background: There is real need for novel therapies to treat bacterial lung infections in cystic fibrosis patients, especially those caused by *Pseudomonas aeruginosa*. Bacteriophages targeted at *P. aeruginosa* have potential as alternatives or adjuncts to conventional antibiotics. This work shows efficacy with a rationally designed bacteriophage mix in a murine *P. aeruginosa* lung infection model.

Methods: Lytic bacteriophages with efficacy against *P. aeruginosa* strain PAK were assayed in liquid cultures of host bacteria, addressing both cross-resistance and apparent antagonism between specific bacteriophages in the development of an optimised therapeutic mixture. Three selected bacteriophages were mixed and used in an in vivo study where infection was established using a luminescent strain of PAK (PAK-lumi). Four groups of eight BALB/C mice were infected intranasally with PAK-lumi and treated 2h later as follows: 1) untreated euthanized (CFU baseline); 2) PBS treated; 3) intra-peritoneal ciprofloxacin treated; 4) intranasal phage treated. Mice were observed for clinical signs and luminescence measured using an in vivo imaging system. At 24h animals in groups 2, 3 and 4 were euthanized and lung CFU / PFU determined.

Results: The bacteriophage mix showed potent activity and no resistance in vitro at 24h. In vivo, phage treated mice showed a marked decrease in luminescence after 6h with greater reduction overall compared with the ciprofloxacin group. This was particularly notable in the nasopharyngeal area, although reductions were also seen in luminescence within the abdominal area. Luminescence in the lungs was broadly comparable, but was markedly reduced with both ciprofloxacin and the bacteriophage mixture. By 24h all phage and antibiotic treated mice survived with ~3 log reduction in lung CFU observed for both groups.

Conclusion: An optimised selection process generated a bacteriophage mixture which was shown to be a highly effective treatment in a *P. aeruginosa* murine nasal/lung infection model, with efficacy comparable or superior to ciprofloxacin in this model.

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IS FLUCLOXACILLIN ANTIBIOTIC PROPHYLAXIS ASSOCIATED WITH DIFFERENTIAL RATES OF INFECTION WITH *STAPHYLOCOCCUS AUREUS* OR *PSEUDOMONAS AERUGINOSA* IN YOUNG CHILDREN?

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Introduction: The use of antibiotic prophylaxis to prevent *Staphylococcus aureus* infection varies between the UK and the USA. Despite UK CF Trust working group recommendations and a recent Cochrane review supporting the use of *S. aureus* prophylaxis, concern regarding the possibility of earlier or more frequent *Pseudomonas aeruginosa* infections prompted the Cystic Fibrosis Foundation to refrain from recommending its use. Our objective was to determine the association of antibiotic prophylaxis for *S. aureus* with positive and negative outcome for patients with CF, in terms of microbiological efficacy (*S. aureus* isolation) and complications (*P. aeruginosa* isolation).

Method: We conducted a longitudinal observational study using data from the UK Cystic Fibrosis Trust Registry (2000-2009) providing entries for 11673 people. Those younger than 4 years (1500 days) at first entry to the registry were eligible. Patients who had variable antibiotic use (over the first three entries), had positive respiratory cultures for *S. aureus* or *P. aeruginosa* or were receiving inhaled tobramycin or inhaled colistin at first entry were excluded. We completed a Cox proportional hazards survival analysis comparing the age at first acquisition of *S. aureus* and *P. aeruginosa* between patients receiving flucloxacillin prophylaxis and those not receiving prophylaxis. Participants entered into the risk set at the age of first registry data entry.

Results: Data were available for 2363 children aged younger than 1500 days at entry to the registry. After excluding those who, at entry to the registry, were receiving inhaled tobramycin or colistin and after those isolating *S. aureus* or *P. aeruginosa* at entry to the registry were excluded, the final analysis consisted of 484 children (262 male) for whom stable antibiotic use could be identified. Median follow up was 2.13 years with 1036 person-years at risk in total. There were no statistically significant differences in baseline characteristics (age at registry entry, gender, BMI z-score) for those receiving flucloxacillin or no prophylaxis and for those included or excluded. In a model including gender, age at first entry to the registry and DNase use, flucloxacillin use was not associated with a reduction in the rate of first acquisition of *S. aureus* (hazard ratio (SE) 1.16 (0.32) 95% CI 0.67, 1.99, p=0.62). There was also no difference in the number of positive cultures of *S. aureus* over time (p=0.29). However, flucloxacillin use was associated with a significant increased hazard of first isolation of *P. aeruginosa* (HR 2.39 (0.51) 95% CI 1.57, 3.64, p<0.001) and more positive cultures of *P. aeruginosa* over time (p<0.001).

Conclusion: In a UK registry study over 10 years, the use of flucloxacillin did not appear to be associated with a reduction in the risk of first acquisition of *S. aureus*, but was associated with an increased risk of first isolation of *P. aeruginosa*, compared to no antibiotic prophylaxis use.

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EFFECT OF MANNITOL UPON BIOFILM FORMATION OF *PSEUDOMONAS AERUGINOSA* ON AN ABIOTIC SURFACE

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Introduction: Chronic *P. aeruginosa* infection is responsible for considerable morbidity and mortality in patients with cystic fibrosis and is mediated, in part, by the biofilm mode of growth. Biofilms confer protection from the host immune system and antibiotic resistance. Mannitol has been shown to affect the behaviour of other Gram negative bacteria and so we were interested in the effect upon *P. aeruginosa*.

Method: The effect of mannitol upon bacterial growth was examined in planktonic culture (LB) using an automated plate reader (TECAN). Concentrations of mannitol considered achievable within the lung (50-200mg/mL) were used. Bacterial attachment and biofilm formation was examined using steel coupons, microtitre well slides and microfluidic flow chambers (Fluxion, US). PAO1 expressing gfp growing in biofilm media (10-20% LB v/v) and nutrient limited media (0.05%NB2 v/v) and imaged using a confocal laser microscope (Zeiss) were used. Swarming was also investigated using standard methods.

Results: In nutrient rich media (100% LB) planktonic growth decreased in response to supplemented mannitol in a concentration-dependent manner (peak (SE) OD: 50mg/mL 0.68 (0.078); 200mg/mL: 0.13(0.02)). Bacterial attachment to steel coupons in nutrient limited media (NB2) (surface coverage relative to control) was increased at the lowest level of supplementation (50mg/mL mannitol – 2.11(0.16)) and inversely proportionately biofilm coverage reduces with incremental mannitol supplementation. A similar pattern of biofilm formation was seen in microtitre well slides with NB2 media. Using a relatively richer nutrient media (10-20% LB) in flow chambers, biofilm formation was increased in response to mannitol supplementation in a positive dose response (control biofilm volume (SE): 793104µm³ (66685); 150mg/mL: 1185456µm³ (41781)). In media containing an optimal sugar source, mannitol does not affect swarming. In media with mannitol as the only sugar source swarming is increased in the presence of mannitol, but to levels lower than those with an optimal sugar source.

Conclusion: In *in vitro* models, bacterial attachment and biofilm formation are increased in the presence of exogenously added mannitol. The pattern of this increase appears to be dependent upon the growth media used.

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SAFETY AND PHARMACOKINETICS OF INHALED VANCOMYCIN IN INDIVIDUALS WITH CYSTIC FIBROSIS

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important emerging CF pathogen. The prevalence of MRSA has quadrupled over the last ten years and is now detected in 25% of US CF patients. MRSA is also associated with a more rapid decline in lung function and decreased survival. One potential therapeutic option for MRSA is inhaled vancomycin, which is being used more frequently in clinical practice but has not been formally studied in CF.

Objective: The primary purpose of this study was to evaluate the sputum pharmacokinetics and safety of inhaled vancomycin (ClinicalTrials.gov Identifier: NCT01509339).

Methods: Single center, open-label, pilot study evaluating a single dose of vancomycin for inhalation (250mg reconstituted in 5cc of sterile water) in 10 adult CF patients. Sputum vancomycin levels were obtained at baseline, 5 minutes, 1, 2, and 6 hours post-inhalation. Pharmacokinetic measurements included maximum and time to peak concentration and area under the curve. Safety outcomes included change in: FEV₁(L) from baseline to 30 minutes post-inhalation, symptoms from baseline to 15 minutes and 4 hours post-administration, sputum cell count/eosinophil count from baseline to 6 hours post-inhalation, peak serum vancomycin level, development of hypoxia, and adverse events.

Results: Three men and 7 women were enrolled, and all 10 tolerated the entire dose and were able to provide sputum at every time point. Mean age of the participants was 36.3 years (range: 27-58 years). Mean baseline FEV₁(L) of the participants was 1.74L (range: 1.17L-2.94L). Mean baseline FEV₁% predicted was 51.2% (range: 40%-74%). 30 minutes after inhaling vancomycin, the mean change in FEV₁(L) was -3.7% (range: -9.9% to +13.7%). Fifteen minutes after inhaling vancomycin, the most commonly reported symptom was increased cough in 6 participants. Nine of 10 patients reported a bitter taste with inhalation. One adverse event of moderate chest pain after coughing was possibly related to study drug and resolved spontaneously. No participant had a 6 hr post-inhalational eosinophil count that increased by more than 10%. Sputum and serum levels of vancomycin are currently being measured and will be presented.

Conclusions: This analysis of 10 adults with CF suggests that a single dose of inhaled vancomycin, 250mg reconstituted in 5cc of sterile water, is well tolerated and safe. Thirty minutes after inhaling vancomycin, all patients achieved at least 90% of their baseline lung function. The increase in cough is similar to reports from previous inhalation therapies. Vancomycin does have a bitter taste and therefore it will be important to mask placebo medications in future trials. These results prepare for future studies of inhaled vancomycin in CF by clarifying safety and pharmacokinetics. Supported by Harry Shwachman Clinical Investigator Award DASENB10A0.

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IS SEROLOGY A USEFUL TOOL FOR PREDICTING INITIAL PSEUDOMONAS RESPIRATORY INFECTION IN CHILDREN WITH CYSTIC FIBROSIS?

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Background: In young CF patients, monitoring for acquisition of *Pseudomonas aeruginosa* (Pa) is typically by oropharyngeal (OP) cultures which are known to have limited sensitivity for lower airway infection. Pa infection undetected by OP culture may place CF individuals at higher risk for subsequent Pa infection. Pa serology could play an important role in clinical care by identifying individuals at low or high risk of developing Pa infection in the future. However, the diagnostic value of Pa serology in this setting is unclear.

Objectives: Among young CF patients from whom Pa has not previously been isolated from an OP culture, to evaluate the sensitivity and specificity of Pa serology in predicting first isolation of Pa from an OP culture in the subsequent year.

Methods: The study cohort included all participants in the U.S. EPIC Observational Study who had no prior Pa-positive respiratory cultures and at least one annual serum sample. Serum IgG antibody titers were evaluated against alkaline protease, elastase, and exotoxin A by semi-quantitative ELISA (Mediagnost, Reutlingen, Germany). Receiver operating characteristic (ROC) curve analysis was used to optimize cut-off titers separately for ages 1 to 6 years and >6 years. Sensitivity and specificity of the ELISA for predicting first Pa positive OP culture in the 6 or 12 months after the serum sample was obtained were estimated according to the calculated cut-off titers.

Results: The cohort consisted of 582 subjects with 2084 serum samples; 261 (44.8%) had initial isolation of Pa from a respiratory culture and 321 (55.2%) remained Pa culture-negative during a mean 44 (SD 19) months of follow up. Area under the ROC curve for initial Pa positive culture in the 12 months following serologic measurement was 0.45 (95% CI 0.40, 0.50) for alkaline protease, 0.56 (95% CI 0.52, 0.61) for exotoxin A, and 0.52 (95% CI 0.48, 0.57) for elastase. Among the 261 OP Pa positive patients, 46.7% had a positive antibody titre against at least one antigen in the previous 12 months, while among the 321 OP Pa negative patients, 34.9% had a positive antibody titre against at least one antigen in the previous 12 months (p=0.005).

Conclusions: In this large cohort of young CF patients with no prior isolation of Pa from a respiratory culture, baseline Pa serology (alkaline protease, exotoxin A or elastase) was positive in more than a third, supporting previous studies suggesting that diagnosis of Pa respiratory infection based on respiratory cultures alone may not be reliable and that serology may be a valuable adjunctive diagnostic tool. In patients with positive serology, a Pa-positive OP culture was significantly more likely in the ensuing 12 months than in those with negative Pa serology. Pa serology may therefore help to define CF patients at higher risk of developing Pa infection.

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LONGITUDINAL ANALYSIS OF THE INTRACOLONIAL DIVERSITY WITHIN *PSEUDOMONAS AERUGINOSA* POPULATIONS DURING CHRONIC CF LUNG INFECTIONS

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Patients with CF suffer from chronic lower respiratory tract infections caused by microbes that are often recalcitrant to clinically prescribed therapies. Previous reports suggest that during exacerbation, the sputum densities of dominant CF pathogens such as *Pseudomonas aeruginosa* (*P. aeruginosa*) remain relatively unchanged over time, resulting from genetic adaptation and microevolution of the initial infecting strains. The potential clinical impacts of this phenomenon remain poorly studied in chronic infections. To examine correlations between intracolony diversity within *P. aeruginosa* populations in the CF lung, adaptive resistance and the specificity of targeted treatments, we have characterized the morphologic, phenotypic and genotypic attributes of sequential bacterial isolates from a CF patient chronically colonized by *P. aeruginosa*. A total of 400 clones were recovered from 11 expectorated sputum samples produced by a single female patient attending the Adult CF Clinic at St. Michael's Hospital (Toronto, ON) between Nov. 2010 and Nov. 2011. Colonies were classified into morphotypes by visual inspection on LB and Columbia + 5% Sheep's Blood agar and screened for phenotypic traits including biofilm formation, motility, antimicrobial susceptibility and pyocyanin overproduction, using standard protocols. Genomes from at least 28 of these clones were sequenced by standard paired-end Illumina sequencing and the reads assembled with the Burrows Wheeler Assembler, a reference-mapping tool. The genome of the PAO1 strain was used as the reference and single nucleotide polymorphisms were identified with SAMTOOLS. Variable regions were identified and characterized with CLC Genomics and in-house JAVA programs. Distinct colony morphologies were documented on a per-sample basis, with up to 3 confirmed mucoid *P. aeruginosa* variants present within a single sample. Sequential morphotype-specific isolates were found to differ in all tested phenotypes, particularly in both swimming and twitching motilities when incubated at either 30°C or 37°C. Furthermore, preliminary inspection of the genomic content of the first sequenced isolates revealed dynamic changes in the genome of *P. aeruginosa* as well as common genetic signatures across all samples. We present an in-depth, longitudinal analysis of adaptive radiation within *P. aeruginosa* populations in the CF lung. Similar to recent studies, we suggest that current methods of susceptibility profiling may underestimate the true resistance potential of CF pathogen populations, leading to possible treatment failure.

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PREVALENCE AND CLINICAL SIGNIFICANCE OF *S. AUREUS* SMALL-COLONY VARIANTS (SA SCVS) IN CHILDREN WITH CF: A COHORT STUDY

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Background: SA SCVs emerge frequently during many chronic infections, including in CF airways. These slow-growing variants, which are frequently missed by clinical laboratories, can be selected *in vitro* by exposure to certain antibiotics and by growth with *P. aeruginosa*. Different SCV types are selected by these different conditions, resulting in different predicted antibiotic susceptibilities. Exactly how SA SCVs are selected during CF infections, their associations with clinical outcomes, and how best to treat them are poorly understood.

Methods: We performed a two-year longitudinal study of 100 children with CF ages 0-18y. Respiratory samples were cultured using techniques we developed to maximize SCV detection. *S. aureus* colonies were classified as SCVs by colony size using standard criteria and typed biochemically. Clonal relationships between isolates within subjects were established using pulsed-field gel electrophoresis. Sputum samples were collected when possible and analyzed for *S. aureus* by species-specific qPCR. Patients maintained logs of all antibiotic use; exacerbations were defined as new inpatient or outpatient antibiotics for respiratory symptoms. Relationships between microbiological characteristics and clinical features of source patients were evaluated.

Results: SA SCVs were found among 24% of children with CF during the study, similar to the prevalence of MRSA (26%). Optimized culture was as sensitive as PCR for *S. aureus*. SCVs were found significantly more frequently among subjects treated with trimethoprim-sulfamethoxazole (TMP-SMX) in the prior quarter, and 94% of the SA SCVs were thymidine auxotrophic, suggesting they had been selected at least in part by TMP-SMX treatment. In contrast, 8% were hemin auxotrophic, suggestive of selection by aminoglycosides. Furthermore, both SCV types were relatively resistant to growth inhibition by *P. aeruginosa*, suggesting that coinfection could either select or stabilize SA SCVs *in vivo*. SCVs were nearly always clonally related to wild-type isolates from the same subjects, indicating that they were generally selected *in vivo*. Analyses related to exacerbation occurrence and decline in FEV₁ during the study among subjects with and without SA SCVs are ongoing.

Conclusions: SA SCVs were detected frequently among children with CF using optimized culture techniques, which were at least as sensitive as qPCR. In our center, as in previous studies from Europe, CF SCVs appear to be frequently selected by treatment with TMP-SMX, indicating that this drug is likely not effective for SCV treatment. Genetic typing data indicate that SA SCVs are selected *in vivo* during chronic CF infection. Results related to respiratory exacerbations and lung function decline among children with SCVs in the study compared with children with *P. aeruginosa* or only normal *S. aureus* will be presented. We are also currently evaluating the optimal treatment for SA SCVs.

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THE MICROBIOME AND VIROME OF CF

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Metagenomic and metatranscriptomic approaches have been widely used to characterize the taxonomical and functional properties of the micro-

bial and viral communities in many environments. However, samples collected from the airways of CF patients often contain large amounts of host-derived nucleic acids that interfere with recovery and purification of microbial and viral nucleic acids. The purpose of this study was to establish a comprehensive protocol that addresses these technical challenges, and validate the methods using fresh expectorated CF sputum collected from adult CF patients at different health status.

Contaminating host material was reduced from 98% to less than 5% in the viral metagenomes, and from more than 99% to as little as 13% in the microbial metagenomes. Comparison of commercially available kits showed that the Ribo-Zero rRNA removal kit (Epidemiology) was the most effective in removing both microbial and host rRNA from the metatranscriptomes.

The data revealed unique microbiomes from individual CF patients and identified viral communities that were dominated by phages that infect major CF pathogens. In some cases, *Pseudomonas aeruginosa* was replaced by other opportunistic bacteria such as *Pseudomonas fluorescens* and *Rothia* spp. *Rothia mucilaginosa* was found in more than 80% of the eighteen microbiome examined. Its prevalence in CF patients is much higher than previously reported and is almost equivalent to *P. aeruginosa* (89%). In addition, this study presents a near complete genome of *R. mucilaginosa* reconstructed from the metagenomic reads of a single microbiome. The genome has allowed in-depth functional analysis.

The draft genome revealed highly adaptable physiological and metabolic properties in this organism, including the acquisition of a gene coding for an alternative L-lactate dehydrogenase. The immune systems against foreign DNA and extracellular stress include phage lysis, rearrangement hotspot (rhs) elements, CRISPRs, and multiple antibiotic resistance mechanisms. High and constant doses of antibiotic usage in CF patients have facilitated the selection of antibiotic resistant strains. The prevalence and pathogenicity of *R. mucilaginosa* in CF lungs can be significant and therefore, routine screening should be implemented to monitor its transmission.

Sequencing of genomic DNA provides taxonomic and functional properties of microbial and viral communities. The metatranscriptomic approach allowed the monitoring of actual microbial activities and their changes during disease progression and in response to perturbations such as antibiotic treatments.

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THE ROLE OF SECOND-GENERATION SEQUENCING TO CHARACTERIZE THE FUNGAL MICROBIOTA IN THE ADULT CYSTIC FIBROSIS AIRWAY, AND ITS CORRELATION WITH STANDARD CULTURE-BASED METHODS AND CLINICAL PHENOTYPE

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Background: The CF airway is predisposed to colonisation and infection with a broad spectrum of organisms, including filamentous fungi. Studies to date reveal a wide variability (range 16-56%) in the prevalence of fungus in the CF airway using traditional culture-based methods. Advances in culture-independent methods allow a more extensive characterisation of the fungal community of the CF lung. The aim of this study is to profile the fungal microbiota of the stable CF airway using a high-throughput sequencing technique, and to correlate this with standard culture-based methods and clinical phenotype.

Methodology: Fifty-five clinically stable adult CF patients prospectively recruited from the Cork Adult CF Centre donated one or more sputum samples. Culture-based methods using fungus-specific growth plates were employed at time of sampling. Fungal DNA was extracted from all samples. High-throughput bar-coded sequencing targeting the internal transcribed spacer (ITS) and small sub-unit (SSU) regions was used to profile the fungal microbiota of all sputum samples. ITS and SSU amplicons were subse-

quently sequenced on a 454 Genome Sequencer FLX platform (The Genome Analysis Centre, UK). Baseline FEV1% predicted, genotype, gender, BMI and *Pseudomonas* status, (as per Leeds guidelines) were recorded by retrospective review of medical notes.

Results: Fifty-five clinically stable adult CF patients were recruited. Forty-one patients donated a single sputum sample at time of recruitment, 9 patients donated a second sample at a later date, with 8 patients subsequently donating a third sample, yielding a total of 83 samples. Culture-based methods detected fungus (*Aspergillus* spp. and *Candida* spp. only) in 13 of the 55 patients. High-throughput bar-coded sequencing identified rich fungal communities in greater than 90% of the patient sputum samples, with over 82% of the species found not detected by culture. Fungi detected included species such as *C. albicans*, *C. dubliniensis*, *Saccharomyces cerevisiae*, *Malassezia* spp., *Fusarium culmorum*, *Acremonium strictum*, *Thanatephorus cucumeris* and *Cladosporium* spp. A preliminary comparison of patient status with diversity and species richness of fungal microbiota identified that lower fungal diversity associates with decreased lung function.

Conclusion: We describe the use of high-throughput sequencing to characterize the fungal microbiota in the largest population of adult patients with CF to date. DNA analysis reveals increased species diversity and richness compared with standard culture-based methods. Preliminary analysis suggests that the level of fungal diversity may contribute to, or be associated with, the variability in clinical phenotype seen in patients with CF.

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SERIAL ANALYSIS OF THE INTERACTION BETWEEN GUT AND LUNG MICROBIOME IN CF IN INFANCY

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Objective: To examine respiratory and intestinal microbiota development in infants with CF from birth to 18 months, their potential interactions, and to assess the impact of dietary factors on the developing intestinal and respiratory microflora. The ultimate aim is to inform treatment strategies to ameliorate colonization with pathogens.

Design/Methods: Prospective longitudinal study of infants with CF from birth to 18 months. Respiratory and gut samples were collected at <1 month followed by 3-month intervals. DNA was extracted followed by 454 pyrosequencing.

Results: Fifty-nine samples from 7 infants with CF were analyzed. Distinct bacteria dominated in the gut compared to lung, yet some bacteria overlapped demonstrating a core microbiota dominated by *Veillonella* and *Streptococci* (p<0.05). At both sites, bacterial diversity increased over time (p<0.05), with evidence of more rapidly acquired diversity in the lungs. A pairwise comparison of intra-class correlation coefficients for all 77 bacterial genera between the lungs and gut revealed greater (p=0.02) inter-subject variability in the gut. Interestingly there was a high degree of concordance between the bacteria that were increasing or decreasing over time in both systems: in particular, 13 of 16 genera that were increasing in the gut were also increasing in the lung (p<0.001). There is evidence that in 8 genera of bacteria, gut colonization presages microbial populations in the lung. Clustering analysis of respiratory samples indicated profiles of bacteria that were associated with breastfeeding (p = 0.08), and for gut samples, introduction of solid foods (p = 0.01) even after adjustment for time.

Conclusions: The developing microbiota of the enteral and respiratory tracts in infants with CF are distinct with an overlapping core, and differentially increase in diversity over the first years of life. There is concordance between the genera in both gut and lung, and a large number of genera that

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are present in the gut prior to colonization of the lung. Furthermore, changes in diet also result in altered respiratory microflora, suggesting a link between nutrition and development of microbial communities in the lung. Our findings suggest that nutritional factors and gut colonization patterns are determinants of the microbial development in the lung in infancy and present opportunities for early intervention in CF.

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MULTICENTER COMPARISON OF THREE TYPING METHODS FOR *PSEUDOMONAS AERUGINOSA* ISOLATES FROM PATIENTS WITH CYSTIC FIBROSIS

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Background: Epidemic *Pseudomonas aeruginosa* strains associated with significantly increased morbidity and mortality have been reported in the cystic fibrosis (CF) population. The optimal molecular typing methodology for assessing the epidemiology of *P. aeruginosa* isolates from CF patients is not yet known.

Methods: This was a multicenter comparison of three typing methods (pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) and multilocus sequence typing (MLST)) for a blinded sample of 48 well characterized CF *P. aeruginosa* isolates. The discriminatory power and congruence between methods were compared using the Simpson's index, Rand index and Wallace coefficient.

Results: PFGE and MLST had the greatest congruence with the highest Rand index (0.697) while PFGE and RAPD had the least congruence with the lowest Rand index (0.418) (Table 1). PFGE had the greatest discriminatory power with the highest Simpson index (0.980) and MLST had the lowest (0.973), although 95% confidence intervals overlapped for all methods. MLST had the greatest predictive value (100%) in labeling strains as unique while PFGE had the greatest predictive value (100%) in labeling strains as clonal.

Conclusions: This study demonstrated the highest level of concordance between PFGE and MLST in genotyping *P. aeruginosa* isolates from CF patients. MLST was the best method for predicting unique strains and has the potential to be a cost efficient, high throughput first-pass typing method. As PFGE had the highest discriminatory power, it would likely remain the gold standard for confirming clonality among strains.

Table 1. Comparison of three typing methods

Method	No. of types	Simpson's index (95% CI)	Adjusted Rand index			Wallace coefficient		
			PFGE	RAPD	MLST	PFGE	RAPD	MLST
PFGE	31	0.980 (0.967-0.992)	1.000	0.418	0.697	1.000	0.478	0.826
RAPD	31	0.975 (0.960-0.990)		1.000	0.530		1.000	0.571
MLST	27	0.973 (0.954-0.992)			1.000			1.000

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IS CHRONIC *STENOTROPHOMONAS MALTOPHILIA* INFECTION ASSOCIATED WITH MORTALITY OR LUNG TRANSPLANTATION IN CYSTIC FIBROSIS PATIENTS?

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Background: Chronic *Stenotrophomonas maltophilia* infection is an independent risk factor for severe pulmonary exacerbations in cystic fibrosis (CF) patients. The goal of this study was to determine the effect of chronic *S. maltophilia* infection on mortality and the need for lung transplantation in a longitudinal study of children and adults with CF.

Methods: This was a cohort study of CF patients from the Hospital for Sick Children and St Michael's Hospital (Toronto, Canada) from 1997 to 2008. A Cox regression model was used to estimate the hazard ratio (HR) to time of death or lung transplantation adjusting for age, gender, genotype, pancreatic status, CF related diabetes (CFRD), forced expiratory volume in 1 second (FEV₁), body mass index, number of pulmonary exacerbations, *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex and chronic *S. maltophilia* infection (≥ 2 positive cultures /yr).

Results: A total of 709 patients were followed over the 12 year study period; 101 patients underwent a lung transplantation (of which 29 died) and an additional 58 patients died (total 159 events). The Kaplan-Meier curve (Figure 1) demonstrated that patients who ever had chronic *S. maltophilia* infection were more likely to die or undergo a lung transplantation than patients who were never infected with *S. maltophilia* ($p < 0.0001$). However, in the Cox regression model, the risk factors for time to death or lung transplantation were lower FEV₁ (HR 1.09, 95% CI 1.07-1.10), homozygous $\Delta F508$ (HR 1.63, 95% CI 1.15-2.31), greater annual number of pulmonary exacerbations (HR 1.21, 95% CI 1.03-1.42) and CFRD (HR 2.25, 95% CI 1.49-3.41). Chronic *S. maltophilia* infection was not a significant risk factor (HR 1.02, 95% CI 0.81-1.30) after adjusting for these other variables.

Conclusions: Although CF patients with chronic *S. maltophilia* infection were more likely to die or undergo lung transplantation than patients who were never infected with *S. maltophilia*, in a multivariate model the independent risk factors were other markers of disease severity such as lung function.

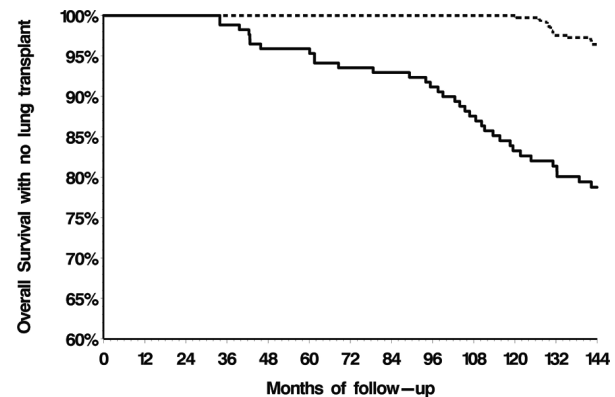


Figure 1. Kaplan-Meier curve of survival with no lung transplant of patients with chronic *S. maltophilia* (solid line) vs never *S. maltophilia* (broken line) infection. Log rank p value < 0.0001 .

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DENTAL POCKETS AS A SOURCE FOR CF LUNG INFECTION

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All CF patients suffer from chronic lung infections caused by a large variety of bacteria. These bacteria may be derived from different environmental sources as well as from the human body itself. Here we investigated if dental pockets may serve as an origin of CF lung infection, and if identical bacteria are present in the oral cavities and the lungs.

We determined in 26 adults and 20 children with CF and a healthy control group of 30 adults and 9 children aerobic and anaerobic bacteria in samples derived from sputum (if available) and dental pockets (up to 4 samples/person). Dental pockets were explored using sterile paper tips. Bacteria in sputum and/or dental pocket smears were identified using the Crystal® (Becton Dickinson, Heidelberg, Germany), the Vitek® (BioMérieux) and the RapID™ ANAII® (Remel, Lenexa, KS, USA) biochemical identification systems. Antibiotic resistance patterns for colistin, azithromycin, cef-tazidime, piperacillin/tazobactam, meropenem, clindamycin and metronidazole were determined using Etest® (AB Biodisk, Solna, Sweden).

The dental pocket microbiome of CF adults and children did not differ from the healthy controls already at an age of 18 months. In 20 out of the 24 adults producing sputum (83.3%) pairs of identical aerobic species and in 17 of them (70.8%) pairs of the same anaerobic strains were found simultaneously as well in sputum as dental pockets. Resistance patterns for all seven antibiotics were identical in at least one aerobic pair in 11 out of 20 patients (55.0%) and at least one anaerobic pair in 10 out of 17 patients (58.8%). In 5 out of 6 CF children with sputum production (83.3%) an identical anaerobic strain could be identified.

Dental pockets of CF patients and healthy controls are contaminated with an abundance of aerobic and anaerobic bacteria. Sputum samples and dental pockets of most CF patients simultaneously are contaminated with the same aerobic and anaerobic species. In most patients these strains do not differ in terms of antibiotic susceptibility between the lungs and the oral cavity. Thus, dental pockets may well serve as sources for CF lung infection.

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BABY BOTTLE STEAM STERILIZERS DISINFECT HOME NEBULIZERS INOCULATED WITH COMMON BACTERIAL RESPIRATORY PATHOGENS

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Background: Contaminated nebulizers are a potential source of infection of the respiratory tract of CF patients. Different methods, including steam-sterilization, have been proposed to disinfect home nebulizers but no single method is universally accepted. We hypothesized that baby-bottle steam sterilizers would be an effective and user-friendly method to disinfect home nebulizers.

Methods: We inoculated three different home nebulizers (Pari LC Plus, eFlow Rapid and Altera), at three different locations with the common CF respiratory pathogens methicillin resistant *Staphylococcus aureus* (ATCC 43300), *Burkholderia cepaciae* (ATCC 25416), *Haemophilus influenzae* (ATCC 25416), *Stenotrophomonas maltophilia* (ATCC 51331) and a non mucoid and mucoid *Pseudomonas aeruginosa* (ATCC 27853 and clinical isolate). The nebulizers were then placed in either the AVENT (Philips) or the NUK Quick & Ready (Gerber) baby bottle steam sterilizer and the machines used with tap water per the manufacturer's instructions. The inoculated sites on each nebulizer were swabbed for bacterial growth after steam treatment. In a control set of experiments, inoculated nebulizers were placed in the machine for the same time period without turning the machine on and the nebulizers were reswabbed for bacterial growth. Steam treatment was tested with a wet drop of bacterial inoculum, dried inoculum, and inoculum mixed with de-identified discarded CF sputum samples.

Results: The AVENT (Philips) and the NUK Quick & Ready (Gerber) steam sterilizers effectively disinfected home nebulizers. With an average inoculum of 4×10^5 – 2×10^6 organisms, we did not recover viable bacteria of any strain under each of the conditions tested (Table 1). After increasing the inoculum of *P.aeruginosa* and *S.aureus* to 3×10^8 we still failed to recover viable bacteria after steam treatment.

Conclusions: Steam treatment is an effective method to disinfect home nebulizers inoculated in vitro with common respiratory CF pathogens justifying future studies of patient nebulizers to confirm the value of steam sterilization in the clinical setting.

Table 1. Baby bottle steam sterilizers effectively disinfect home nebulizers*

Inoculum Condition	Nebulizer	<i>H.influenzae</i>		<i>B.cepacia</i>		<i>S.maltophilia</i>		<i>S.aureus</i>		Non- mucoid and mucoid <i>P.aeruginosa</i>	
		Mock	Steam	Mock	Steam	Mock	Steam	Mock	Steam	Mock	Steam
Wet or dry	Pari LC Plus	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG
	eFlow Rapid	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG
	eFlow Altera	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG	>10 ⁵ CFU	NG
	Pari LC Plus	3+	NG	3+	NG	3+	NG	3+	NG	3+	NG
Sputum [^]	eFlow Rapid	3+	NG	3+	NG	3+	NG	3+	NG	3+	NG
	eFlow Altera	3+	NG	3+	NG	3+	NG	3+	NG	3+	NG

*Identical results were obtained using the NUK Quick & Ready and the AVENT machines for all inoculum sites tested.

[^] Organism recovery was based on a semi-quantitative 4 quadrant streaking of agar plates. 3+ indicates bacterial growth into the third quadrant.

CFU= colony forming units, NG = no growth

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STOPPING THE SPREAD OF MRSA: EFFECTIVE USE OF INFECTION CONTROL MEASURES IN A PEDIATRIC CF CENTER

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Background: The prevalence of MRSA colonization in cystic fibrosis patients has risen dramatically over the past decade, and recent evidence links MRSA colonization with lower lung function and worse prognosis. MRSA prevalence at our CF center has been well above the national average, ranging from 40 to 42.5% of our population over the past five years. In 2007, our center revised our infection control policy and began cohorting infants to a separate clinic day in an effort to decrease the spread of MRSA. We are now able to report follow-up data five years after implementing these changes.

Methods: A uniform infection control policy for CF patients in all settings (inpatient, outpatient, home care) was instituted. All patients were placed on contact isolation during clinic visits. Spirometry was performed in patient exam rooms rather than in the PFT lab. Patients with MRSA and other resistant organisms were placed on contact isolation during hospitalizations. Infants and children <2 years of age were cohorted to separate clinic days from older patients. Patients were provided with individual bottles of alcohol-based hand gel as well as disposable pens on admission to clinic.

Results: Review of the 56 MRSA positive patients in our pediatric population for whom data was available showed median age of acquisition of 3.3 years (range 0.08 years to 16 years) since 2007 compared to a median age of 8 years (range 1 mo to 18 yrs) from 1997-2006. The incidence of new MRSA cases in our population has declined from an average of 7.3 new cases per year in the 3 years preceding the above changes to an average of 2.6 new cases per year over the past 3 years (Fig 1). Overall incidence of MRSA at our center has increased 2.5% in comparison to a 6.7% increase in the national average (Fig 2).

Conclusions: MRSA colonization rates continue to be high at our center. However, we have seen a decline in the rate of acquisition of MRSA in our pediatric population after instituting a strict infection control policy and cohorting infants to a separate clinic day. Median age of MRSA acquisition has also decreased, possibly due to an influx of younger patients diagnosed by newborn screening. MRSA colonization rates at our center have remained relatively stable in comparison to the national average, which has continued to rise. Given the association between persistent MRSA colonization and increased rate of decline in lung function, we must continue to work toward controlling the spread of MRSA in the CF population through careful attention to infection control.

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EXPERIMENTAL PHAGE THERAPY OF THE BURKHOLDERIA CEPACIA COMPLEX

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Recently, in situations where a bacterial pathogen is also highly antibiotic resistant, the efficacy of bacteriophage therapy has come under increasing scrutiny. In this regard, bacteriophage therapy may be especially applicable to infections caused by the *Burkholderia cepacia* complex (Bcc) since members of the Bcc are highly antibiotic resistant. Bcc infections can significantly reduce the life expectancy of CF patients and in severe cases can lead to "cepacia syndrome," which is characterized by a rapid deterioration of pulmonary function. A therapy that utilizes bacteriophages (or phages) to specifically target Bcc species would complement current chemical antibiotic therapeutic regimens. Phages are bacterial viruses that exclusively attack and lyse particular species or strains of bacteria. Here we demonstrate effective phage therapy for different strains of the Bcc in an aerosol mouse model, including important improvements over previous descriptions of Bcc phage therapy. Using a Nose Only Inhalation Device, we show in an immune-compromised murine infection model that aerosol phage therapy is safe and effective. Using 5 different phages specifically active against two different *B. cenocepacia* strains, we observe three to five log decreases in bacterial numbers in the lungs, with treatment delays of between 24 and 48 hours post-infection. In addition, we observe in vitro that some Bcc bacteriophage can provide a dual role in combating the growth of *Pseudomonas aeruginosa*. Finally, we examine the possibility of phage loss from the lungs, which could result in a reduction of phage therapy effectiveness. Our data suggests that intranasal aerosol delivery of phages to the bacterial site of infection in lungs is more effective than intraperitoneal delivery of the phages. Our results suggest that Bcc phage therapy can effectively clear bacterial infections from lungs by a magnitude of up to 5 log units and with a treatment delay of up to 48 hours in this infection model.

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PALIVIZUMAB IMMUNOPROPHYLAXIS EFFECTIVENESS IN CHILDREN WITH CYSTIC FIBROSIS

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Background: Respiratory syncytial virus (RSV) is the primary cause for lower respiratory tract infections and a significant contributor to morbidity and mortality among infants and young children. For patients with CF, RSV infection, like other viral infections is implicated in causing respiratory symptoms. While no vaccination is available, prophylaxis with palivizumab (Synagis®, MedImmune), a monoclonal antibody has been approved. In infants with CF, American Academy of Pediatrics (AAP) qualifies the recommendation for palivizumab immunoprophylaxis because of the absence of clinical trials evidence.

Methods: We utilized Medicaid Extract files from 27 states from 1999-2006 linked to the CF Patient Registry data to establish a cohort of children 0-2 years with CF diagnosis. Eligible children entered the cohort after CF diagnosis and after RSV season onset, and were followed until season end, 2nd birthday, death, or hospitalizations for reasons other than the study outcome. Two outcomes were examined: hospitalization for RSV infections (RSV-ha), or hospitalization for acute respiratory infections (ARI-ha). Palivizumab exposure was defined based on pharmacy or procedure claims as current (claim date plus 30 days), former (day 31-60 after a claim), and no exposure (days before the first or > 60 days after any claim). Both outcomes were examined in a Cox regression model, adjusting for RSV risk factors and CF severity via exposure propensity score.

Results: The matched cohort included 1974 infants (2875 infant-seasons), who experienced 32 RSV-ha and 145 ARI-ha (3.9 and 17.9 per 1000 season-months, respectively). Significant determinants of exposure were presence in the registry, failure to thrive, newborn screening status, bronchopulmonary dysplasia, use of inhaled tobramycin, DNase, pancreatic enzymes, inhaled bronchodilator or steroid and the presence of acute respi-

ratory illness. Compared to periods of no use, the adjusted hazard ratio for current use was 0.57 (95%CI 0.20-1.60). ARI-related hospitalizations showed a HR=0.79 (95% CI: 0.50-1.23). Each month of increasing age reduced the ARI-ha by 5.8%.

Conclusion: RSV hospitalization incidence was low suggesting either little contribution of the virus to acute respiratory infection pathogenesis in CF patients or lack of RSV testing and diagnosis. Hospitalizations for acute respiratory illness with possible RSV contribution showed no or minimal association with palivizumab prophylaxis. Age greatly affected infection risk with incidence rates for 1-2 year olds reduced to half compared to 0-1 year old infants.

We acknowledge the CF Foundation for the provision of CFPR data.

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MICROARRAY ANALYSIS OF THE EFFECT OF FOSFOMYCIN AND TOBRAMYCIN AGAINST PSEUDOMONAS AERUGINOSA FROM CF PATIENTS

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Introduction and Aims: There is a clear unmet need for new antibiotics or combinations of antibiotics that possess activity against CF pathogens such as *Pseudomonas aeruginosa* (PA). Since micro-aerophilic and anaerobic niches exist in CF sputum, the efficacy of these agents against bacteria grown in low oxygen conditions merits investigation. We have previously demonstrated that a 4:1 (w/w) combination of fosfomycin/tobramycin (F:T), currently in development for treatment of bacterial respiratory infections, has good activity against CF pathogens, including PA. Enhanced F:T activity was observed after growth of PA in anaerobic, compared to aerobic conditions. This study aimed to use transcriptional analysis of PA cultured anaerobically and aerobically, to investigate possible mechanisms of F:T activity.

Methods: A clinical CF PA (n=1) isolate was grown under aerobic and anaerobic conditions in sub-inhibitory concentrations of fosfomycin, tobramycin and F:T. RNA was extracted from log phase culture using Trizol® reagent and further purified prior to use (Qiagen RNeasy®). mRNA was applied to the Affymetrix PAO1 genome array, in accordance with the manufacturer's instructions. Data was analysed using Partek genomics suite and normalisation was carried out using RMA. Gene expression was compared using ANOVA with a p-value of ≤0.05 indicating significant differential expression. Genes with a fold change of ≥1.5 compared to control were included in this analysis.

Results: A number of genes showed a ≥1.5-fold change in expression under aerobic (n=192) and anaerobic (n=87) conditions after exposure to F:T. In general, expression of genes involved in protein synthesis were down-regulated following exposure to tobramycin and F:T in both aerobic and anaerobic conditions. Expression of *narG*, considered essential for anaerobic growth, was down-regulated (2.7 fold) in F:T anaerobically vs aerobically. Genes involved in the uptake of tobramycin (*phnA* and *phnB*) were up-regulated in response to F:T under anaerobic conditions (1.8- and 1.6-fold respectively). In contrast, hydrogen cyanide synthase (*hcnA*), associated with blocking aminoglycoside uptake, was down-regulated. Expression of *murA*, a target for fosfomycin, was down-regulated in response to tobramycin and F:T following aerobic vs anaerobic growth.

Conclusions: Growth of PA in anaerobic vs aerobic conditions, and exposure to F:T results in differential gene expression which may result in (i) increased targets for fosfomycin (*murA*), (ii) decreased capacity for anaerobic growth (*narG*); (iii) increased uptake of tobramycin (*phnA*, *phnB*) and (iv) decreased blockage of uptake of aminoglycosides (*hcnA*). These studies appear to confirm initial investigations which observed that F:T was more effective against PA grown anaerobically and may provide a rationale for future clinical studies. Further work is currently underway to confirm these findings in gene knockout models.

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POLYMICROBIAL DIVERSITY IN CYSTIC FIBROSIS PATIENT SPUTUM SAMPLES

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Diverse polymicrobial microbial communities chronically colonize cystic fibrosis patients' lungs. Pyrosequencing of 16S rRNA amplicons was used to profile the bacterial communities of sputum samples collected from 22 clinically stable patients (outpatients) and 13 patients during an acute exacerbation (inpatients). We employed qPCR as an independent approach to confirm the detection of *Pseudomonas aeruginosa* and *Streptococcus* in patient samples, and HOMIM analysis to speciate the streptococci identified by pyrosequencing. We show that outpatient sputum samples have significantly higher bacterial diversity than inpatients. Interestingly, treatment with tobramycin did not significantly impact community diversity. The fractional representation of *Streptococcus* was significantly higher in the outpatient cohort than the inpatient cohort and was the strongest predictor of clinically stable lung disease. The most prevalent streptococci detected in this patient cohort included species typically associated with the oral cavity (*S. salivarius* and *S. parasanguis*) and the *Streptococcus milleri* group species. The increased relative abundance of these species of *Streptococcus* in outpatients may promote community diversity in the cystic fibrosis lung and, thus, facilitate patient stability.

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EFFECTS OF AZITHROMYCIN ON REGULATION OF METALLOPROTEASES RELEASED BY PSEUDOMONAS AERUGINOSA (PA) CLINICAL STRAINS

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Background: Modulation of *Pseudomonas aeruginosa* (Pa) virulence factors was suggested as mechanism for azithromycin (AZM) beneficial effects in cystic fibrosis (CF) patients.

Aims: Our work was aimed to study the regulation of matrix metalloproteases (MMPs) released by Pa clinical strains following AZM treatment.

Methods: We analyzed pro-inflammatory markers in a CF human airways epithelial cell line untreated and pretreated with AZM and then incubated for 4 hours in presence of conditioned media (CM) from laboratory strain PAO1 and Pa clinical strains. Bacteria were grown in absence or presence of AZM. mRNA analysis of cytokines expression was performed by RT-PCR. Gelatinase activity was investigated in CM from chronic and sporadic strains by zymography technique as functional assay.

Results: Pretreatment with AZM significantly reduced the expression of IL-8 and TNF alpha mRNA in CF cells exposed to CM of Pa clinical strain (25% and 45% decreases, respectively) while it had no statistically significant effects on IL-6 mRNA expression.

CM from Pa clinical isolates induced an increase of IL-8, TNF alpha and IL-6 mRNA in CF airway epithelial cells. This induction was reduced, except for IL-6, when the clinical isolates were grown in the presence of AZM: range of reduction was from 17% to 45%. The zymography assay revealed that the metalloproteases were poorly expressed by late clinical strain AA43 while early AA2 strain expressed more proteases and decreased their expression after exposure to AZM.

We tested clinical isolates from CF patients followed at the Cystic Fibrosis Center of Verona. In 45 of the 66 sporadic strains we detected gelatinase activity (68%) while this was true only for 26 of 67 (39%) among the chronic strains. Thirty-nine of 45 (87%) of sporadic strains decreased gelatinase activity following exposure to AZM while the same occurred only

for 12 chronic strains among 26 analyzed (45%) ($p < 0.0001$, Fisher's exact test).

Conclusions: The correlation among gelatinase activity/ MMPs expression and clinical conditions suggest that MMPs might play a role in the clinical manifestations of Pa infection while the response of the Pa clinical isolates to AZM can contribute to better explaining the variable response to Pa infection and to AZM treatment known to occur in CF patients.

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CHARACTERIZING BACTERIAL AIR CONTAMINATION DURING CF OUTPATIENT VISITS

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Objective: While droplet transmission is recognized to result in patient-to-patient spread of respiratory tract (RT) pathogens in CF clinics, little is known about factors that facilitate such transmission. This study was performed to determine potential factors associated with air contamination (AC) in the office setting.

Methods: We performed a multicenter study (7 study sites at 4 CF centers) to assess the frequency of AC with CF pathogens during outpatient visits. Eligible subjects were attending clinic for routine or sick visits and were infected with *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA), *Stenotrophomonas maltophilia* (SM), and/or *Burkholderia* species (BS). For this study, spirometry was performed in a separate room from the remainder of the encounter. Air samples were collected with a single stage impactor (Andersen 1-STG) that drew ambient air (rate of 28.3 L/min) onto a blood agar plate (Becton Dickinson). The impactor was positioned ≥ 6 ft from the subject and air was collected for 10 min per sample. The following samples were obtained for each subject: during spirometry, 30 min after spirometry, and in the exam room. Plates were processed at a core laboratory using selective media, and bacterial isolates were characterized using Clinical and Laboratory Standards Institute recommendations. The proportion of subjects with the same species isolated from ≥ 1 air sample(s) and historic and/or current RT specimens was determined. The air exchange rate (AER) in each room was measured by the carbon dioxide decay technique.

Results: In all, 219 subjects were enrolled (162 adult; 57 pediatric) who harbored the following pathogens: 115 non-mucoid PA, 87 mucoid PA, 108 SA, 51 MRSA, 43 SM, and 10 BS; 28 had protocol-defined pulmonary exacerbations (PE). The mean bacterial density observed from air samples was 15.1 colony forming units/M³. During spirometry, the AC rate was 5.5% (11/219; 95% confidence interval 5.4%-5.6%), and for 10 of these 11, the species were concordant with subjects' RT pathogen(s). The AC rate decreased to 0.9% (95% CI 0.89%-0.91%) 30 min post spirometry and only half were concordant with relevant RT pathogens. The AC rate in exam rooms was 0.9% (95% CI 0.89%-0.91%), though some subjects wore masks. None of these samples were concordant with RT specimens. The overall AC rate was not influenced by the presence or absence of PE (7.1% vs. 7.9%, respectively). AC increased with lower AER, but this difference was not statistically significant.

Conclusion: The overall AC rate was similar to previous findings (1). AC was more frequent during spirometry than during the remainder of the office visit, but 30 min after spirometry, most AC had cleared. Further analysis will explore possible associations of AC with AER, patient mask use, carriage of specific RT pathogens, and cough frequency.

References: 1) J Cystic Fibros 2009; 8:186-92.

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CLINICAL EFFECTS OF SINUS SURGERY — A ONE YEAR PROSPECTIVE FOLLOW-UP STUDY OF 106 PATIENTS WITH CYSTIC FIBROSIS

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Background: The paranasal sinuses are often colonized with lung pathogenic bacteria (*Pseudomonas aeruginosa*, *Achromobacter xylosoxidans*, *Burkholderia multivorans*) in patients with cystic fibrosis (CF). The paranasal sinuses may be a bacterial reservoir for initiation or maintenance of deleterious pulmonary infections. Whether sinus surgery has any impact on colonization/infection of the lower airways is controversial.

Aim: Various clinical outcomes of extensive sinus surgery followed by two weeks of intravenous antibiotics and six months of local antibiotics by nasal rinsing were assessed.

Materials: Danish CF patients with extensive sinus surgery performed during 2007–2011, n=106.

Methods: Data were prospectively ascertained and observations before and after surgery was compared: Six months and one year pre- and post-operative frequencies of lower airway cultures positive for CF-pathogenic Gram-negative bacteria, lung function tests (FEV1 and FVC), BMI, and IgG serum antibodies to *P. aeruginosa*; questionnaires on symptoms of chronic rhinosinusitis (SNOT-22), questionnaires on general disease-specific health-related quality of life (HRQOL), and finally surgical complications were assessed.

Results: Sinus surgery and nasal irrigations accomplished reduction in frequencies of pulmonary cultures with CF-pathogenic bacteria irrespective of pre-operative pulmonary infection status (38% vs. 30%, $p<0.01$). Furthermore, sinus surgery and nasal irrigations decreased self-reported symptoms of chronic rhinosinusitis (SNOT-22) observed 3 and 12 months post-operatively (score 20 vs. 11, $p<0.01$ and 20 vs. 14, $p<0.01$), and increased HRQOL observed 6 months ($p<0.05$) and twelve months (non-significant) post-operatively. No irreversibly surgical complications were seen. Sinus surgery had no effects on lung function, BMI or specific antibodies to *P. aeruginosa*.

Conclusion: Combined extensive sinus surgery and post-operative antibiotic treatment reduce the frequency of pulmonary samples positive for CF-pathogenic bacteria six months and one year after surgery. In addition, we substantiate that sinus surgery relieves symptoms of chronic rhinosinusitis in CF patients and improves quality of life.

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PSEUDOMONAS AERUGINOSA MODULATES THE PHOSPHORYLATION CHANGES OF SCAFFOLDING PROTEINS AND APICAL EXPRESSION OF CFTR IN AIRWAYS

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Although therapies have augmented the life span of cystic fibrosis (CF) patients, chronic infection and inflammation represent key pathogenetic events of lung damage and respiratory insufficiency.

P. aeruginosa infections of the host airway cell have been shown to decrease apical expression of both wild-type (wt) and F508del CFTR (Swiatecka-Urban A. et al. 2002, Guerra L. et al. 2005, Kwon SH. et al. 2007). Recently, a factor secreted by *P. aeruginosa* called CFTR inhibitor factor (Cif) has been identified which has an inhibitory effect on a deubiquitinating enzyme (Bomberger J.M. et al. 2011).

The host cell intracellular molecular mechanism(s) underlying this *P. aeruginosa* infection-dependent inhibition of CFTR membrane recycling could involve other pathways. Recent work has shown that the PDZ domain adaptor protein, NHERF1, finely regulates both wt and F508del CFTR membrane recycling through its association with the C-terminal PDZ-binding motif of CFTR (Favia M. et al. 2010).

Here, we investigated *in vitro* and *in vivo* the effect of *P. aeruginosa* on the expression of NHERF1, ezrin and CFTR by Western blot in wt respiratory cells and in murine lung upon exposure of the cells and mice.

In both bronchial epithelial cells and in the lungs of the mice, *P. aeruginosa* induced a reduction of CFTR expression and increased NHERF1 molecular weight (Mr) together with a reduction of phospho-ezrin levels. The increase of NHERF1 Mr was partially caused by its hyper-phosphorylation, because exposure of the cell extracts to increasing levels of alkaline phosphatase (AP) reduced the high Mr band of NHERF1. Because a certain amount was resistant to AP treatment, we tested for the presence of ubiquitin in the infected cell extracts and observed an ubiquitinated protein at the same Mr of the shifted NHERF1. Importantly, while reducing NHERF1 expression by knock-down reduced the ability of *P. aeruginosa* infection to down-regulate mature CFTR expression, the increase of CFTR expression driven by NHERF1 overexpression did not render it insensitive to the negative effects of the infection.

The molecular identification of the pathological mechanisms responsible for these effects could identify novel targets to block this process and also block the *P. aeruginosa* interference with the efficacy of drugs capable of increasing CFTR apical membrane expression.

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HYPERSENSITIVITY TO ANTIBIOTICS IN PATIENTS WITH CYSTIC FIBROSIS

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Background: Clinical observations and several cohort studies have demonstrated that hypersensitivity reactions to parenterally administered antibiotics (HRPA) are a substantial problem in the management of cystic fibrosis (CF), particularly in advanced CF. Due to a dramatic increase in life

expectancy most of the published data about HRPAs in CF do not reflect today's patients and treatment regimens. Therefore we conducted this study with focus on the prevalence of reactions to specific antibiotics, the reaction's characteristics and the predisposing risk factors.

Methods: We collected data with a semi-open, structured questionnaire documenting age, sex, latest FEV1, dF508 genotype, age at diagnosis of CF, bacterial colonisation, allergy history and history about past antibiotic exposure as well as past HRPAs. We verified and expanded each interview's findings by analyzing the medical records.

Results:

Prevalence of reactions: We documented 188 HRPAs with 378 symptoms in 100 patients. 65% of the HRPAs led to a change of antibiotic or discontinuation of therapy. 60% of our patients had at least one HRA, 46% had two and 31% three or more. We saw 70% of HRA to beta-lactams and 30% to non-beta-lactams. Both penicillins and cephalosporins accounted for 35% of the HRA, carbapenems for 10% and both quinolones and polypeptides for 4% of the HRA.

Relative risk for hypersensitivity to antibiotics: The relative risk for reactions (reactions per administration of a given drug) was highest for tetracycline with 27%, followed by amphotericin B with 24% and ofloxacin with 18%. Among standard antipseudomonal drugs the highest relative risk was observed for ceftazidime with 12%, followed by piperacillin/tazobactam with 11% and aztreonam with 8%.

Characteristics of HRA: 52% of the symptoms were dermal, 10% drug fever, 9% bronchopulmonary, 8% neurologic, 6% gastrointestinal, and 5% cardiovascular.

3% of the HRA were classified as life threatening. 53% of the HRA occurred while infusing the drug, 35% within one hour of the infusion and 12% more than one hour later. 81% of the HRA occurred between days one and four of the treatment course.

Risk factors: We found low FEV1, duration of *P. aeruginosa* colonisation and number of years with intravenous therapy as significant risk factors. dF508 genotype, gender and markers for atopy such as in vitro screening tests for inhaled allergens and total IgE concentration were not significant risk factors for HRA.

Conclusions: Our results demonstrate that HRA are highly relevant. The prevalence of 60% for HRA was higher than in most previous studies. This underlines the observation of many CF clinicians that HRA have become a crucial issue in the management of advanced CF. 88% of all the HRA occurred while or within one hour of the infusion and 81% of all the HRA occurred during the first four days of the antibiotic course. These findings suggest immediate type reactions as underlying cause. Facing the high prevalence and severity of HRA, the insecurity of attending physicians and the consecutive limitations of therapeutic options, we see an urgent need for further identification of HRA's immunology and the development of diagnostic algorithms for clinical use.

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FILAMENTOUS FUNGAL COLONIZATION AFFECTING ADULTS WITH CYSTIC FIBROSIS

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Background: Cystic fibrosis (CF) patients acquire chronic colonization by infectious organisms in their airways. Most common pathogens related to lung infections are bacterial in origin, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Previous reports have demonstrated that colonization by *Aspergillus fumigatus* correlates with a significant progression of pulmonary disease. The purpose of this study was to determine the effect of intermediate and persistent colonization with filamentous fungi on lung function and exacerbation rates.

Methods: Retrospective chart review of all adult CF patients at the Keck Hospital University of Southern California (USC) was conducted during a 4-year period from 2008 through 2011 under an IRB-approved protocol. Patients who were not able to provide a respiratory culture or perform reproducible spirometry during the study period were excluded. Patients who produced 2 positive sputum cultures for each observational 6-month period were assigned to the Persistent Colonization (PC) cohort. Those who produced a single positive culture were assigned to the Intermediate Coloniza-

tion (IC) cohort. Those without growth of any mold were assigned to the No Colonization (NC) cohort. The major species identified were *Aspergillus* spp., *Scedosporium* spp. and *Penicillium* spp. Linear regression was used to trend pulmonary function tests and to determine the rate of exacerbation.

Results: A total of 124 out of 200 CF patients were eligible to participate in this study. Median age was 28.5 years with 47.6% women; 77.4% (96 pts) were Caucasian, 18.5% (23 pts) Hispanic, 0.8% (1 pt) African-American and 3% (4 pts) were of "other" ethnicity. Median BMI was 21.6 kg/cm². Baseline median FEV1 was 57% predicted and average number of exacerbations per patient was 0.15 (range 0-3). Thirty-five pts (28%) were PC, 35 pts (28%) IC and 54 pts (44%) NC. With respect to changes in the three groups, the median FEV1 change over the 4-year analysis was a decline of 11% in NC, 7.8% in IC and 13% in PC cohorts. After controlling for age, FEV1 and gender, the incidence of hospitalization increased >1.5-fold in the IC and 2.3-fold in the PC group. There were no changes in exacerbation rates in the NC group. *Aspergillus* (69.5%) and *Scedosporium* (20.9%) accounted for the majority of fungi isolated. The incidence of exacerbation rate increased >4-fold with *Scedosporium* (p<0.01), while *Aspergillus* had a trend for a higher number of exacerbations (p=0.09).

Conclusion: We have demonstrated that development of persistent, but not intermittent, colonization by filamentous fungi is related to an increase in the number of hospitalizations in CF patients despite minimal differences in FEV1 changes. While a high rate of pulmonary exacerbations with the presence of *Aspergillus fumigatus* was identified, colonization of *Scedosporium* had a 4-fold incidence of exacerbation rate.

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EARLY GENETIC ADAPTATION ASSOCIATED WITH CHRONIC PSEUDOMONAS AERUGINOSA AIRWAYS INFECTION

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P. aeruginosa (PA), widespread in nature, can successfully colonize the airways of cystic fibrosis (CF) patients and subsequently develop "a chronic phenotype" caused by adaptive mutations in the genome. In young CF patients antibiotic therapy can effectively eradicate *P. aeruginosa* from the lungs, but in many patients recurrent colonization with the same initial clone is observed, and it was speculated that the bacteria could hide in one or more protected niches in the body. We have therefore investigated the early genetic adaptive changes in PA colonizing the paranasal sinuses and lungs of young CF patients to elucidate whether the sinuses constitute protected niches providing foci for adaptation.

We studied PA cultured from sputum samples from 31 intermittently colonized patients (median age 14 years, range 7-29 years) of whom 22 patients also had PA in their sinuses after surgery. PA was genotyped by pulsed-field gel electrophoresis and antibodies against PA alginate and sonicate were measured using an ELISA method. We also looked at morphotype diversity, motility and antibiotic resistance.

Of the 22 patients PA positive in both sinuses and lungs, 91% had identical PA genotypes in both locations. Saliva IgA against PA sonicate and alginate was 15 and 39 times higher than in serum indicating a local production of IgA in the upper airways (p<0.001). We found that the overlap of morphotypes between the sinuses and the lungs was high; on average more than 3 identical types per patient (approximately 60% of all morphotypes). Motility expressed as twitching, swimming and swarming decreased over time in both locations. Sinus isolates demonstrated the highest level of resistance against ciprofloxacin.

We have shown that identical genotypes exist comparing PA in the sinuses and lungs supporting the hypothesis that the sinuses may serve as foci in early colonization, when bacteria diversify and develop antibiotic resistance. High levels of non-inflammatory secretory IgA in the sinuses probably impede the polymorphonuclear leukocytes from being recruited, and therefore local and systemic inflammation and recognition of the

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microorganisms is prevented. The sinuses are protected niches, due to less efficient antibiotic function. As well, significantly reduced immune function facilitates the establishment of chronic PA infection in the lower airways in association with genetic adaptation.

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MOLECULAR COMPARISON OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM PATIENTS WITH CF AND NON-CF BRONCHIECTASIS

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Introduction: Chronic infection with *P. aeruginosa* (PA) is common in both CF and bronchiectasis not related to CF (BE). Although well characterized in CF, less is known regarding the nature of strains involved PA infection in BE, and whether strains, known to be more virulent in CF patients [e.g. Liverpool epidemic strains (LES)] are also common in other respiratory conditions. This study aimed to use molecular methods to compare PA isolates from both CF and BE, and also to characterize PA isolated pre- and post-antibiotic treatment of an exacerbation.

Methods: PA from CF [n=7, LES; n=8 Belfast, (BFS) isolates] were compared to PA from BE (n=16 isolates from 10 patients). BE PA isolates obtained pre- (n=5) and post-antibiotic (n=5) treatment of a pulmonary exacerbation were included, together with two distinct PA morphotypes (n=6 isolates) from one patient. Isolates were characterized by whole genome restriction enzyme digestion (*SpeI*) followed by Pulsed Field Gel Electrophoresis (PFGE) of resulting fragments. Dendrograms of PFGE patterns were constructed using UPGMA analysis and DICE coefficient using GelCompar II software. BE strains were additionally typed by a modified 5-locus multi-locus VNTR analysis (MLVA).

Results: PFGE identified 6 main groups, each with >70% homology: Groups A (n=5 BE PA), B (n=7 LES PA), C (n=2 BFS PA), D (n=4 BE PA), E (n=4 BE PA) and F (n=2 BFS PA). The remaining isolates (n=9) were genotypically distinct, as determined by PFGE. Isolates taken pre- and post-antibiotic treatment in one BE patient were >70% similar (group A), with the exception of 1 post-treatment isolate which shared <45% homology (group E). Different morphotypes isolated from the same patient were found to be genotypically identical (>95% homology). Within each PFGE group, isolates had a minimum of 4/5 identical MLVA repeats. Identical MLVA patterns were observed in isolates with >95% homology by PFGE.

Conclusions: PA isolates from a BE patient pre- and post-antibiotic treatment were identical, with one exception. This genotypically distinct isolate may have initially been below detection limits or may represent a new colonization. In general, PA from CF and BE formed distinct PFGE groups, suggesting genotypic differences between isolates cultured from CF and BE patients. Grouping of isolates according to MLVA type yielded results which were concordant to PFGE; however, as MLVA is rapid, less subjective and as discriminatory for typing PA, it is a potentially more useful method than PFGE for large-scale surveillance and epidemiological studies of PA in the respiratory clinic. Future work will determine if any phenotypic differences (e.g. antibiotic sensitivities) are observed between groups and further isolates from both CF, BE and other respiratory conditions are currently under investigation.

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THE TOXIC *P. AERUGINOSA* EXOPRODUCTS CYANIDE AND PYOCYANIN INHIBIT CILIARY FUNCTION OF HUMAN RESPIRATORY EPITHELIA VIA DIFFERENT MECHANISMS

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P. aeruginosa is one of only a few bacterial species capable of producing the virulence factor hydrogen cyanide (HCN). HCN is detectable in the sputum of CF patients at concentrations >200 µM and its presence is negatively correlated with lung function (FEV₁ 1% CN- negative: 46.0 ± 6.7%; CN- positive: 26.8 ± 3.8%; p<0.01). Our work explores the possible mechanisms for this association, focussing on the effect of physiologically relevant concentrations of HCN on human respiratory epithelial cell function. Concentrations of HCN similar to those found in sputum were found to inhibit ciliary beat frequency (CBF) of healthy human nasal epithelium (n=6) after 60 min (150 µM: 47% fall, p=0.021; 75 µM: 32% fall, p=0.0450). Samples from CF patients (n=3) showed similar results (150 µM: 55% fall, p=0.02). The decrease was not due to loss of viability (ns, p=0.2036) and was reversible, in that CBF rapidly recovered to control levels when HCN was removed. The toxic *P. aeruginosa* exoproduct pyocyanin is also detectable in sputum at concentrations >75 µM. Pyocyanin has previously been shown to inhibit CBF in an ATP-dependent manner. We looked at the combined effect of pyocyanin and cyanide on CBF. Ciliated strips of human nasal epithelium were obtained from the inferior turbinate of healthy volunteers with a cytology brush and CBF in the presence or absence of cyanide, pyocyanin, or a combination of both, was measured at 37°C using light microscopy. CBF measurements were also carried out on airway liquid interface cultures (ALIs). ATP measurements were performed on 16-HBE cells. After 60 min, the addition of either cyanide (75 µM: 42% fall p=0.025) or pyocyanin (75 µM: 27% fall p=0.025) decreased the CBF of nasal brushings (n=3). The effect of treatment with cyanide and pyocyanin was significantly greater than that with pyocyanin alone (51% fall p=0.024). Preliminary CBF measurements on the ALIs (n=2) support this finding (KCN 75 µM: 21% fall, pyocyanin 75 µM: 5% fall, KCN 75 µM + pyocyanin 75 µM: 63% fall). ATP measurements confirmed that pyocyanin depletes cellular ATP levels (75 µM: 55% fall, p<0.0005; 150 µM: 60% fall p<0.0005). Interestingly, KCN did not cause a fall in ATP (ns at 75 µM and 150 µM). Combined treatment with cyanide and pyocyanin did not decrease ATP levels more than pyocyanin alone (ns, p=0.256). These results suggest that cyanide and pyocyanin inhibit ciliary beat through different mechanisms. In addition, the presence of cyanide in the lung appears to exacerbate the effect of pyocyanin alone, causing a marked decrease in CBF *in vitro*. If this was of a sufficient magnitude to impair mucociliary clearance *in vivo*, it could provide an explanation for the link with increased disease severity observed in CF patients with detectable levels of cyanide in the airway.

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EXTENDED SUSCEPTIBILITY TESTING FOR *STENOTROPHOMONAS MALTOPHILIA* (SM): A CENTER'S EXPERIENCE

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Background: SM is a common organism isolated in the sputum of cystic fibrosis (CF) patients. Studies have failed to show a clear clinical impact of this organism on lung function, and thus there is no optimum therapeutic approach in treatment. Patients with decreased FEV₁ may need antimicrobial therapy. First line antibiotic therapy is high dose trimethoprim/sulfamethoxazole (TMP/SMZ). Others include levofloxacin, ceftazidime and ticarcillin/clavulanate. Since 2008, our institution has experienced a decrease in susceptibility to TMP/SMZ from 100% to 94%. Susceptibilities to other antibiotics have also decreased and we had several CF patients with allergies to alternative agents thus making treatment challenging. We worked with our microbiology department to expand susceptibility testing for SM for other options (outpatient and inpatient treatment). We expanded

the current panel of TMP/SMZ, levofloxacin, ticarcillin/clavulanate and cef-tazidime to add minocycline, colistin and tigecycline. As part of this quality improvement initiative, we performed outcome studies assessing retrospective evaluation on the effectiveness of extended susceptibility testing and impact on treatment outcomes at our center.

Methods: We identified all CF patients who grew *SM* from January 2011 to December 2011. We evaluated susceptibility patterns, treatment with oral antibiotic regimens, hospitalization rates, changes in lung function and other organisms in sputum culture. We evaluated FEV₁ before and after treatment when available as well as total decrease from the year personal best prior to therapy.

Results: Thirty-three patients (6 months to 18 years of age, 9.1 ± 5.8 years) grew *SM* 64 times in their sputum culture during the study period and were included in the analysis. This represents 21% of patients. Fifty-eight percent of these cultures also grew *Staphylococcus aureus* (SA) and 30% *Pseudomonas aeruginosa*. Cumulative *SM* susceptibility was as follows: TMP/SMZ 80%, levofloxacin 67%, ceftazidime 36%, ticarcillin/clavulanate 47%, minocycline 98%, colistin 81% and tigecycline 64%. Fifty-eight percent of patients required treatment with oral antimicrobials. The patients treated received TPM/SMZ 54% of the time, fluoroquinolone 38% of the time, and minocycline 8%. Twenty percent of all patients who grew *SM* were hospitalized after failing oral therapy, and they received appropriate intravenous antibiotics. FEV₁ was $12 \pm 8.9\%$ lower than their personal best for the year at the start of therapy. In patients who were treated with antimicrobials, FEV₁ improved by $10.8 \pm 6\%$ from the start of therapy. In seven instances, the patient was too young to perform pulmonary function test.

Conclusion: Our data showed a variation in susceptibility to TMP/SMZ from our CF center of 80% compared to 94% from our hospital antibiogram. We also saw lower susceptibility to other first line therapy including ceftazidime, ticarcillin/clavulanate and levofloxacin. Of note, our extended panel showed that 98% of the strains tested were susceptible to minocycline. This has provided an inexpensive, oral therapy for treatment at home when needed for both *SM* and SA.

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THE IMPACT OF *P. AERUGINOSA* AND *S. AUREUS* INFECTIONS ON INFLAMMATORY BIOMARKERS IN ADULT CYSTIC FIBROSIS PATIENTS

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Background: *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) are the most common pathogens in CF airway infections. PA infects up to 80% of adult patients, causing airway inflammation and tissue destruction, which leads to lung function decline and increased mortality. SA infects up to 30% of adult CF patients, but its role in lung disease and inflammation is still poorly understood. Sagel *et al* found that PA and SA co-infections have an additive effect on lung inflammation in young children (Sagel 2009). The objective of our study is to assess the effects of PA and SA infections on the clinical status of adult CF patients, as well as their inflammatory status using the biomarkers C-reactive protein (CRP) and calprotectin.

Methods: Adult CF patients from the McGill University Health Center CF clinic were enrolled in a prospective cohort study. Sputum and serum samples were collected at baseline during a stable clinical state. Sputum samples were analyzed by standard CF microbiological techniques, as well as quantitative measurement of PA and SA. Plasma CRP and calprotectin were measured by ELISA. ANOVA and T-tests are used for comparison between different groups.

Results: Our study included 48 patients. The patient characteristics and infection status (PA alone, SA alone, PA+SA, neither) are described in Table 1. Patients in all infection groups were similar in gender, age and BMI. There is a trend towards progressively lower clinical scores in patients without PA/SA infections compared to those infected with SA alone, PA+SA and PA alone (lowest clinical score). Patients infected with PA alone had higher levels of CRP compared to those infected with SA alone ($p < 0.01$), as well as those co-infected with both PA+SA ($p = 0.05$). Calprotectin levels were

higher in patients with PA compared to those without PA ($p < 0.05$), but they did not differ significantly based on SA infection status.

Conclusions: PA infections are associated with increased plasma CRP and calprotectin levels. In contrast to children, our results show that PA + SA co-infections are associated with lower CRP than infection with PA alone, suggesting that SA co-infections does not elicit an additive inflammatory response in adults. Patients infected with PA alone may have more advanced and active disease.

Table 1

Infection Status	PA alone	SA alone	PA + SA	Neither PA/SA	p value
n	21 (43.8)	10 (20.8)	10 (20.8)	7 (14.6)	-
Male gender	52.4	55.6	60	40	0.924
Age, in years	32.7 (13.4)	35.7 (14.2)	33.1 (14.7)	36.1 (16.4)	0.915
Body Mass Index	21.9 (5.3)	22.9 (2.7)	21.7 (3.7)	21.8 (2.9)	0.920
FEV ₁ % predicted	55.3 (30.1)	74.3 (28.9)	59.2 (28.0)	74.3 (17.5)	0.228
Clinical Score	34.2 (4.0)	37.9 (3.5)	36.1 (4.5)	40.3 (3.6)	0.006
CRP	10.8 (7.9)	4.3 (3.0)	5.3 (4.0)	2 (2.6)	0.003
Calprotectin	183.4 (177.7)	102.5 (122.8)	213.7 (185.5)	53.8 (66.9)	0.131

Values expressed in: n (%) or mean ($\pm$ standard deviation).

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IMMUNE GLOBULIN FROM EGG YOLK (IGY) - AN ALTERNATIVE TO ANTIBIOTICS

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Antibiotic resistance: Today one of the largest threats against health is the increasing number of antibiotic-resistant bacteria. Each year 3 million patients are affected and approximately 50,000 die by infections with such bacteria - only in Europe. The cause is excessive use of antibiotics. Thus there is a tremendous need to find alternatives to antibiotic treatment.

CF represents the disease associated with the highest rate of microbial resistance towards antibiotics and among the different bacterial organisms *Pseudomonas aeruginosa* (PA) is most frequently reported to develop resistance (meta analysis: Fish *et al* 1995). Infections by multiresistant PA result in severe adverse outcomes - including death.

Specific IgY - Immune globulin from egg yolk - is an alternative to treat infectious diseases. Hens vaccinated with bacteria or other microbes produce specific IgY against these organisms. A single hen produces ~ 40 g IgY per year in their egg yolk. IgY antibodies decrease bacterial adhesion to epithelial cells and neutralize toxins. Solutions with IgY are prepared from egg yolk diluted in water - no other additives. Efficacy: Specific IgYs have proven efficacy against several bacteria, viruses and fungi in animal and human studies. It is effective also against antibiotic resistant microbes. Safety: Orally given immune globulins are not absorbed from the gastrointestinal tract. Orally administered IgY neither activates the human complement system nor reacts with any cell activators or mediators of inflammation. Solutions with IgY diluted in water are generally recognised as safe for oral use, including gargling and swallowing. Individuals, who are allergic to eggs, cannot use IgY drugs. There is no risk that pathogens develop resistance against IgY.

IgY for CF: A study of gargling with a solution of anti-*Pseudomonas* IgY in a group of CF patients has been running in Sweden for more than 17 years on a license granted by the Swedish MPA. An open study on CF patients was conducted during the first 12 years of this study. Seventeen CF patients were gargling anti-*Pseudomonas* IgY and 23 Danish CF patients, who were not gargling, served as controls. In the treated group only 2.3 cultures per 100 treatment months were positive for PA compared to 7.0/100 months in the control group ($p = 0.028$). In the IgY treated group only two patients (2/17) became chronically colonized with PA compared to seven patients (7/23) in the control group. In the treated group no cultures were positive for *B. cepacia*. Other gram negative bacteria and *A. fumigatus* appeared only sporadically. There was no decrease in pulmonary functions in either of the groups ($p = 0.730$). There were no adverse events due to IgY. In 2006 anti-*Pseudomonas* IgY was granted orphan drug designation for patients with CF by EMA. A multicentre, randomized, placebo controlled phase III study was started in Europe in 2011.

In conclusion: Anti-*Pseudomonas* IgY has great potential to prevent PA infections in CF patients. IgY also offers great opportunities to treat other specific infectious diseases and thereby diminish the need for treatment with antibiotics.

Fish D *et al*. Pharmacotherapy 1995;15(3):279-91.

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PYOCYANIN MODULATES IRON-LIMITING INNATE IMMUNE DEFENSES IN MACROPHAGES

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Background: Cystic fibrosis (CF) patients develop chronic bacterial infections and *Pseudomonas aeruginosa* is the most common bacterial isolate that persists in the CF lung contributing to declining lung function. The inability of CF patients to resolve airway infections points to dysregulated innate immune defenses. Since iron is essential for bacterial growth, upon infection the host limits the bioavailability of iron by up-regulating expression of hepcidin, the master iron regulating hormone, which limits iron uptake from the gut and retains iron in macrophages. This host defense strategy is known as the iron-limiting innate immune defense. Pyocyanin (PCN), a major virulence factor secreted from *P. aeruginosa*, is a cytotoxic redox-active small molecule that exerts detrimental effects on host cells. The cytotoxic effects of PCN on CF bronchial epithelial cells are well established. However, the effect of PCN on iron-limiting innate immune defenses in macrophages is not known. The aim of this study is to investigate the effect of PCN on hepcidin and other iron-regulated proteins in macrophages.

Methods: Peripheral monocyte-derived macrophages as well as macrophage cell lines THP-1 and RAW264 were treated with PCN (low doses of 2 or 6 µg/ml did not affect macrophage viability) with or without TLR ligands for 16 hrs. Cellular responses were assessed for autophagy induction and release of ROS, nitric oxide, and cytokines. Expression of iron-regulating genes was assessed by qRT-PCR.

Results: We found that PCN drastically modulated macrophage innate immune responses. Upon phagocytosis of invading pathogens, the respiratory burst is triggered as a defense mechanism that leads to ROS release required for the oxidative killing of invading pathogens. PCN inhibited, in a dose-dependent manner, ROS release from macrophages triggered by phagocytosis of opsonized zymosan. PCN also inhibited TLR ligand-induced nitric oxide release from macrophages. In contrast, PCN amplified autophagy formation induced by TLR ligands. This may be due to PCN's ability to inhibit cellular respiration in mitochondria, since damaged mitochondria are recycled via autophagy. The data suggest that PCN's modulatory effects on macrophages support *P. aeruginosa* survival in the host. Further, PCN induced expression of hepcidin, which causes iron retention in macrophages. Increased labile iron in macrophages is toxic and leads to oxidative stress. PCN also up-regulated expression of NRAMP1 in human and murine macrophages. NRAMP1 controls iron export from phagosomes and late endosomes and therefore plays a very important role in cellular iron homeostasis and host defense.

Conclusions: Pyocyanin helps *P. aeruginosa* to persist in the CF host by modulating macrophage innate immune defenses.

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THE EFFECTS OF CFTR AND CAPSULAR PHENOTYPE ON SUSCEPTIBILITY AND INNATE IMMUNE RESPONSES IN A MOUSE MODEL OF PNEUMOCOCCAL LUNG DISEASE

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Streptococcus pneumoniae (SP) is a respiratory pathogen and a leading cause of morbidity and mortality worldwide, but its role in cystic fibrosis (CF) has not been well studied. An unexpectedly frequent number of SP were recovered from the sputum of pediatric CF patients, with a surprisingly high incidence of highly mucoid SP phenotype. Other mucoid bacteria, predominately *Pseudomonas aeruginosa*, have been directly associated with CF respiratory decline, although the adaptive significance of mucoidy is not fully understood. It is also unclear whether mucoid SP contributes to respiratory failure among patients with CF. We investigated susceptibility to mucoid and non-mucoid SP and associated inflammation, using a mouse model of pneumococcal infection in CF. We hypothesized that highly mucoid SP would cause more severe lung disease than non-mucoid SP, and do so more readily in CFTRm1Unc-/- (FABPhCFTR) (CF-mice) than in

CFTRm1Unc+/+FABPhCFTR) (WT-mice). Congenic CF-mice and WT-mice were infected intranasally with 5x10⁵ CFU SP. CHB756 (a highly mucoid capsular serotype 3 isolate), WU2 (a less mucoid capsular serotype 3 isolate), and CHB1058 (a non-mucoid capsular serotype 19A isolate) were compared. Bronchial alveolar lavage (BAL) fluid, lung homogenates, and blood were collected from mice 5 days post-infection. CF-mice infected with CHB756 were more susceptible to infection than their wild type counterparts as indicated by higher bacterial burden from lung tissue and BAL fluids and/or higher mortality rates (P=0.0196); however, no significant difference in susceptibility was observed between CF- and WT-mice infected with either WU2 or CHB1058. Among CF-mice, infection with CHB756 resulted in significantly more profound disease than did infection with WU2 (P=0.0008) or CHB1058 (P≤0.001). In a separate set of experiments, CF and WT-mice were infected intranasally with 1x10⁵ CFU highly mucoid CHB756. BAL was collected 24 hours post-infection and TNF-α quantified. CF-mice had a significantly higher bacterial burden than WT-mice (P=0.0026); however they also produced significantly lower TNF-α per unit CFU compared to WT-animals (P=0.0069). Our results indicate that highly mucoid serotype 3 SP may cause more severe lung disease than non-mucoid SP, and does so more readily in the lungs of CF-mice than in the lungs of WT-animals. Infection with mucoid SP in CF-mice is also associated with decreased production of TNF-α. The results of this study support the association of highly mucoid SP with poor prognosis in a murine model of CF, and provide a better understanding of the pathogenic mechanism that favors mucoidy in cystic fibrosis airways.

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RISK FACTORS FOR MRSA SUBTYPES IN CF

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Introduction: Studies on MRSA types among patients with CF have shown mixed genetic background in the U.S. [Gilman D et al. 2008 and Muhlebach M et al. 2011.] Based on typing using the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) the majority are SCC*mec* II, the type historically originating in hospitals. About 30% are SCC*mec* IV, historically seen in community based infections and most are the endemic strain USA300. Risk factors for SCC*mec* II MRSA in non-CF subjects include contact with the medical system, recent use of antibiotics, and indwelling lines.

Aim: Patients with CF meet many of all of these risk factors but carry different MRSA SCC*mec* types. Here we evaluate subject characteristics and risk factors of MRSA acquisition based on SCC*mec* typing.

Methods: STAR-CF is a prospective, observational study at 7 CF centers in the U.S.A. The MRSA isolates are obtained from routine clinical cultures and typed by PCR assessment for SCC*mec* type and PVL presence [Goodrich J et al. 2009]. Clinical information is obtained from the CF Patient Registry. Statistical analyses are performed in SAS.

Results: Clinical information and MRSA isolates from 274 patients are included, of these 196 are SCC*mec* II, 45 SCC*mec* IV PVL+, and 35 SCC*mec* IV PVL-. Mean age at initial detection is 6.5 ± 4.2 yrs. and not different between the 3 groups. A higher proportion (29%) of subjects in the SCC*mec* IV PVL+ had BMI < 10% compared to the SCC*mec* IV PVL- (6%) and SCC*mec* II (15%), p=0.04. In the year of MRSA acquisition, no differences were seen in use of DNase, hypertonic saline, or chronic macrolides but overall use was low during this time frame and in these young patients. Inhaled antibiotics were most frequent in SCC*mec* IV PVL+ (31%) compared to SCC*mec* II (15%) and SCC*mec* IV- (3%), p=0.001. Microbiology in the 12 months preceding the initial MRSA positive culture, showed chronic MSSA in 42-48% of patients and did not differ in the 3 groups. *P. aeruginosa*, defined as "any positive culture per year" was higher in patients with SCC*mec* II MRSA (58%) vs. SCC*mec* IV PVL+ or PVL- (41% and 33%, respectively), (p=0.019), however chronic *P. aeruginosa* was not different (p=0.07). Healthcare utilization measured by number of clinic visits, was higher in the SCC*mec* II group in the 6 months preceding initial MRSA than in patients with SCC*mec* IV PVL+ or PVL- MRSA: 2.5 ± 1.8; 1.7 ± 1.4; 1.8 ± 1.2, p=0.016. This was not significant when extend-

ing to 12 months prior to initial documented MRSA. Hospitalization or IV antibiotics were rare but not different among the 3 groups.

Discussion: Increased number of clinic visits in patients with SCCmec II may indicate worsening clinical status prior to the first MRSA isolation, possible higher use of antibiotics but also being exposed to the hospital setting more often. However, use of inhaled antibiotics was not higher in these patients. Patients with SCCmecIV PVL + had the highest rate of poor nutrition. It deserves further analyses if this may be explained by social status which can also be a risk factor for acquisition of MRSA in the community or could be linked to PVL.

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DETERMINATION OF HYDROLASE ACTIVITIES IN BRONCHOALVEOLAR FLUID OBTAINED FROM CYSTIC FIBROSIS PATIENTS

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Background and Objective: Surfactant produced by type II alveolar epithelial cells contains numerous hydrolases which play a regulatory role in antimicrobial pathways and maintenance of alveolar homeostasis. These hydrolases may alter key properties of microbial cell surfaces and modify specific host-pathogen interactions. The role hydrolases play in acute and chronic CF airway infection and inflammation is poorly understood. The objective of this study was to compare surfactant hydrolases in bronchoalveolar lavage fluid (BALF) taken from CF patients to BALF from healthy individuals.

Methods: BALF was obtained from CF patients undergoing bronchoscopy for clinical reason and compared to BALF obtained from healthy donors. A colorimetric method based on the cleavage of p-nitrophenol from a specific substrate upon enzymatic activity was used to measure the hydrolase activities present in the samples. Up to 25 µl of substrate (2.5 mM) and 25 µl of concentrated BALF were loaded in a 96-well plate. After 1 h incubation at 37°C with gentle shaking, reactions were stopped by adding 65 µl of 1 M sodium carbonate and the optical density (OD) read at 450 nm.

Results: BALF from a total of 6 CF patients was obtained. There were 4 females and 2 males with mean age of 13.5 years (range 7-28). Of the 6 patients, 3 (50%) had infection with MRSA and 2 (33%) had chronic infection with *Haemophilus influenzae*. The BALF was neutrophilic in nature with mean cell differential of 70% neutrophils, 26% macrophages, 3% lymphocytes, and 1% eosinophils. The activities of 17 hydrolases from BALF are summarized in Figure 1. CF BALF had significantly higher levels of acid phosphatase, α-mannosidase, β-galactosidase, α-glucosidase and phospholipase C than healthy controls.

Conclusions: This study demonstrates that using a colorimetric approach we can identify the basal activity of several hydrolases in BALF, where we found higher activity levels of several surfactant hydrolases in CF patients compared to healthy individuals. Hydrolases with the most abundant catalytic activity and the most significant differences from healthy controls were acid phosphatase and α-mannosidase. The focus of future studies will be to determine whether these hydrolases remodel the *P. aeruginosa* and *S. aureus* cell wall and how this impacts the outcome of microbial infections observed in CF patients.

Hydrolase content in the CF BAL (normalized to 1 mg/ml of phospholipid) CF patients, n=6 by duplicate Healthy donors, n=12 by triplicate Student t-test CF vs. healthy donors *p<0.05; **p<0.005; ***p<0.0001

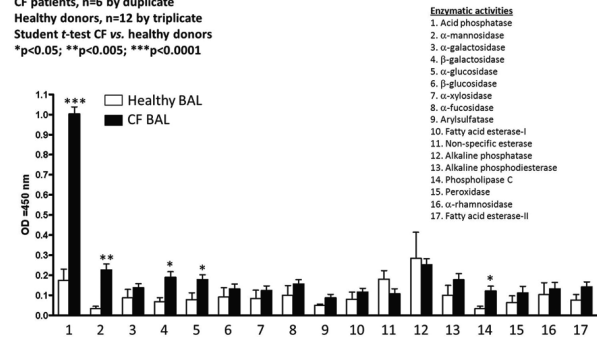


Figure 1

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FIRST GROWTH PSEUDOMONAS AERUGINOSA ERADICATION - A 16 YEAR PERSPECTIVE

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Introduction: Cystic fibrosis (CF) patients infected with *Pseudomonas aeruginosa* (Psa) are likely to have increased pulmonary disease and a more rapid decline in pulmonary function compared to those who are Psa culture and antibody negative. In recent years, the eradication of Psa at its initial culture appearance has become a treatment goal and standard of care with several protocols currently in practice. Since 1996 our pediatric CF clinic has had a *Pseudomonas* eradication protocol that includes an initial 2 weeks of IV antibiotics (piperacillin and tobramycin), followed by 3 weeks of oral ciprofloxacin and 6 months of nebulised colymycin.

Patients and Methods: CF patients (n=135 in 2011, ages newborn to 18 years) are routinely seen in the CF clinic every 3-4 months or more frequently if newborn or otherwise clinically indicated. Provincial-wide CF newborn screening was implemented in November 2009 in British Columbia. Comprehensive review of growth, nutrition, pulmonary function (for > or = 6 years of age), physiotherapy technique and cough swabs or sputum cultures are obtained at every clinic visit. Anti-*Pseudomonas* antibodies have been measured since 2008 using *Pseudomonas*-CF IgG antigen ELISA testing kit from Statens Serum Institut, Denmark. Clearance of Psa is defined as 3 consecutive negative cultures for Psa over a 6 month period (Leeds criteria).

Results: During the 16 years that we have followed this eradication protocol at our clinic, 95 patients had "first growth" Psa cultures (i.e. all previous cultures were negative for Psa) and were treated with the described protocol. The mean age at first growth of Psa was 7 years of age (range 6 months to 16 years of age). The average number of patients with a first growth Psa was 7 per year (range 2-14 per year). Psa clearance success rate in this group of patients was 96%. At the time of first growth Psa, anti-Psa antibody levels were available in 16 first growth patients, 14 (87%) were antibody negative. All Psa were classic strains. Since the institution of this early eradication protocol, the percentage of CF children attending our clinic who are chronically colonized with *Pseudomonas aeruginosa* has steadily declined from 44% in 1995 to 14% in 2011.

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THE EFFECT OF BURKHOLDERIA COLONY MORPHOLOGY IN CYSTIC FIBROSIS (CF) PATIENTS WITH CHRONIC BURKHOLDERIA SPECIES IN A RANDOMIZED TRIAL OF AZTREONAM FOR INHALATION SOLUTION (AZLI)

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Objectives: Examination of mucoid exopolysaccharide (EPS) production among *Burkholderia* spp. (Burk) has demonstrated an inverse correlation with rate of decline in lung function in CF patients (pts) with CF. A randomized, blinded placebo (PBO)-controlled trial of continuous AZLI therapy in addition to standard care (SOC) in CF pts with chronic Burk airway infection has been completed in 100 pts (AZLI: 48, PBO: 52). No statistically significant differences were detected between AZLI and PBO-treated patients in change from baseline in FEV1 % predicted, as measured by the time-adjusted area under the curve (AUCave). A post-hoc analysis was performed to evaluate the effect of Burk morphology on lung function and changes in lung function relative to baseline for all study participants.

Methods: In this trial, pts ≥6 yrs old with protocol-defined chronic Burk infection (>50% of respiratory cultures confirmed as Burk positive by a reference lab in past year) and stable pulmonary disease were enrolled. Pts were randomized to receive 24 wks of AZLI 75 mg or PBO TID every day via a Pari Investigational eFlow® Nebulizer System, followed by 24 wks of open label AZLI 75 mg TID daily. Investigator-determined SOC, including use of systemic or non-study inhaled antibiotics was allowed. At the completion of the trial, Burk isolated at baseline or at the next available subsequent visit were recovered from freezer stocks and subcultured onto yeast extract medium (YEM), streaking for individual colonies. Plates were incubated for 48 hours at 35°C and scored for EPS production. Mucoidy was scored as non-mucoid (NM), partially mucoid (PM) and frankly mucoid (either 2+ or 3+). Control strains included J2315 and LMG13010 (NM), 249-2 and PC184 (PM), CEP509 and LMG14086 (2+), and ATCC25416 and J415 (3+). Pts were sub-grouped according to their most mucoid Burk isolate. Comparisons in lung function (baseline and change from baseline) were made between NM and frankly mucoid (3+) Burk subgroups.

Results: Isolates were available from 98 subjects; 14 had only NM and 40 had at least one frankly mucoid (3+) Burk at baseline. Equal number of ALZI (n=7) and PBO (n=7) pts were infected with only NM Burk. The median baseline FEV1 % predicted (42 vs 66) was lower in pts infected with only NM Burk compared to pts infected with at least one frankly mucoid (3+) Burk. The subgroup of pts with only NM Burk exhibited similar decreases in lung function over a 6 month period compared to pts with frankly mucoid Burk.

Conclusions: In this PBO-controlled trial, baseline differences in lung function between pts with NM Burk and frankly mucoid (3+) Burk suggest that Burk colony morphology may be associated with lung function. However, Burk mucoidy was not associated with rate of decline during the 6 month PBO controlled segment of this trial.

Supported by Gilead Sciences.

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AN NIH-FUNDED MECHANISM TO PROVIDE CYSTIC FIBROSIS CLINICAL BACTERIAL ISOLATES TO ACADEMIC RESEARCHERS

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Clinical isolates of *Pseudomonas aeruginosa* and other CF pathogens are of value to researchers studying CF microbiology in both academic and industry settings. Organisms with specific phenotypes including mucoidy and multiple antibiotic resistance are critical to the evaluation of potential

new therapeutic agents. Serial isolates from individual CF patients offer the opportunity to study the genotypic and phenotypic variability that evolves over time in the CF airways. Previously funded by the CF Foundation, the CF Isolate Core at Seattle Children's Research Institute is now funded by an NIH P30 grant (PD: Bonnie Ramsey, MD) as a part of the Microbiology Core (PI: E. Peter Greenberg, PhD). Developed over the past 2 decades, the CF Isolate Core has a well-established archived collection of *P. aeruginosa*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, *Achromobacter* spp., methicillin-susceptible and -resistant *Staphylococcus aureus* isolated at the clinical laboratory at Seattle Children's, University of Washington Medical Center and the Therapeutics Development Network (TDN) Center for CF Microbiology. Isolates have been stored at -70°C and cataloged. Many of the isolates that are available have linked clinical data that can be used for epidemiologic studies and to evaluate clinical status at the time isolates were obtained. The process for researchers to obtain these isolates is initiated by an email to the CF Isolate Core at Seattle Children's (CFIsolateCore@seattlechildrens.org). Researchers will be asked to provide some basic information on planned studies and the characteristics of isolates requested. Subsequently, a Materials Transfer Agreement will be established and the isolates retrieved from the archive and shipped to the requester. Some clinical data can be provided in a de-identified fashion. However, if additional information is needed, an IRB application must be submitted to Seattle Children's. Isolates from research studies conducted through the TDN can also be obtained for specific studies along with linked study data. There is a separate process for these requests which should be submitted to the TDN. Industry investigators may also use the CF Isolate Core, but commercial use is not an NIH-funded activity and additional charges will apply. Supported by NIH P30 DK089507.

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NONTUBERCULOUS MYCOBACTERIUM IN CHILDREN WITH CYSTIC FIBROSIS: A SINGLE CENTER EXPERIENCE

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Background: Cystic fibrosis patients are susceptible to various bacterial infections, including nontuberculous mycobacterium (NTM). Various studies have shown an increased prevalence of NTM lung infections in CF patients, with *M. avium*-complex (MAC) being the most commonly cultured. There is limited published data on NTM infections in CF. Our goal was to describe our experience with NTM lung disease in pediatric patients with CF.

Methods: We conducted a retrospective review of pediatric patients with CF who have received care at Oregon Health & Science University (OHSU) utilizing Cystic Fibrosis Foundation Registry as well as hospital databases to identify patients who had a positive NTM culture. Clinical and microbiological data was abstracted from available electronic and written data sources. Sputum clearance was defined as having ≥2 consecutive negative cultures after initiation of treatment regimen.

Results: Eleven pediatric patients with CF had at least one positive culture for NTM from 2003-2012 (82% were female). Average age at first positive NTM culture was 13.4 years old. Average FEV1 at time of first positive culture was 90% predicted. Two patients (18%) had single positive NTM culture while 9 (82%) had >3 positive NTM cultures. Two patients (18%) were smear negative while the rest were smear positive. Nine (82%) patients grew *M. abscessus* complex, 2 (11%) grew MAC and a single patient grew both. History of CF surveillance culture pathogens in the 2 years prior to positive NTM culture; 10 (91%) grew MSSA, 9 (82%) grew *S. maltophilia*, 7 (64%) grew *P. aeruginosa*, and none grew MRSA. Ten patients (91%) fulfilled ATS criteria for NTM lung disease and at the time of this abstract; 1 (10%) stopped growing NTM without treatment with stable lung disease, 3 (30%) were under evaluation for NTM antibiotic therapy, 1 (10%) completed NTM antibiotic regimen and 5 (50%) are currently being treated. The medications used were: amikacin (100%), azithromycin (100%), tigecycline (67%), clofazamine (33%), cefoxitin (17%), ethambutol (17%), and rifampin (17%). Tigecycline 50mg IV daily dosing was utilized at OHSU. Average time on parenteral NTM therapy was 5.3 months. Four patients (67%) had stable FEV1 after NTM treatment was initiated while 2 (33%) had significant decline in FEV1 after NTM treatment. Five (83%) had sig-

nificant improvement in CT findings after NTM treatment. None of the patients treated for NTM had sputum clearance of NTM.

Conclusion: At our center, *M. abscessus* was the most commonly cultured NTM, which differs from previously published data. The majority of patients had history of infection with *S. maltophilia*, *P. aeruginosa* and *MSSA* but none with *MRSA*. After initiation of NTM antibiotic regimen, most patients had stable FEV₁ and improved CT findings but none had sputum clearance. Additional studies are needed to better understand the microbiology of NTM infections, indications for treatment and NTM treatment regimens.

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LUNG FUNCTION IN CYSTIC FIBROSIS (CF) PATIENTS WITH CHRONIC *BURKHOLDERIA* (*BURK*) SPECIES INFECTION OVER THE COURSE OF A PROSPECTIVE, RANDOMIZED TRIAL OF AZTREONAM FOR INHALATION SOLUTION (AZLI)

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Objectives: *Burk* spp. infections cause significant morbidity/mortality in CF patients (pts). *Burk*-infected CF pts have been excluded from prior inhaled antibiotic trials. Thus, no comprehensive clinical trial datasets exist on CF pts with *Burk* spp infection. A 48 wk randomized, blinded placebo (PBO)-controlled 2-part trial of continuous AZLI therapy in addition to standard care (SOC) in CF pts with *Burk* airway infection has been completed. One hundred pts were randomized (AZLI: 48, PBO: 52) and 75 pts completed 48 wks of study treatment. Baseline FEV₁% predicted varied widely (mean 57%; range 16%-115%). *B. cenocepacia* and *B. multivorans* accounted for 40% and 27% of infection, respectively. *P. aeruginosa* was isolated in 30% of pts. More than 90% of pts received additional systemic or inhaled antibiotics for respiratory exacerbations or maintenance therapy. No statistically significant differences were detected between AZLI and PBO-treated patients in change from baseline in FEV₁% predicted, as measured by the time-adjusted area under the curve (AUCave). A post-hoc analysis was performed to further evaluate lung function changes relative to baseline for all study participants.

Methods: In this recently completed trial, pts ≥6 yrs old with protocol-defined chronic *Burk* infection (>50% of respiratory cultures confirmed as *Burk* spp. positive by a reference lab in past year) and stable pulmonary disease were enrolled. Pts were randomized to receive 24 wks of AZLI 75 mg or PBO TID every day via a Pari Investigational eFlow® Nebulizer System, followed by 24 wks of open label AZLI 75 mg TID daily for all pts. Investigator-determined SOC, including use of systemic or non-study inhaled antibiotics was allowed. Pts were identified as having lung function maintenance or improvement, defined as AUCave of percent change from baseline FEV₁% predicted of ≥0 or ≥5, respectively, for both the 24 wk comparative phase and for the 48 wk study overall.

Results: During 48wks of SOC + PBO and/or AZLI, 40% of patients maintained and 17% improved FEV₁.

Conclusions: Subsets of *Burk*-infected CF pts maintained or improved FEV₁ over the course of this 48-wk study. Further analyses are ongoing to attempt to identify factors that may differentiate patients who maintained or improved lung function from those who experienced loss of FEV₁.

Supported by Gilead Sciences.

Patients who maintained or improved FEV₁

FEV ₁ AUCave	Comparative Phase (weeks 0-24)		Overall* (weeks 0-48)	
	≥ 0	≥ 5	≥ 0	≥ 5
AZLI	21/47 (45%)	6/47 (13%)	21/47 (45%)	5/47 (11%)
Placebo	22/52 (42%)	10/52 (19%)	19/52 (37%)	12/52 (23%)
Total	43/99 (43%)	16/99 (16%)	40/99 (40%)	17/99 (17%)

*all patients received AZLI in open label phase (weeks 24-48)

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OPTIMIZING VIRAL RNA ISOLATION AND IDENTIFICATION FROM CYSTIC FIBROSIS SPUTUM DURING EXACERBATIONS: SUPPORT FOR VIRAL SURVEILLANCE

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Background: Viral infections of the respiratory tract are common in the general population and are usually not associated with long-term complications. Persons with cystic fibrosis (CF) acquire viral infections at similar rates with greater risk of long-term effects on respiratory health. It is presumed that viral infections frequently trigger CF exacerbations leading to aggressive antibiotic treatment. However, the role of respiratory viruses in contributing to CF exacerbations and lung disease progression is not well understood. Nasopharyngeal (NP) or oropharyngeal swabs are often used to identify respiratory viruses, but their representation of lower respiratory tract illness is poorly defined.

Objectives: To optimize a viral RNA isolation method from expectorated CF sputum and identify respiratory viruses by using two methods of identification, a novel viral PCR ligation detection reaction multiplex assay and a commercial PCR viral identification kit.

Methods: CF sputum was spiked with various concentrations of rhinovirus 39, and RNA was extracted using column- and solution-based commercial kits. Extraction with Trizol (Invitrogen Corp) gave the highest recovery of total RNA and was further optimized to improve RNA quality. From a large bank of CF sputum specimens recovered as discarded samples from University of Michigan clinical microbiology laboratory (UMCML), samples were identified for which UMCML viral results were available from an NP specimen collected within two weeks of the sputum sample. RNA was isolated from these samples and subjected to the viral multiplex assay.

Results: Viral multiplex assay analysis of 59 sputum samples from 35 CF patients (mean age 24 years) collected between September 2009 and April 2012 identified twelve specimens positive for rhinovirus, seven positive for influenza A, three positive for parainfluenza and one each positive for influenza B, coronavirus, human metapneumovirus and co-infection of influenza A/rhinovirus by any of the three methods of identification. UMCML viral results from the NP samples associated with these 25 sputum specimens detected three samples with influenza A, including the co-infected sample, and one sample with influenza B. UMCML viral tests also identified parainfluenza in an additional NP sample not detected by viral multiplex assay in the associated sputum sample.

Conclusions: We optimized a viral isolation method and have developed a low cost system for surveillance of the most common upper respiratory viruses. This PCR based assay detected virus in CF sputum samples that have been stored frozen for several years. The presence of virus in CF sputum during exacerbations may be more prevalent than expected. The role of viruses in CF exacerbations should be further elucidated in future research.

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CLINICAL CHARACTERISTICS AND OUTCOMES IN CHILDREN WITH CYSTIC FIBROSIS INFECTED WITH *MYCOBACTERIUM ABSCESSUS* COMPLEX SUBSPECIES: A CASE SERIES

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Background: Previous studies have reported poorer clinical outcomes in adults with *Mycobacterium abscessus* (recently reclassified as *M. abscessus* subsp. *abscessus*) lung disease compared to those infected with *M. mas-*

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siliense (reclassified as *M. abscessus* subsp. *massiliense*). Whether this translates to the pediatric cystic fibrosis (CF) population is unknown.

Objective: To evaluate differences in clinical characteristics and outcomes in children with CF infected with *M. abscessus* subsp. *abscessus* versus subsp. *massiliense*.

Methods: We retrospectively identified all CF patients less than 21 years old at our center with at least 2 positive sputum or bronchial cultures for *M. abscessus* complex during the years 2000-2011. We performed molecular identification of stored isolates to allow for comparisons of clinical characteristics and outcomes between patients with probable pulmonary infection with *M. abscessus* subsp. *abscessus* and those with *M. abscessus* subsp. *massiliense*.

Results: We identified 10 patients with at least 2 positive cultures for *M. abscessus* complex and clinical evidence of pulmonary infection for which isolates for molecular identification were available. Half of the patients were *M. abscessus* subsp. *abscessus* positive. Nine of the patients received antimycobacterial therapy. We found no significant differences in clinical characteristics or outcomes between patients infected with *M. abscessus* subsp. *abscessus* versus those with positive cultures for *M. abscessus* subsp. *massiliense* (Table).

Conclusions: We did not find major differences in clinical characteristics or outcomes in a modest single-center cohort of children with CF infected with *M. abscessus* subsp. *abscessus* versus those infected with *M. abscessus* subsp. *massiliense*.

Clinical characteristics at first positive culture and selected outcomes

	Subsp. <i>abscessus</i> (n=5)	Subsp. <i>massiliense</i> (n=5)
Age, years*	12 (4)	14 (1)
Female, n [†]	4 (80)	2 (40)
Body mass index, percentile for age*	33 (17)	39 (8)
Genotype, n [‡]		
ΔF508 homozygous or heterozygous	4 (80)	3 (60)
Other	1 (20)	2 (40)
FEV1, percent predicted*	78 (6)	84 (12)
Hemoptysis, n [‡]	2 (40)	1 (20)
AFB smear positive, n [‡]	5 (100)	3 (60)
Received anti-mycobacterial treatment, n	5	4
Microbiologic response, n [‡]		
Sputum conversion to negative without relapse	1 (20)	2 (50)
Sputum conversion to negative with relapse	0	0
Persistently positive sputum culture for AFB	4 (80)	2 (50)
Follow-up, months [‡]	155 (108, 181)	125 (123, 142)

Data presented as *mean (SD), [†]number (%), [‡]median, (interquartile range)

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THE BACTERICIDAL EFFECT OF COLISTIN ON *PSEUDOMONAS AERUGINOSA* IS INDEPENDENT OF HYDROXYL RADICAL FORMATION

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Background: Chronic lung infection with *Pseudomonas aeruginosa* is the most severe complication in CF. This is in part due to the low susceptibility of *P. aeruginosa* to antibiotic treatment. It has recently been demonstrated that the bactericidal effect of several major types of antibiotics is dependent on oxidative stress in metabolically active cells resulting from formation of toxic amounts of hydroxyl radicals. The formation of hydroxyl radicals implies a need for oxygen and, since *P. aeruginosa* may reside in oxygen-depleted areas of CF airways, we speculated that the killing effect of colistin, an antimicrobial peptide whose bactericidal activity does not rely on bacterial metabolic activity, is not dependent on formation of hydroxyl radicals.

Methods: The effect of colistin and ciprofloxacin treatment on killing curves and formation of hydroxyl radicals was compared in cultures of *P. aeruginosa* with aerobic and anaerobic metabolic activity as well as in cultures with reduced metabolic activity.

Results: When hydroxyl radical formation in *P. aeruginosa* cultures was prevented by growth in oxygen-depleted media or by abolishing metabolic activity by culturing in 0.9% saline the susceptibility to colistin was

increased while the susceptibility to ciprofloxacin was decreased. Accordingly, formation of hydroxyl radicals was significantly increased only when aerobically growing cultures were treated with ciprofloxacin. In addition, no formation of hydroxyl radicals in *P. aeruginosa* killed by colistin was seen by flow cytometric real-time analysis. Furthermore, prevention of hydroxyl radical formation and scavenging of hydroxyl radicals could not rescue *P. aeruginosa* from killing by colistin.

Conclusion: Our results demonstrate that the bactericidal activity of colistin is not caused by oxidative stress. Therefore, antimicrobial peptides might be useful for treatment of *P. aeruginosa* existing at low oxygen tension such as in the endobronchial mucus and paranasal sinuses in CF or in biofilm.

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ELEVATED LEVELS OF ANTIBODIES AGAINST *MYCOBACTERIUM ABSCESSUS* IN CYSTIC FIBROSIS PATIENTS IS ASSOCIATED WITH ACTIVE LUNG DISEASE

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Background: The clinical presentation of *M. abscessus* lung disease in patients with cystic fibrosis (CF) varies from sporadic colonization to invasive debilitating disease. Diagnosis and monitoring disease progression during treatment can be notoriously difficult. A serological method could potentially guide treatment, a principle which has previously been shown clinically useful with other CF pathogens. We present a multi-antigen method for investigating antibody activity against *M. abscessus*.

Objective: To test an enzyme-linked immunosorbent assay (ELISA) method for measuring immunoglobulin G (IgG) levels in serum from CF patients in order to improve the understanding of antibody responses to mycobacterial antigens.

Methods: Mycobacterial antigens were produced by X-pressing and sonication of a *M. abscessus* obtained from a CF patient. Antimycobacterial antibody levels were determined by enzyme-linked immunosorbent assay (ELISA). Micro-titer wells were coated with the diluted *M. abscessus* antigen, washed and blocked with buffer to reduce nonspecific binding. Serum samples were then applied to wells coated with mycobacterial antigen. Rabbit anti-human IgG conjugate was added and after rewashing, substrate was applied. The reaction was measured, using an ELISA reader.

Results: A total of 305 CF patients and 12 healthy controls were screened cross-sectionally for *M. abscessus* IgG levels. Results are shown in the Table. Longitudinal determination of IgG levels was subsequently performed on 15 of the patients who fulfilled ATS criteria with > 1 positive *M. abscessus* culture. The median observation time was 12 years with an average of one serum sample per patient per year. IgG kinetics were well correlated with time of first positive culture and number of positive cultures. Mean peak IgG was 155 ELISA units during time of disease. Treated patients who cleared infection showed sharp drops in IgG levels. The study is still in progress.

Conclusion: Elevated levels of IgG antibodies against *M. abscessus* correlate to invasive disease. Further investigation will reveal the diagnostic and prognostic value of the method.

Table 1. Median IgG levels of 305 cystic fibrosis patients and 12 healthy controls

	Number of subjects	IgG level (ELISA units) median (IQR)
Patients with active <i>M. abscessus</i> disease at time of screening	7	123 (58 - 160)
Patients with > 1 positive <i>M. abscessus</i> culture at any time	19	41 (8 - 115)
Patients with only 1 positive <i>M. abscessus</i> culture at any time	5	22 (7 - 29)
Patients with ≥ 1 <i>M. avium</i> culture at any time	17	13 (8 - 32)
Patients with no positive mycobacterial culture	264	9 (4 - 20)
Healthy controls (non cystic fibrosis hospital staff)	12	7 (2 - 15)

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CYSTIC FIBROSIS-ADAPTATION OF *PSEUDOMONAS AERUGINOSA* MODULATES INNATE IMMUNE SYSTEM DETECTION AND TISSUE DAMAGE CONTROL

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CF lung disease is characterized by transient airway *P. aeruginosa* infections and excessive neutrophil-dominated inflammation early in life followed by permanent chronic infection that causes persistent respiratory symptoms and decline in lung functions. As the disease progresses, strain variants can be distinguished from the strain initially acquired. However, the contribution of *P. aeruginosa* adaptation to lung disease is not completely understood. Comparing sequential strains isolated from a CF patient in multi-host pathogenesis system, including non-mammalian and mammalian hosts, our previous works showed that *P. aeruginosa* early strains were lethal, while late adapted clonal isolates were attenuated in acute virulence but retained their capacity to persist in murine lungs (Bragonzi, AJRCCM 2009; Cigana, PlosOne 2010; Lorè, PlosOne 2012). To dissect the contribution of *P. aeruginosa* patho-adaptive strains during the progression of lung disease, we report the host-response in murine models of acute and chronic infection. As a model system in studying long term and persistent CF infections, the agar beads mouse model was used in a time course for up to three months in C57Bl/6 mice and *Cftr*^{tm1UNC}TgN(FABPCFTR) mice. Bacterial count, inflammation and lung pathology were evaluated. During acute infection, cytokines and chemokines amounts in the whole lungs by bioplex assay were lower after infection with late patho-adaptive strains compared with early strain, confirming that late strains have evolved the capacity to evade innate immune system detection. After one month of chronic infection with *P. aeruginosa* late strains, cytokines/chemokines involved in leukocytes recruitments (IL-1 β , IL-6, KC, MIP-1 α and MIP-2) were lower than after acute infection. Impressively, MCP-1, IL-17, IFN- γ and TNF- α , correlated to tissue damage control, were maintained during acute and chronic infection, indicating a continuous and elevated triggering of specific pathways. In addition, high amounts of sulphated glycosaminoglycan in lung homogenates, detected by a dye-binding assay, and TGF- β production, measured by bioplex, were found in chronic infection, suggesting that late adapted strains may induce damage to airways. Histopathological examination of lung tissue sections showed that late adapted strains induced mucin-positive goblet cells metaplasia, collagen deposition and apoptosis, typical hallmarks of damage in the airway of CF patients. *P. aeruginosa* immunofluorescence staining showed that the persisting bacterial cells were localized in the bronchial lumen both in the beads and in biofilm-like structures.

These results suggest that during chronic infection *P. aeruginosa* patho-adaptive variants revisit their interaction with host attenuating innate immune system detection, while modulating tissue damage. Our findings, obtained through murine models, can be translated to an improved understanding of CF human disease.

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BAX/BAK REQUIREMENT FOR *PSEUDOMONAS AERUGINOSA* HOMOSERINE LACTONE-STIMULATED APOPTOSIS AND INFLAMMATION RESPONSES IN FIBROBLASTS

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P. aeruginosa accumulate and form biofilms in the lungs of cystic fibrosis (CF) patients. *P. aeruginosa* uses N-(3-oxododecanoyl)-homoserine lactone (C12) as a quorum-sensing molecule to control bacterial gene expression, including production of biofilms and virulence factors. Because C12 may be present in high concentrations in biofilms (estimates range to 100 μ M) and C12 has high lipid solubility, multiple cell types are likely to be exposed to C12 in CF lungs, including fibroblasts, epithelia, endothelia, macrophages and mast cells. C12 induces apoptosis and alters inflammatory responses in multiple types of host cells, though effects on inflammatory processes appear to depend on cell type and the specific response being

measured. Biofilms elicit C12-like, proapoptotic effects on mitochondrial membrane potential, Ca²⁺ signaling and IL8 secretion of airway epithelia (Schwarzer Cell Micro 14:698-709, 2012). C12 also triggers the unfolded protein response (Kravchenko, JBC 281:28822-30, 2006). The present work tested whether bax and bak were involved in the effects of C12 on fibroblasts. In wild type mouse embryo fibroblasts (wt MEF's) C12 (50 μ M) elicited, within 60 mins, typical apoptosis-associated effects, including blebbing of plasma membranes, condensation of nuclei, release of cytochrome C from mitochondria into the cytosol and activation of caspase 3/7. C12 also caused, within 3-15 mins, Ca²⁺ to be released from the endoplasmic reticulum (ER) into the cytosol and mitochondria to depolarize. C12 caused none of these effects in bax^{-/-}/bak^{-/-} (dko) MEF's. C12 had contradictory effects on NF- κ B in wt MEF's, causing NF- κ B p65 to enter the nucleus from the cytosol during the first 45 mins of treatment (immunofluorescence), but inhibiting NF- κ B-regulated activity (luciferase expression) measured at 4 hrs. C12 also had contradictory effects on proinflammatory cytokine genes in wt MEF's: C12 stimulated expression (QPCR) but inhibited secretion (ELISA) of IL6 and KC in both untreated and in TNF α -treated wt MEF's. In contrast, C12 had no effect on either activity of NF- κ B or expression/secretion of IL6 and KC in either untreated or in TNF α -treated dko MEF's (all at 4 hrs). Thus, C12-triggered apoptosis and inflammation responses all required bax/bak. It is proposed that C12 regulates bax/bak (or closely associated protein), which orchestrates a wide array of cellular events and signaling in host cells: (i) depolarization of mitochondrial membrane potential; (ii) release of cytochrome C (followed by activation of caspase 3/7 and apoptosis); (iii) activation of IP₃ receptor (followed by release of Ca²⁺ from the ER into the cytosol); (iv) activation of NF- κ B (and expression of proinflammatory cytokine genes); (v) inhibition of cytokine production and secretion resulting from effects of C12 to trigger ER stress and inhibit protein synthesis. These C12-triggered, bax/bak-dependent responses share many characteristics with those triggered by pattern associated molecular pattern receptors (e.g., TLR's and NODs).

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***PREVOTELLA* SPECIES ISOLATED FROM CF PATIENTS: ANTIBIOTIC SUSCEPTIBILITY AND PRESENCE OF ANTIBIOTIC RESISTANCE GENES**

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Introduction and Aims: *Prevotella* spp. are the predominant anaerobe detected in CF pulmonary samples and may be a target for future antibiotic treatment. Therefore, the aims of this study were to determine and compare (1) *in vitro* antimicrobial susceptibility of *Prevotella* isolates cultured from CF (US and UK) and non-CF (UK) patients to a range of antibiotics and (2) the presence of antibiotic resistance genes.

Methods: The susceptibility of *Prevotella* isolates (UK CF, n=43; US CF, n=24; Non-CF, n=51) to 12 antibiotics (Graph) was determined by Etest. Four PCR assays were developed and used to detect genes encoding for resistance to penicillins and broad-spectrum cephalosporins (*cfxA/cfxA2*), metronidazole (*nim*), lincosamides, macrolides and streptogramins (*ermF*) and tetracyclines (*tetQ*).

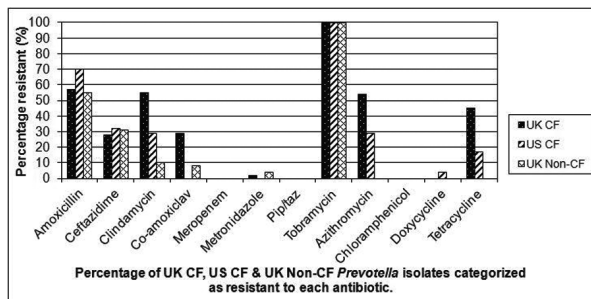
Results: All isolates tested were resistant to tobramycin but susceptible to piperacillin, meropenem and chloramphenicol. Amoxicillin, ceftazidime, metronidazole and doxycycline resistance was similar between the groups. In contrast, clindamycin resistance was greater among the CF *Prevotella* isolates and co-amoxiclav resistance was more common among the UK CF *Prevotella* isolates (Graph). Overall, 53/111 (48%) isolates were *cfxA/cfxA2* positive with the majority of *cfxA/cfxA2* positive isolates having reduced susceptibility to amoxicillin (n=49/53; 92%). Two of 109 (2%) isolates were *nim* positive but were susceptible to metronidazole. Twenty-six of 108 (24%) isolates were *ermF* positive with 20/26 (77%) and 9/9 (100%) isolates also resistant to clindamycin and azithromycin, respectively. Furthermore, 23/101 isolates were *tetQ* positive with 5/10 (50%) *tetQ* positive CF isolates also resistant to tetracycline.

Conclusions: The target genes were common among the *Prevotella* isolates and can be associated with antibiotic resistance. There were differences in antibiotic resistance between CF and non-CF isolates as well as between UK and US CF isolates. Based on these findings, if *Prevotella* are

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contributing to infection in the CF lung meropenem, ptp/taz and metronidazole would be the most appropriate treatment options.

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GENETIC BACKGROUND OF MRSA COLLECTED FROM CYSTIC FIBROSIS (CF) PATIENTS VERSUS MRSA COLLECTED FROM INTENSIVE CARE UNIT (ICU) PATIENTS: DOES ANY DIFFERENCE EXIST?

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Background: Nowadays MRSA represents a worldwide public health issue, playing an important role in CF patients. It has been recently demonstrated that the detection of MRSA in the respiratory tract of CF patients is associated with worse survival; moreover, MRSA is a leading pathogen in ICUs also. Based on their genetic background, and in particular the genetic mobile element Staphylococcal Chromosome Cassette mec (SCCmec), it is possible to distinguish between community-associated MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA). This distinction is important due to the high hospitalisation rate of both CF and ICU patients. Although several epidemic clones have been already described worldwide, data concerning genetic background of both CF and ICU patients are often scanty. The purpose of this study was to supply data concerning genetic background and antimicrobial susceptibility of MRSA coming from respiratory samples of CF and paediatric ICU patients to highlight any difference.

Materials and Methods: MRSA strains were collected from respiratory samples of ICU patients and CF patients during a two-year period of time (2010-2011). Antibiotic susceptibility will be evaluated by assessing the activity of a large panel of drugs by means of the disk diffusion test on Mueller-Hinton agar (Kirby Bauer). SCCmec typing was performed on each strain, as previously described by Oliveira and de Lancastre in 2002. Multi Locus Sequence Typing (MLST) (Enright et al, 2000) was the tool used to define the global epidemiology picture of collected strains.

Results: Fifty-one MRSA strains were isolated from 24 ICU patients, CF patients supplied 54 MRSA clinical isolates, one strain/year. Antimicrobial susceptibility patterns of the two groups appeared to be different. CF isolates were more resistant to trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, erythromycin, clindamycin, and gentamicin. ICU patients' isolates showed higher resistance against rifampin and doxycycline. ICU specimens were characterized by 17% of HA-MRSA, ST228-MRSA I (Southern Germany clone) being the most represented genotype. CF patients showed 48% of HA-MRSA, and several different genetic backgrounds: Southern Germany clone, ST8-MRSA IV PVL-negative, ST239-MRSA III (Brazilian-Hungarian clone), ST1-MRSA I (Archaic clone).

Conclusions: These data highlight some differences between MRSA isolated from ICU and CF patients. Antimicrobial resistance patterns appeared different, as well as the diffusion of already known clones. These

findings could be explained considering different therapeutic schedules and patient management.

This work was supported by Italian Cystic Fibrosis Research Foundation (grant FFC#11 2009) with the contribution of Angelini.

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COMPARATIVE IN VITRO ACTIVITY OF TEMOCILLIN AGAINST BURKHOLDERIA CEPACIA COMPLEX MEMBERS AND BURKHOLDERIA GLADIOLI

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Background: *B. cepacia*-complex (Bcc) strains are often multidrug-resistant due to innate and acquired mechanisms of resistance, but few data are available about the antibiotic susceptibility patterns of different genomovars (gvs) in cystic fibrosis (CF) patients. Recently, *Burkholderia gladioli* (BG) has also been considered an important pathogen in CF lung infections. Data concerning *in vitro* activity of antimicrobial agents on different gvs and BG are still poor, in particular concerning the efficacy of the orphan drug temocillin.

Materials and Methods: Fifty isolates were collected from 50 CF patients during a 15-year period of time (1997-2012). The minimum inhibitory concentrations (MICs) were determined by E test. The genomovar (gv) status was determined by *recA*-based PCR assays and DNA sequencing; the presence of genetic elements characteristic of epidemic lineages such as BCESM, IS 1363, and *cblA* was evaluated by specific PCR tests.

Results: Seven out of fifty (14%) belong to gv I, 4 (8%) to gv II, 27 (54%) to gv III, 3 to gv IV, one to *B. metallica* and 8 were BG. All the selected strains belonging to the same gv showed different susceptibility profiles. Temocillin demonstrated the higher *in vitro* efficacy, while other antimicrobial agents showed different resistance percentages depending on gvs. Ten out of 27 gv III strains were BCESM positive, and one was IS 1363 positive, suggesting the presence of strains belonging to epidemic lineages.

Conclusion: Temocillin appeared the only antimicrobial agent tested during this study efficacious against all the examined strains when compared to the other tested drugs.

Table1. Antimicrobial resistance patterns of collected strains (number of resistant isolates).

gv	Temocillin/Meropenem	Ticarcillin/clavulanic acid	Tobramycin/Ceftazidime	Trimethoprim/sulfamethoxazole	Chloramphenicol		
I (n=7)	0	0	7	5	2	2	
II (n=4)	1	2	3	4	0	1	2
III (n=27)	2	9	19	25	12	15	11
IV (n=3)	2	2	3	3	1	2	3
BG (n=8)	1	2	5	1	4	4	5

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CYSTIC FIBROSIS TH17/TREG IMBALANCE TO *ASPERGILLUS FUMIGATUS*: INSIGHTS FOR NOVEL ANTI-INFLAMMATORY STRATEGIES AND IMMUNOGENETIC SCREENING

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Aspergillus fumigatus is a frequent colonizer and pathogen in cystic fibrosis (CF). Although allergic bronchopulmonary aspergillosis (ABPA) is associated with deterioration of lung function, the relative contribution of *A. fumigatus* colonization versus host Th17-driven inflammation to the disease is not clear. We evaluated parameters of adaptive Th1/Th17/regulatory T (Treg) cell immunity to *A. fumigatus* in Cfr tm1Unc (Cfr-/-) mice, reported to mimic, to some extent, human CF as well as in human CF. Despite visible signs of inflammatory pathology in the lung parenchyma, and in the face of the heightened inflammatory response, only a moderate fungal growth in the lungs of Cfr-/- mice was observed. High levels of inflammatory cytokines, IL-17A and TNF- α and neutrophils could be detected in these mice. Differences were observed in indoleamine 2,3-dioxygenase (IDO) gene expression and protein in Cfr-/- mice as well as human CF compared to the corresponding controls. The tryptophan kynurenin pathway was down-regulated in experimental CF and correlated with an imbalanced Th17/Treg cell responses in the lungs. SNP analysis confirmed IDO deficiency in CF patients. Exogenous supply of kynurenins or IFN- γ , or prevention of Th17 cell activation restored protective immunity to the fungus and improved lung inflammation and pathology in experimental CF. These data are a proof-of-concept demonstration that targeting the Th17/IDO/Treg imbalance and tryptophan kynurenin metabolism could have therapeutic and immunorestitution potential in CF.

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IDENTIFYING BIOMARKERS OF *P. AERUGINOSA* ANTIBIOTIC RESISTANCE

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Background and Objectives: A primary cause of morbidity and mortality for CF patients is chronic *Pseudomonas aeruginosa* (*P.a.*) lung infection, and the acquired *P.a.* phenotypes of mucoidy, antibiotic (Ab) resistance, and quorum sensing deficiency. We hypothesize that specific volatile biomarkers exist for the mutations that confer these phenotypes, making it possible to detect the *P.a.* phenotype in the lung via breath analysis. In this work we have identified the putative biomarkers for the Ab-resistance mutation, *mexA*, using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS) and artificial neural network analysis (ANN), and validated them using *P.a.* clinical isolates bearing this hallmark mutation for Ab resistance.

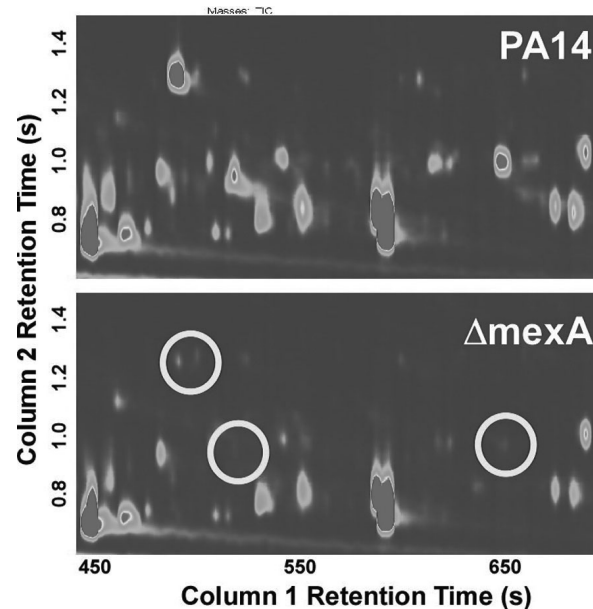
Methods: *P. aeruginosa* strain PA14 wild-type (WT) and $\Delta mexA$ were cultured in synthetic CF medium (SCFM), and the headspace volatiles of the bacterial cultures were identified using GCxGC-TOFMS. ANN analysis was used to identify putative biomarkers for *mexA* mutation, and the specificity and selectivity of these biomarkers were measured against clinical *P.a.* isolates harboring additional mutations in multiple genetic backgrounds.

Results: Using GCxGC-TOFMS and ANN we have identified putative volatile biomarkers for Ab resistance conferred by *mexA* mutation (Figure), and validated these biomarkers against a diverse collection of clinical *P.a.* isolates from chronic CF lung infections.

Discussion and Conclusions: Our data demonstrate that *P.a.* mutations that commonly arise during chronic CF lung disease give rise to putative volatile biomarkers. Tracking the biomarkers for *P.a.* mutations via breath analysis could allow physicians to detect mutations such as antibiotic resistance or mucoid conversion in the lung prior to the successful culture from

sputum or BAL isolates, which would facilitate the implementation of earlier, better-targeted treatments for *P.a.* infection.

Funding: This project is supported by a Cystic Fibrosis Foundation Fellowship and by grants from the NIH-NCRR and the NIH-NIGMS.



GCxGC chromatograms of PA14 WT (top), and $\Delta mexA$ (bottom) in SCFM. The circles indicate volatiles that are significantly reduced in the headspace of the *mexA* mutant, and may serve as biomarkers for Ab resistance.

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THE *PSEUDOMONAS AERUGINOSA* TRANSCRIPTION FACTOR, SPHR, DETECTS HOST-DERIVED SPHINGOSINE TO REGULATE A DEFINED SET OF TRANSCRIPTS INCLUDING CERAMIDASE

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Our long term goal is to understand how *Pseudomonas aeruginosa* detects and responds to the host, particularly in relation to the mammalian lung. We have used the *P. aeruginosa* response to pulmonary surfactant and human respiratory epithelial cells to identify transcripts induced in these conditions. Using this strategy, we identified a set of transcripts, *PA5325*, *PA5328-PA5326*, and ceramidase (*PaCD*), which are strongly and coordinately induced in response to these host-related environments. We noted that the *PA5325* gene was divergently transcribed from an uncharacterized AraC-family transcription factor, *PA5324*. Our goal in this study was to determine the signal that induced these transcripts and determine if the *PA5324* transcription factor was the direct regulator of transcriptional induction.

To measure regulation and induction of *PA5325*, *PA5328*, and *PaCD*, translational and transcriptional fusions to *lacZ* and a *P. aeruginosa* codon-biased SNAP tag were used. Binding of *PA5324* to the *PA5325*, *PA5328*, and *PaCD* promoters was demonstrated by EMSA. Fractionation, heat stability, size exclusion, and a small screen led us to the signal required for transcriptional control of the *PA5325*, *PA5328-PA5326*, and *PaCD* transcripts.

We determined that the *PA5324* transcription factor controls *PA5325*, *PA5328-PA5326*, and *PaCD* transcription in response to host-derived sphingosine. Control is due to direct and specific binding of *PA5324* to the regulated promoters. *PA5324* could control transcription in response to sphingosine induction in an *E. coli* heterologous system, supporting a direct detection of sphingosine by *PA5324*. Based on these results, we have renamed *PA5324* the Sphingosine Regulator (SphR). The SphR-controlled transcripts are induced in response to lung surfactant, respiratory epithelial cells, and

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during contact with macrophages. Deletion of *sphR* eliminates ceramidase activity and reduces hemolysis, phenocopying an alkaline ceramidase mutation.

This is the first identification of a sphingosine-sensitive transcription factor in bacteria. We predict that SphR transcriptional regulation is important in response to many eukaryotic infection sites. This response to sphingosine may be of specific importance in the CF lung where ceramide accumulation would provide abundant precursor material to generate sphingosine. We are currently investigating the importance of this regulation in mouse models of lung infection.

This project was supported by grants from the National Center for Research Resources (5P20RR021905-07) and the National Institute of General Medical Sciences (8 P20 GM103496-07) from the National Institutes of Health.

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THE TREATMENT EFFECT OF CIPROFLOXACIN ON *PSEUDOMONAS AERUGINOSA* IN CYSTIC FIBROSIS PATIENTS

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Objectives: Ciprofloxacin (CIP) is used to treat CF patients and is partly metabolised in the liver by the CYP3A4 enzyme. Earlier studies has shown that CIP concentration can vary a lot from patient to patient, which can result in treatment failure. This variation may partly be explained by the differences in CYP3A4 enzyme activity. We used a Monte Carlo simulation to predict treatment outcome with one or two or three doses a day of oral 500 mg CIP on sensitive *P. aeruginosa* strains. And we investigated the correlation between the enzyme activity and the metabolism of CIP.

Methods: We included 22 CF patients (21-53 yr) with chronic *P. aeruginosa* lung infections. CIP (500mg) was given orally and blood samples were collected every half hour the first 3 h and at 6 and 12 h. The concentrations of CIP were calculated by using a biological assay and AUC, T_{max} and C_{max} were calculated. The Monte Carlo simulation was based on the MIC of 12 sensitive *P. aeruginosa* strains and the pharmacokinetic data from the 22 patients. The clinical breakpoint of CIP on *Pseudomonas* spp. was: $S \leq 0.5\text{mg/L}$ after EUCAST clinical breakpoints and the target at the simulation was an AUC/MIC ratio of 125 or more.

The CYP3A activity was measured using the erythromycin breath test (ERMBT). ERMBT was measured by giving 0.15 MBq [¹⁴C-N-methyl] erythromycin intravenously. Afterwards, the patients exhaled into a glass with liquid collecting the CO₂ every 10 min for one hour. The ¹⁴C activity was measured using liquid scintillation.

Results: A 14-fold variation, median (range), in AUC (mg*min/liter); 473.5 (138-1880) for CIP, a 30-fold variation in C_{max} (mg/liter) 2 (0.25 - 7.6) and a 3 fold-variation in T_{max} (min); 91 (46-153) was found.

The Monte Carlo simulation showed that with one dose of 500 mg oral CIP a day 10% of the patients would be able to eradicate the bacteria, with two daily doses 27% would be able to eradicate the bacteria and with three daily doses 40% would be able to eradicate the bacteria.

We found an 8-fold variation in the CYP3A4 activity, with a median (range) of 0.8 (0.3 - 2.0), but no correlation with CIP metabolism was found.

Conclusion: We found a large variation in the metabolism of CIP. The Monte Carlo simulation showed that only 10-40% of the patients could eradicate the *P. aeruginosa* due to low AUC/MIC ratio. These patients might therefore be in risk of treatment failure, and development of resistance.

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IN VITRO ACTIVITY OF TEMOCILLIN ON MULTIRESTANT *P. AERUGINOSA* ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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Background: Chronic pulmonary disease is characterized by a cycle of inflammation and infection and it is the major cause of morbidity and mortality. It is known that one of the major pathogens for the CF lung is *Pseudomonas aeruginosa* (Pa). Once chronic Pa infection is established, elimination of the microorganism from the airways is difficult, while eradication of Pa by early treatment of the first colonization has been documented. One of the most concerning consequences of treating these infections is the emergence of resistant organisms. Therefore there are limited therapeutic options available. Temocillin (TMO) is an orphan drug resistant to most β -lactamases and is therefore considered a useful alternative to carbapenems in infections caused by several resistant Gram-negative pathogens.

Aims: The objective of the present study was to compare in vitro activity of TMO due to multiresistant (MDR) Pa to other frequently used antimicrobial agents for treatment of pulmonary infections associated with CF: imipenem (IMP), meropenem (MEM), ticarcillin/clavulanate (TCC), cefepime (FEP), ceftazidime (CAZ), ciprofloxacin (CIP), tobramycin (TM) and amikacin (AN).

Methods: Multiresistance was defined as resistance to all agents in two or more of the following three antimicrobial categories: β -lactams, aminoglycosides and fluoroquinolones. The antimicrobial activity effect was investigated by E-test on 42 unique MDR Pa bacteria isolated from CF patients attending two Italian CF Centers, Florence and Verona, over a period of 10 years (2000-2010). All isolated strains were identified with commercial (API20NE, bioMérieux) biochemical tests. Box PCR were performed to exclude strains representing the same clones. Determinations of the MICs of the tested agents were performed according to the manufacturer's recommendations.

Results: The bacterial strains examined were highly resistant to antibiotics (see Table). TMO was the most active agent inhibiting 42.9% of tested MDR Pa strains followed by AN, IMP, MEM, TCC, CAZ, TM, FEP and CIP inhibiting respectively: 17.5%, 14.3%, 14%, 14%, 14%, 11.6%, 7.1% and 4.7%.

Conclusions: TMO was the most active antibiotic against strains of MDR Pa compared to other antibiotics tested. These data suggest that TMO may have potential therapeutic use in CF patients infected with MDR Pa.

Tab 1. The MIC₅₀, MIC₉₀ and range for antibiotics used

Antibiotics	MIC ₅₀ µg/mL	MIC ₉₀ µg/mL	RANGE µg/mL
TMO	128	1024	3-1024
IMP	32	32	0.03-32
MEM	32	32	0.13-256
TCC	256	256	2-256
FEP	256	256	3-256
CAZ	256	256	2-256
CIP	12	32	1.5-32
TM	32	256	1-256
AN	96	256	4-256

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FACTORS INFLUENCING TIME TO FIRST ACQUISITION OF *STENOTROPHOMONAS MALTOPHILIA* INFECTION IN CYSTIC FIBROSIS PATIENTS

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Background: *Stenotrophomonas maltophilia* is one of the most common multi-drug resistant organisms isolated from the respiratory tract of cystic fibrosis (CF) patients. However, little is known about the factors influencing initial pulmonary infection with *S. maltophilia*. The aim of this study

was to characterize the risk factors for initial *S. maltophilia* infection in a longitudinal cohort of CF patients.

Methods: CF patients followed at the Hospital for Sick Children and St Michael's Hospital (Toronto, Canada) from 1997 to 2008 were included in the study. The data for this study was extracted from the Toronto Cystic Fibrosis Database. Patients with *S. maltophilia* infection prior to 1997, who could not produce sputum, who were unable to perform reproducible spirometry or who had received a lung transplant, were excluded. Cox Proportional Hazards models were used to estimate risk factors for acquisition of *S. maltophilia* infection.

Results: A total of 164 of the 800 patients (20.5%) had *S. maltophilia* infection during the follow-up period. The majority of infections (69.5%) were in children <18 years of age. Overall, patients positive for *S. maltophilia*, were: more often diagnosed with CF at a younger age (< 1 yr), homozygous for $\Delta F508$, pancreatic insufficient, underweight and had a previous infection with *P. aeruginosa*. Baseline lung function did not differ between patients acquiring *S. maltophilia* and those who did not. In a multi-variable model, *S. maltophilia* acquisition was more likely in those with lower FEV1 z-scores (HR 1.47, 95% CI 1.27-1.69) and in younger patients (HR 1.03, 95% CI 1.01-1.05). Prior pulmonary exacerbation requiring hospitalization and antibiotic treatment was a strong risk factor for *S. maltophilia* acquisition and was associated with a ten-fold increased risk of *S. maltophilia* infection in CF patients less than 18 years of age (HR 9.69, 95% CI 4.67-20.05) and (HR 4.09, 95% CI 2.03- 8.23) in adults. Of note, prior *P. aeruginosa* infection was a significant risk factor for *S. maltophilia* infection in adults with CF (HR 4.70 95% CI 1.69; 13.08) but was a protective factor (HR 0.46, 95% CI 0.28-0.76) in children. Antibiotic use, recorded as cumulative dose prior to first infection, although significant on univariable analysis, was not an independent predictor of first *S. maltophilia* infection.

Conclusions: CF patients, particularly children with worse lung disease, are at increased risk of *S. maltophilia* acquisition. Cumulative antibiotic use is not an independent risk factor for *S. maltophilia* acquisition in the CF lung but may simply be a marker of increased disease severity. Further studies, particularly in a pediatric population, need to investigate the timing of exposures to other infections and antibiotic use, to better understand the mechanism behind acquisition of *S. maltophilia*.

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THE GUT MICROBIOTA IN CYSTIC FIBROSIS: A COMPLEX COMMUNITY UNVEILED BY META-OMICS APPROACHES

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In cystic fibrosis (CF) patients the normal community of commensal bacteria in the gut can be affected by intestinal exocrine malfunction, antibiotic usage and swallowing of infected respiratory mucus. However, CF-related gut dysbiosis has only recently been subjected to detailed investigation. Herein we report on the integrated metaproteomic, metagenomic and metabolomic workflow for monitoring gut microbiota composition. Twenty-four faecal samples were collected from cystic fibrosis young patients (age range 0-6 years) and 6 faecal samples from healthy children. All samples were processed for peptide, DNA and metabolite analysis. Protein content was analyzed with a shotgun metaproteomic approach by a nano-scale LC-MS2 set-up (Bruker amaZon-ETD) and filtered for operational taxon

units (OTUs) assignment. OTUs were corroborated by hybridization of the small subunit ribosomal RNA (SSU rRNA) gene variable regions against the human intestinal tract microarray platform HITChip. In order to evaluate the volatile metabolites a gas-chromatographic (GC)-mass spectrometer (MS)/solid-phase microextraction (SPME) analysis was performed on Agilent Hewlett-Packard 6890 GC

gas-chromatograph equipped with a MS detector 5970MSD (Hewlett-Packard, Geneva, Switzerland). ¹H-NMR spectra were acquired on a Varian 400 MHz Mercury-plus NMR. Pre-analytical steps of the metaproteomics workflow were devised to improve hit coverage, while the OTUs repository was guaranteed by metagenomics entries. ¹H-NMR and GC-MS/SPME were used to identify and quantify fecal metabolites.

The application of integrated meta-omics-based approaches to the characterization of the membership and dynamics of the polymicrobial communities colonizing the gut is expected to have important predictive potential in translational medicine, specially in CF-related gut dysbiosis.

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MALDI-TOF MS PROTEOMIC PHENOTYPING BOOSTS CF PATIENT-TAILORED PROTOCOLS IN DIAGNOSTIC MICROBIOLOGY

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Cystic fibrosis (CF) patients are currently at high risk for fungal opportunistic infections. Identification (ID) of the increasing diversity of fungal pathogens by conventional methods is often difficult, time-consuming and frequently, for unusual fungi, inconclusive. We designed and set up a MALDI-TOF MS-based assay to identify the most isolated and emerging therapy-refractory/uncommon fungi from CF and immunocompromised patients. The aim was achieved by generating a dedicated engineered library of reference spectra from solid Sabouraud agar (SDA), used in the diagnostic ID workflow. A growth time-course at 30°C was performed for 22 morphotypes belonging to 10 different genera and 2 phyla (the ascomycetes *Aspergillus*, *Emericella*, *Fusarium*, *Geosmithia*, *Neosartorya*, *Penicillium*, *Pseudallescheria*, *Scedosporium*, *Talaromyces* and the basidiomycete *Fomitopsis*) at 48, 72, 96 and 120 hour time points to address the best conditions for full recovery of multithyphal structure producing either conidia or asci, depending on morph status, and to address spectra reproducibility.

The 120 hour time point was selected for generating the best phenotype spectra, based on correlation by unsupervised hierarchical clustering (Pearson similarity measure); fingerprinting classification by statistical Wilcoxon/Kruskal-Wallis test; classification robustness by recognition capability (RC) and cross-validation (CVA), achieved by genetic algorithm (GA), support vector machine (SVM), supervised neuronal network (SNN) and quick classifier (QC) model generation. Classifiers were applied to 200 clinical isolates tested for external validation.

Proteome profiling-based assay may be an optimal diagnostic approach to overcome traditional ID methods, to encompass multiple fungal genera, to reflect the variety of morphotypes, growth phase and fungal microenvironment.

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NOVEL TARGETS FOR ANTIMICROBIAL MOLECULES IN PSEUDOMONAS AERUGINOSA: IDENTIFICATION AND CHARACTERIZATION OF AN ESSENTIAL MEMBRANE EUKARYOTIC-LIKE TRANSGLUTAMINASE

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Pseudomonas aeruginosa is a highly adaptable bacterium which thrives in a broad range of ecological niches and can infect multiple hosts as diverse as plants, nematodes and mammals. In humans, it is an important opportunistic pathogen in compromised individuals, such as patients with cystic

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fibrosis, severe burns and impaired immunity. This bacterium is also noted for its resistance, often multiple, to many antibiotics. This urgently calls for novel strategies for treatment and prevention of *Pseudomonas aeruginosa* infections. In this context, the discovery of novel essential genes or pathways, not yet targeted by common antibiotics, play a key role in the development of new antimicrobial molecules.

We used the technology of interfering antisense RNAs to screen novel essential functions in *Pseudomonas aeruginosa*. Among several positives to our screenings, we focused on a protein of 688 aa, that we named PtgC. Firstly, the essential role of the *ptgC* gene was validated both by insertional and conditional mutagenesis. Then, we concentrated on functional aspects of PtgC, for which bioinformatic analysis, listed in the *Pseudomonas* Genome Database (PGD) (www.pseudomonas.com), indicated is an inner membrane protein endowed with 6 transmembrane helices and a large periplasmic domain encompassing a highly recognizable structural TG sub-domain belonging to the transglutaminase-like superfamily. Unfortunately, PtgC was annotated in PGD as "hypothetical protein," i.e. no experimental evidence of in vivo expression was available and thus its existence had only been predicted during bioinformatic genome analysis. To provide evidence of PtgC expression, we isolated membrane fractions, digested them with trypsin and identified the resulting tryptic peptides through Multidimensional Protein Identification Technology (MudPIT). Among the peptides identified by this approach, we found 6 peptides belonging to the PtgC periplasmic domain. These results validate PtgC expression and strongly suggest that PtgC localizes in membrane compartments. To assess whether the conservation of the structural TG domain correlated with transglutaminase activity, an N-(His)10-PtgC periplasmic domain (PtgCPD) was purified and tested through a colorimetric microassay, using purified guinea pig transglutaminase as positive control. PtgCPD displayed a positive transglutaminase test, with a specific activity that was about 45% of that of the guinea pig counterpart. We plan to design and test inhibitors of PtgCPD transglutaminase activity, which will be further assayed for antimicrobial effects on *P. aeruginosa*, both on cultured bacteria and in animal models of lung infection. Moreover, we plan to test in animal models the effectiveness of peptide nucleic acids (PNAs), targeting the *ptgC* mRNA, which were shown to elicit growth inhibition of cultured *Pseudomonas aeruginosa*.

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ANALYSIS OF CELL ENVELOPE PROTEOME OF *PSEUDOMONAS AERUGINOSA* BY CAPTURE OF SURFACE-EXPOSED PROTEINS ON ACTIVATED MAGNETIC NANOPARTICLES

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Surface-associated proteins play a key role in bacterial physiology and pathogenesis. Given these roles, identification and characterization of such proteins may lead to novel antibacterial targets. In addition, since surface proteins face the host immune system, they may be basic elements of effective vaccines. We report on specific magneto-capturing followed by Multidimensional Protein Identification Technology (MudPIT) for the analysis of surface-exposed proteins of *Pseudomonas aeruginosa*. In this study, magnetic nanoparticles (NPs) were activated making them able to establish covalent bonds with proteins. Given their chemical composition, size (average diameter = 70-90 nm), and negative charge, these NPs were expected to be atoxic for bacterial cells. Indeed, viable counts of bacterial cells incubated with and without NPs indicated no lethality. Transmission electron microscopy evidence also indicated that NPs were unable to get through the cell envelope and distributed instead over the cell surface. Furthermore, we demonstrated that only activated NPs could collect proteins from cells whereas no proteins were detected in controls experiments carried out with NPs inactivated prior to exposure to cells. These features supported the development of a method for magneto-capturing surface-exposed proteins that greatly improves sensitivity and specificity of previous methods such as surface "shaving" with proteases. Briefly, activated NPs were incubated with bacterial cells and, subsequently, their reactive groups were inactivated. Cells were disrupted, and NPs were magnetically separated from cell extracts, washed several times, and tested for protein and cell membrane content. Consequently the NPs bound to cell envelope structures (NP-Envelope)

lopes) were digested by trypsin to shave protruding protein domains. Tryptic peptides shaved from NP-Envelope were identified by MudPIT analysis. Alternatively, heat and detergents were used to remove from NP-Envelope all envelope material but proteins covalently bound to NPs, which were identified by MudPIT analysis. Shedding of proteins and proteins released by spontaneous cell lysis were monitored through control experiments.

As a result, 92 proteins were identified as associated to NP-Envelope and 63 proteins were those directly bound to NPs. A relevant number of proteins with high localization confidence in outer membrane, along with pilin and FlhD (a flagellar hook-associated protein) or outer membrane porin family proteins, were identified. A feature of this approach that is relevant to vaccine development is the possibility to obtain information about protein topology by the direct identification with MudPIT of domains exposed from membrane layers. Therefore, our results might contribute to accelerating the process of vaccine target identification and development.

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SPUTUM GALACTOMANNAN IN CYSTIC FIBROSIS PATIENTS COLONIZED WITH *ASPERGILLUS FUMIGATUS*

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Background: *Aspergillus fumigatus* is a ubiquitous fungus found worldwide. Up to 50% of the cystic fibrosis (CF) population is colonized in lung by *A. fumigatus*. The detection of galactomannan (GM) performed in respiratory specimens is a new valuable test for the diagnosis of probable invasive pulmonary aspergillosis (IPA), even in non-neutropenic high risk populations. CF patients rarely develop IPA, while a subset of them could develop various *Aspergillus* events (AE) such as allergic bronchopulmonary aspergillosis (ABPA), aspergillomas and chronic pulmonary aspergillosis (CPA). The aim of this study was to determine if CF patients with chronic *Aspergillus* colonization have different galactomannan levels in sputum compared to patients with intermittent or no *Aspergillus* colonization.

Methods: Sputa from 42 CF adult patients in stable respiratory conditions were tested for the presence of GM between July 2011 and February 2012. All subjects were classified according to their respiratory culture status in the previous yr; (1) chronic colonization (CC): ≥ 2 positive cultures for *A. fumigatus* in a given yr, (2) intermittent colonization (IC): 1 positive culture for *A. fumigatus* in a given yr or a previous positive culture in the last 3 yrs, (3) never colonization (NC): no cultures positive for *A. fumigatus*. Baseline demographic, clinical and microbiological data were recorded and included at the time of sputum collection. The GM antigen test was performed by sandwich ELISA (PlateliaTM *Aspergillus*, BIO-RAD-Marnes-La-Coquette, France) and the results were interpreted according to the manufacturer recommendations. The samples with an optical density index (ODI) >0.5 were considered positive.

Results: The main results are shown in the Table.

Conclusions: There was no difference in GM values between the 3 groups. Paradoxically the CC group had lower GM values than the others. No AE were reported in CC group ($p < 0.05$), while 6 AE were reported in the IC group (4 ABPA, 1 CPA and 1 aspergilloma) and 1 (ABPA) in NC group. In CF high levels of *Aspergillus* GM on sputum could indicate a possible marker of pulmonary AE, above all in IC patients. Given the small population, further studies are needed to establish the role of sputum GM in CF.

	CC (13)	IC (16)	NC (13)
Male (%)	69.2	62.5	53.8
Age (yrs)	34.2 (23 - 46)	33.7 (18 - 57)	35.3 (16 - 48)
FEV1 (%)	44 (23 - 87)	54 (26 - 94)	58 (33 - 115)
Chronic <i>P. aeruginosa</i> (%)	61.5	62.5	77
Exacerbations/yr	4.8 (2 - 8)	3.9 (0 - 10)	2.3 (0 - 5)
ABPA (%)	0	25	7.7
AE (%)	0	37.5	7.7
GM positive (%)	15.3	25	15.3
GM levels	0.116 (0.008 - 0.592)	0.604 (0.011 - 3.581)	0.195 (0.008 - 0.981)
<i>Aspergillus</i> in sputum (%)	61.6	14.3	0

CC, chronic colonization; IC, intermittent colonization; NC, never colonization

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INFECTION AND COLONISATION WITH
SCEDOSPORIUM SP. IN CHILDREN WITH CYSTIC
FIBROSIS

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Background: *Scedosporium* sp. is an emerging fungal pathogen which colonizes lungs of cystic fibrosis (CF) patients. Its prevalence is underestimated and its role in the evolution of the CF lung disease is not known. Moreover, its pathogenicity in the pediatric CF population has never been studied. We compared the clinical status, lung function and laboratory data before and after colonization with *Scedosporium* sp. in CF children.

Methods: All CF children who had at least one sputum culture positive for *Scedosporium* sp were investigated and the following data were collected the year before 1st isolation, and every year following isolation: lung function, number of exacerbations, associated bacterial or fungal colonization, nutritional status (Zscore-BMI), level of IgE and eosinophils, systemic or inhaled steroids, inhaled antibiotics, evolution after antifungal therapy. *Scedosporium* species were identified using MALDI-TOF mass spectrometry.

Results: Eleven patients out of the 250 (4.4%) investigated had a chronic colonization. The median age at colonization was 14.4 years (2.2-19.8 years). Nine patients were F508del homozygotes. Median follow up after colonization was 40 (18-68) months. Species identified were *S. apiospermum* (n=4), *S. aurantiacum* (n=2), *S. prolificans* (n=1), *S. boydii* (n=1), *Scedosporium* sp. (n=3). The bacterial microorganisms isolated in the sputum were *Staphylococcus aureus* and/or *Pseudomonas aeruginosa* (respectively 9 and 8 patients).

During the year before *Scedosporium* sp. isolation, all the patients had been treated with inhaled antibiotics, and 10 with inhaled steroids. Ten patients were colonized with *Aspergillus fumigatus*. Three of those had been treated for allergic bronchopulmonary aspergillosis the previous year, including at least 6 months of systemic steroids.

After colonization, 7 out of 9 patients with available respiratory function tests, had a decrease in FEV1 (median decrease: -29% (3-51%), p=0.01; comparison between the mean values of the year before colonization and the last value at follow-up). Similarly, 7 patients had an increase by 1.3 fold (0.14-5) in the number of the exacerbations requiring antibiotics (p=0.01). Nutritional status worsened for 5 patients as assessed by the median decrease in Zscore-BMI (-0.71 (-1.67-0.27); p=0.06).

IgE and eosinophil counts increased significantly the year after colonization in respectively 4 and 5 of patients (n=9).

Treatment with voriconazole initiated in 4 patients always failed to eradicate.

Conclusion: Colonization with *Scedosporium* sp. worsens lung CF disease in children. Steroid therapy might be a risk factor for *Scedosporium* sp. colonization.

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LONGITUDINAL MICROBIAL ECOLOGY IN CYSTIC
FIBROSIS

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Background: Molecular, culture-independent techniques have greatly enhanced the characterization of the microbial communities in the lungs of patients with cystic fibrosis (CF), and have helped to identify new potential pathogens. We hypothesized that specific changes in microbial diversity, evenness, and species abundance would correlate with changes in clinical status.

Methods: Respiratory samples (swab, sputum, and bronchoalveolar lavage) from CF patients ages 0 to 20 years are being collected prospective-

ly at every clinical encounter over a 3 year period that began in February 2010. Data on the current clinical status including FEV1, clinical findings, and use of chronic medications such as azithromycin, inhaled tobramycin, aztreonam, pulmozyme and acute antibiotic treatment are documented. We use two different small subunit rDNA-based, culture-independent techniques (Sanger sequencing and 454 pyrosequencing) to carefully characterize, and cross-validate the microbial diversity in samples. Every sample is also compared to duplicate culture data obtained from our clinical microbiology laboratory.

Results and Conclusions: To date, 160 patients have been enrolled in the study with 160 initial isolates and over 500 repeat specimens (projected total >900 specimens). We have completed 16S pyrosequencing for approximately 450 samples. Preliminary data suggests that exacerbations are associated with only modest changes in overall microbial diversity. However, samples collected during periods of relative have lower levels of intersample diversity (beta diversity) and show high levels of ecosystem similarity, suggesting that exacerbations are represented by deviations from a stable equilibrium community. In addition, our results underscore the importance of anaerobic bacteria in cystic fibrosis. Greater than 92% of specimens are positive for organisms from the genus *Prevotella*, and greater than 89% are positive for *Veillonella*. By comparison, only approximately 40%-50% are positive for *Pseudomonas* or *Staphylococcus* species. These data suggest that anaerobes are abundant and important members of the CF respiratory microbiota.

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PHASE I CLINICAL STUDY OF VANCOMYCIN
HYDROCHLORIDE INHALATION POWDER
(AEROVANC) IN HEALTHY VOLUNTEERS

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Persistent methicillin-resistant *Staphylococcus aureus* (MRSA) lung infection has become increasingly common in CF patients, with a prevalence of almost 30% in the US. MRSA lung infection has been associated with increased use of IV antibiotics and hospitalizations, faster decline of lung function, and shortened life-expectancy compared to CF patients without MRSA. Savara Inc. has developed a vancomycin hydrochloride inhalation powder (AeroVanc), with the intent of developing the product as a treatment of persistent MRSA lung infection in CF patients.

A Phase I pharmacokinetic study was performed in healthy volunteers to evaluate the tolerability and safety of AeroVanc, and to evaluate the bioavailability and pharmacokinetics of vancomycin after pulmonary administration of single AeroVanc doses of 16, 32, and 80 mg, compared to an intravenous infusion of 250 mg of vancomycin hydrochloride. The study was an open-label single escalating dose study, in which six subjects received each AeroVanc dose (18 subjects in total), and two subjects from each AeroVanc group (six subjects in total) received intravenous vancomycin as a reference treatment.

The main pharmacokinetic parameters after pulmonary or intravenous administration of vancomycin are shown in Table 1. C_{max} and AUC values were both closely dose proportional between the pulmonary doses (R=0.99 for AUC_(0-inf)). The mean absolute bioavailability of all pulmonary doses was 49 (SD 8) %, with no clear difference between the doses.

Four subjects experienced mild respiratory tract adverse events that were classified to be probably related to AeroVanc. Two of the subjects had cough, one had throat irritation, and one had bronchoconstriction with throat irritation. All symptoms resolved spontaneously in 15-60 minutes from the onset. No clinically meaningful changes from baseline were observed in the spirometry tests at 30 minutes post dose, although three subjects in the high dose group had a slight (7-11 %) reduction in FEV₁. This suggests that in future studies, bronchial responsiveness should be carefully monitored, and bronchodilator pre-treatment considered as an element of the AeroVanc treatment regimen.

In conclusion, AeroVanc was very well tolerated and safe after single dose administration. The product showed a favorable pharmacokinetic profile, with a relatively slow pulmonary absorption phase, followed by elimination comparable to the intravenous administration. The results indicate that AeroVanc is a promising product candidate for the treatment of persistent MRSA infection in CF patients.

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Mean (SD) pharmacokinetic values of single pulmonary (p) or intravenous (iv) vancomycin dose

Dose	T _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC ₍₀₋₄₎ (h*ng/mL)	AUC _(0-inf) (h*ng/mL)
16 mg (p)	8.5 (2.0)	2.1 (0.8)	109 (33)	1,210 (238)	1,461 (257)
32 mg (p)	8.6 (0.5)	1.8 (0.6)	232 (90)	2,380 (975)	3,051 (959)
80 mg (p)	8.0 (1.3)	1.3 (0.4)	618 (230)	6,258 (1,507)	7,136 (1,458)
250 mg (iv)	7.2 (1.1)	0.9 (0.2)	10,028 (1,768)	41,028 (2,696)	44,356 (3,623)

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ANTI-PSEUDOMONAL BACTERIOPHAGE COCKTAIL REDUCES INFLAMMATORY RESPONSE IN THE MURINE LUNG

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Background: As antibiotic resistance increases, there is growing need for novel therapies to target infection in cystic fibrosis (CF). Bacteriophages are naturally occurring viruses that specifically target and infect bacteria and, unlike antibiotics, are able to multiply at infection sites and adapt to resistant bacteria.

Method: Two strains of *Pseudomonas aeruginosa* (Pa) were chosen to be assessed in an *in vivo* murine model: a) a clinical strain from an adult patient with CF and b) a bioluminescent strain generated by transformation of the laboratory strain PA01 with the *lux* CDABE genes (kindly provided by Dr. S. Nelson, UWE, Bristol) in preparation for non-invasive bioluminescent monitoring. Both bacterial strains were shown to be sensitive to a novel anti-pseudomonal bacteriophage cocktail on a standard plaque assay to a dilution of 10⁻⁶.

Adult BALB/c mice were inoculated intranasally with 50µl of either Pa strain followed by 20µl of bacteriophage cocktail (treated n = 12) or SM buffer (control n = 12). All mice were well at 48hrs post-infection when they were sacrificed; bronchoalveolar lavage (BAL) was obtained and the spleen removed. BAL was serially log diluted and cultured at 37°C. Non-quantitative splenic cultures were also performed. Remaining BAL was centrifuged and supernatant stored at -80°C for later analysis of soluble inflammatory markers. Cell pellets were resuspended in 200µl buffer and total cell counts determined using a haemocytometer.

Results: At the 48 hour time point mice had, in general, cleared the Pa infection; very few colonies were grown from BAL or spleen with either strain. However, mice infected with the clinical CF Pa strain and treated with bacteriophage demonstrated significantly fewer inflammatory cells in BAL compared with controls (median 899 x 10⁴/ml (range 568 – 1172) vs. median 1824 x 10⁴/ml (range 1386 – 2772), p < 0.01). A similar trend was seen in the mice infected with the bioluminescent Pa (median 1376 x 10⁴/ml (range 1184 – 2120) vs. median 1874 x 10⁴/ml (range 1712 – 3056), p = 0.132). Differential cell counts and measurements of soluble inflammatory mediators are underway.

Conclusion: At this dose, Pa infection was successfully cleared by the mice but led to an acute cellular inflammatory response. The reduction in cell number following co-administration of a bacteriophage cocktail to which the organisms were sensitive suggests that the bacteria may have been cleared earlier and more effectively in the phage-treated animals. Work is ongoing to explore the effect of this intervention in a chronic Pa infection model using bioluminescence as a read out of bacterial burden.

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AN IN VITRO SYSTEM HAS IDENTIFIED A FACTOR RELEASED ONLY BY *PSEUDOMONAS AERUGINOSA* BIOFILM AND NOT PLANKTONIC BACTERIA THAT INTERACTS WITH A549 LUNG EPITHELIA AND LL-37 PRE-TREATMENT OF BIOFILMS MINIMIZES ITS EFFECTS

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Cystic fibrosis is a disease that is newly diagnosed in 1000 children each year. The number one cause of morbidity and mortality in cystic fibrosis disease is through bacterial infection in the lungs. *Pseudomonas aeruginosa* is a key player in acute and chronic lung infections. There is growing evidence that chronic infections involve the biofilm form of the bacteria. Thus far, *in vitro* research into bacterial-mammalian interactions have focused on planktonic bacteria, which is not reflective of chronic infections. Studies examining the interaction of biofilm with host cells are sparse and have involved infecting mice with specific biofilm-mutants and examining the disease outcome; or through the isolation of bacterial species from the sputum of infected patients and examining their *in vitro* characteristics. These studies are not only expensive and time-consuming but they also cannot interpret specific cell interactions during the infection. This research program has established an *in vitro* system for the co-culture of biofilm or planktonic *Pseudomonas* bacteria with human lung epithelial A549 cells that reflects key aspects of clinical chronic and acute lung infections. This system involves the growth of biofilm on a 96-peg lid, which can subsequently be transferred into plates containing A549 cells. With this system, we established that planktonic exposure produced pronounced cell rounding and cytoskeletal dysregulation as well as bacterial internalization but not from biofilm exposure, which is consistent with clinical data for acute and chronic lung infections. Biofilm stimulated an increase in epithelial metabolism, bringing the cells out of their quiescent state, as well as stimulating an increase in IL-8 production, which is reflective of chronic infections. We also found that pre-treatment of the biofilm with the innate immune peptide LL-37 caused the biofilm to stimulate an increase in IL-8 production by A549 cells, a potentially unfavorable result. Using this co-culture system we were able to determine that biofilm secretes a soluble factor that causes morphological A549 cell changes and this factor is absent in planktonic co-culture. Interestingly, growing the biofilm in the presence of LL-37 obliterates the release of the factor. This secreted factor in biofilm *P. aeruginosa* infections could serve as a target for therapeutics to reduce inflammation and cell damage in chronic infections. Based on the data summarized, the value of this co-culture system for the delineation of key differences in biofilm or planktonic bacterial interactions with lung epithelia has been clearly demonstrated.

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30 YEARS OF *BURKHOLDERIA CEPACIA* COMPLEX INFECTIONS IN VANCOUVER AND BRITISH COLUMBIA, FROM 1981 TO 2011

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Our laboratory has been collecting clinical isolates from patients attending the paediatric and adult cystic fibrosis clinics in Vancouver, BC, Canada since 1981. Since 1994 we have also housed the Canadian *Burkholderia cepacia* complex research and referral repository (CBCRRR). During this period the taxonomy of *Burkholderia cepacia* complex (BCC) species has

changed considerably, as have approaches to both treatment and infection control. When our culture collection was first started all the bacteria now known as the BCC were classified as *Pseudomonas cepacia*; subsequent advances in taxonomy have defined at least 17 different species belonging to the BCC. Furthermore, an understanding of the potential for patient-to-patient spread of the BCC has led to the isolation of all patients that become infected with any members of the BCC. The objective of this study is to review BCC infections in Vancouver over the 30 years between 1981 and 2011. These data show that, between 1981 and 2011, BCC infections in our centres were predominantly caused by either *B. cenocepacia* (55/130 cases) or *B. multivorans* (54/130 cases). Prior to the institution of infection control measures in 1995, new infections with BCC were primarily caused by epidemic clones of *B. cenocepacia* belonging to the RAPD 01, 02, 04 and 06 lineages. Following the introduction of infection control measures, the acquisition of epidemic clones of *B. cenocepacia* almost completely ceased; however, new infections with other BCC bacteria have continued. The majority of new infections after 1995 were identified as non-clonal isolates of *B. multivorans* indicating a shift in the epidemiology of BCC in CF, consistent with observations at other centres. Infection with BCC organisms other than *B. cenocepacia* or *B. multivorans* occurred at a low rate, with small numbers of *B. vietnamiensis*, *B. stabilis*, *B. cepacia*, BCC Taxon K, *B. anthina*, *B. metallica* as well as the non-BCC bacterium *B. gladioli*. An analysis of survival, following infection with BCC, showed that infection with *B. cenocepacia* was associated with a significantly worse outcome than infection with *B. multivorans* over the 30 years of this study.

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SHORT CHAIN FATTY ACID RECEPTOR OVEREXPRESSION IS ASSOCIATED WITH INCREASED IL-8 PRODUCTION IN CF BRONCHIAL EPITHELIAL CELLS

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Objective: There is now emerging evidence on the important role of the microbiome in the CF airway. Anaerobic bacteria including *Prevotella melaninogenica* have been detected in abundant numbers in recent studies. Their role, interaction and virulence with other bacteria are not fully known. Short chain fatty acids (SCFA) are known to be produced as metabolic by-products of anaerobic bacteria in the intestine and GPR 41 and 43 are known SCFA receptors. The aim of this study was to investigate whether GPR 41 and 43 were expressed in human bronchial epithelial cells and the inflammatory impact of anaerobic bacterial derived SCFA signalling.

Methods: In this study we examined the expression of GPR 41 and 43 in CF (CFBE 41o) and normal human bronchial epithelial cells (16HBE14o) using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting. We also investigated the effect of SCFA on pro-inflammatory interleukin-8 (IL-8) production by ELISA.

Results: Examination of GPR 41 and 43 gene expression by RT-PCR revealed upregulation of both receptors in quiescent CF bronchial epithelial cells ($p < 0.05$, $n = 3$). Significant over-expression of GPR 41 and 43 was confirmed by Western blotting (4-fold increase in CF vs controls, $n = 3$, $p < 0.05$). We have demonstrated that exposure of cells to sodium butyrate, a prevalent anaerobic bacterial derived SCFA, resulted in increased IL-8 production by CFBE compared to HBE cells (2-fold increase in CF vs controls, $n = 3$, $p < 0.05$).

Conclusions: Our findings report for the first time, that GPR 41 and 43 are constitutively overexpressed in CF airway epithelial cells and that SCFA signalling results in increased production of the potent neutrophil chemokine IL-8. This mechanism represents a novel intrinsic defect that may contribute to the neutrophil-dominated immune response in the CF airway.

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BURKHOLDERIA CEPACIA COMPLEX AND LIMITED CUTANEOUS VASCULITIS: A 20-YEAR EXPERIENCE AT A LARGE ADULT CF CENTRE

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Background: Limited cutaneous vasculitis in cystic fibrosis (CF) is described in the literature and typically affects the lower limbs. This has been described in respiratory infection including *Pseudomonas* and *Staphylococcus aureus*, with antibiotics and with pancreatic enzyme replacements. IgA and IgG anti-bactericidal permeability proteins have been implicated. To our knowledge vasculitis in association with *Burkholderia cepacia* complex (Bcc) has not been reported. Here we characterize the experience of Bcc infection and cutaneous vasculitis in our large adult cohort.

Methods: We retrospectively studied case notes from patients with Bcc and vasculitis, identified through on-site and national UK CF registries. Details on presentation and various clinical features were documented from paper and electronic records.

Results: 7 patients with Bcc infection and a clinical diagnosis of vasculitis were identified between 1992-2012. All patients presented with rash in forms that included maculopapular lesions, purpura, morbilliform rash and even blisters with few other symptoms. 6/7 patients presented with joint pains, 1/7 with mild proteinuria (known diabetic). Presentation FEV1 was low in all cases (18-55% predicted) and typically reduced when compared to pre-morbid values although respiratory symptoms were not common. The patients also demonstrated the following features: increased erythrocyte sedimentation rate (19-99mm/hr) with 6/7 cases demonstrating an ESR at least twice that of the C-reactive protein; normal serum eosinophils; raised serum IgG (18.2-38.6g/L) and IgA (3.8-6.7g/L) but not IgM; negative anti-nuclear factor and anti-nuclear cytoplasmic antibody (ANCA) in all but 1 patient in whom cANCA was transiently positive; normal C3 and C4 complement levels (1 patient borderline C4 at 0.15mg/L). Other tests inconsistently performed were negative where carried out and included HBsAg (3/3 cases), cryoglobulins (2/2), ASO titre (2/2). Rheumatoid factor was positive in 1/5 patients at 1/640 titre. Despite similar clinical appearances in rash, 4/7 patients that underwent biopsy showed varied histology; 2 showed leucocytoclastic vasculitis, 1 capillaritis and 1 erythema nodosum. Specific treatment was given in 5/7 cases and included prednisolone (4 cases), azathioprine (2), chloroquine (1), cyclosporine (1), dapsone (1) and non-steroidal anti-inflammatory drugs (1). 2/7 were not treated with one self-limiting and the other responding consistently to intravenous antibiotics for chest infection. Treatment response was variable and modest. One person responded well to prednisolone, 1 eventually with combinations of prednisolone, azathioprine and cyclosporine and 1 with azathioprine after prednisolone. 5/7 cases had spirometry 1 year after presentation that either remained decreased or had declined further.

Conclusion: We present our experience of Bcc infection and vasculitis in which we have seen variable responses to treatment. Presentation has been associated with a drop in spirometry, raised inflammatory markers and serum immunoglobulins, and typically negative ANCA. We recommend careful assessments of those presenting with vasculitis in order to better characterize this process and so guide better treatments.

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MICROBIOLOGIC RESULTS IN A LARGE NEWBORN CF CLINIC AND POSSIBLE CLINICAL CORRELATION

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Background: Methicillin sensitive *Staphylococcus aureus* (MSSA), *H. influenzae*, and non-mucoid *Pseudomonas aeruginosa* (NM-Pa) are the most common organisms found in younger patients with CF. Of these three only Pa has been currently recognized as causing potential long-term changes in the airways after chronic exposure. This has led to near universal agreement of attempts to eradicate or suppress growth after first positive culture.

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Methods: This study is an ongoing observational review of routine screening throat cultures obtained in the Newborn CF clinic (n=67) at Nationwide Children's Hospital in Columbus Ohio. The first incidence of organism growth and age at which positive culture was first obtained was reviewed. Newborn screening was started in Ohio in 2006 and since then 67 infants with CF have been enrolled in this clinic.

Results: With the advent of newborn screening in Ohio since 2006 and subsequent earlier diagnosis of CF, we have found in our center that the previously mentioned organisms are not the only organisms being grown regularly from these infants. We have found frequent growth of a variety of other organisms in our patients. The majority of first incidence of positive culture occurred within the first year of life. Most common organisms cultured included *Klebsiella* (57%), MSSA (55%), *E. coli* (43%), NM *Pseudomonas* (42%), *Enterobacter* (42%), *Acinetobacter* (31%), *Stenotrophomonas* (25%), fungal species (30%), *Serratia* (22%), *Chryseobacterium* species (19%) and MRSA (13%).

Although a few of these organisms are enteric in origin which could be related to secondary diagnoses such as GERD, timing of throat culture samples such as after a feed, or poor parental hygiene, others are thought to be more opportunistic organisms, not necessarily seen in the airways. Other possible causes for the appearance of these organisms in culture results include increased environmental exposure, improved sensitivity of identification or the rapid changes in microbiologic taxonomy. Despite the cause, further investigation is warranted to identify the potential long term effects of all organisms on the future morbidity of these young patients.

Conclusions: Currently there are no guidelines as to whether treatment of first or recurrent growth of organisms other than Pa is beneficial. To begin the process of establishing treatment guidelines for new CF pathogens in young patients, the first step would be to identify the most common organisms and investigate clinical correlations. This will be important in ultimately determining appropriate treatment guidelines. In the initial part of our study, we identified the most common organisms found on first culture results of patients 0 to 4 years of age. We will next gather important clinical data such as number of exacerbations, lung function and CT results, to decide which organisms deserve attention for attempted early eradication.

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THE ROLE OF THE THERAPEUTICS DEVELOPMENT NETWORK CENTER FOR CF MICROBIOLOGY IN CLINICAL TRIALS IN THE US

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Clinical trials of new drugs in cystic fibrosis (CF) are an important focus of the CF Foundation and have had a significant impact on improving the lives of individuals with CF. Many of the agents being developed are treatments that either directly or indirectly impact the microbiology of the CF airway. Approval of a drug for use in CF patients requires a high standard of safety, including determining the impact on sputum bacterial density, antimicrobial susceptibility, and emerging pathogens in the CF airway. The Therapeutics Development Network (TDN) Center for CF Microbiology (CCFM) is a national resource center that uses state of the art CF microbiology methods to perform the microbiology for clinical trials in CF. The CCFM has been involved in studies for many agents in the CFF Drug Pipeline, including TOBI®, azithromycin, Cayston®, Arikace®, Kalydeco®, and KB001. CCFM technologists are very experienced, performing thousands of CF cultures each year for almost two decades. This experience has led to many discoveries and innovations in CF microbiology including a proposal to eliminate sputum gram stains for CF samples, recommendations for a molecular identification schema for non-lactose-fermenting gram negative bacilli, and detection of small colony variant (SCV) *Stenotrophomonas maltophilia*. We routinely perform quantitative bacterial cultures using multiple serial dilutions plated onto up to 10 different selective media, a technique that is not available in most clinical or commercial laboratories, because it is very labor intensive. This method enables detection and isolation of even fastidious bacteria, as well as quantitation of specific organisms. Decrease in sputum bacterial density has been both a primary and a secondary endpoint in many studies. Cultures for fungi and acid fast bacteria are performed using CF-specific protocols including the use of a double decontamination technique (initial decontamination by N-Acetyl-L-cysteine – NaOH, followed by a second decontamination of the sediment by oxalic acid) for isolation of non-tuberculous mycobacteria. Organisms

are identified in the CCFM laboratory using state of the art techniques including limited biochemical testing, polymerase chain reaction (PCR) and matrix-associated laser desorption/ionization with detection by a time of flight mass spectrometer (MALDI-TOF). The laboratory has also developed methodology and standards for detection and classification of bacterial small colony variants of *Staphylococcus aureus*. Both classical and molecular testing methods are used to determine antimicrobial susceptibility, as appropriate for study needs. Additional services provided by the CCFM include initial consultation for proposed studies to identify the key microbiology testing components necessary, development of study-specific microbiology specifications, and archiving of study isolates that, with permission, may become a part of the TDN specimen bank and can be linked to study data. The infrastructure for the CCFM is funded by the CF Foundation, to enable the laboratory to maintain high standards and experienced staff. Costs for specific studies are based on testing requested and are borne by the study sponsors.

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ANTIBIOTICS INFLUENCE *PSEUDOMONAS AERUGINOSA* BIOFILM PHENOTYPES THROUGH THE VIRULENCE MODULATOR *MGTE*

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The Gram-negative bacterial pathogen *Pseudomonas aeruginosa* establishes lifelong infections in the lungs of individuals with cystic fibrosis (CF). These infections are enabled by formation of biofilms, antibiotic-tolerant aggregations of bacteria. *P. aeruginosa* biofilm formation in the CF lung is a complex process leading to a decrease in production of factors typically associated with virulence, such as toxins, and an increase in production of secreted polysaccharides. Greater understanding of the molecular mechanisms governing this switch to biofilm formation in the CF lung will aid the identification of novel therapies to thwart these actions. We have recently discovered a magnesium transporter in *P. aeruginosa*, called MgtE, that inhibits the expression of the bacterial Type III Secretion System (T3SS). Because a decrease in T3SS production is one hallmark of the chronic CF biofilm phenotype, factors that induce expression of the *mgtE* gene might influence biofilm formation in the CF lung. We have previously found that the antibiotic tobramycin enhances *mgtE* transcription. Our objective in this current study was to determine whether other antibiotics also influence *mgtE* transcription. Using quantitative and semi-quantitative RT-PCR, we tested the effect of 12 antibiotics from 9 different antibiotic classes on *mgtE* transcription. Nine out of 12 of these antibiotics induced transcription of *mgtE*. To determine whether this effect was part of a general stress response, we screened a mutant library for mutants in genes connected with stress pathways in *P. aeruginosa*. We found potential regulatory interactions between MgtE and AlgR. Importantly, AlgR also regulates synthesis of the exopolysaccharide alginate, which is produced in large amounts in CF-related *P. aeruginosa* biofilms. Our results suggest that antibiotics in the CF lung environment can enhance *mgtE* gene transcription, which in turn can inhibit T3SS and possibly influence alginate production through AlgR. Through this and additional studies, we can obtain a better understanding of how signals present in the CF lung environment influence formation of *P. aeruginosa* biofilm formation and chronic infection.

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DOES LOW MAGNESIUM IN CYSTIC FIBROSIS CONTRIBUTE TO BACTERIAL PATHOGENICITY?

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Background: Magnesium is an essential element in many cellular functions for both humans and bacteria. Therefore, bacteria living within a host (as in the case of lung infection) possess the capacity to respond to fluctuating conditions within the host's system, such as changes in nutrient availability. A bacterial species especially well-equipped for this purpose is *Pseudomonas aeruginosa*, one of the most formidable pathogens in the treatment of cystic fibrosis (CF). When *P. aeruginosa* encounters variations in nutrient availability, as may be the case in CF lung infections, it responds

by altering expression of a number of virulence factors, including biofilm formation, motility, toxin release, and antibiotic resistance.

Objectives: Here we hypothesize that *P. aeruginosa* modulates virulence factors in response to magnesium fluctuations without an overall change in the amount of bacterial growth. Our objective is to conduct experiments in varying magnesium levels in order to observe the effect on bacterial virulence factors and growth.

Methods: Assays were conducted for motility, biofilm formation, minimal inhibitory concentration (MIC), and cytotoxicity toward cultured cells derived from the CF bronchial epithelium. Experimental media included a range of physiologically relevant magnesium levels (up to 2.0mM magnesium as $MgSO_4$). *P. aeruginosa* laboratory strains PA14 and PAO1 were used, as well as strain GGA52, which carries a mutation in the gene encoding for the magnesium transport protein MgtE. Bacterial growth was measured by spectrophotometry and by count of colony forming units (CFU).

Results: Within the range of 0.4-1.4mM Mg^{2+} (as $MgSO_4$), PAO1 and PA14 demonstrate similar growth at all concentrations. Within this same range, *P. aeruginosa* cytotoxicity levels reflect sensitivity to fluctuations in magnesium. Swarming motility increases with increased magnesium in the range of 0.1-2.0mM. In the presence of 1µg/mL gentamicin and varying magnesium (0, 0.5mM, 1.0mM, and 2.0mM $MgSO_4$), PAO1 growth is more robust with increasing magnesium.

Conclusions: Results suggest that *P. aeruginosa* responds to fluctuations in magnesium that vary as little as 0.2mM. This response is not simply a change in growth, but rather a more complex response which enables the bacterium to adapt to the fluctuating nutrient levels it encounters within the lungs of a person with CF. We anticipate that our research will elucidate the relationship between magnesium and *P. aeruginosa* pathogenicity and potentially lead to improved treatments for people living with CF.

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DO PARENTS OF CHILDREN WITH CYSTIC FIBROSIS CARRY *PSEUDOMONAS AERUGINOSA* IN THEIR UPPER AIRWAY?

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Background: Parents of children with cystic fibrosis (CF) are obligate carriers of a CF mutation, and as a result, may have a higher incidence of chronic rhinosinusitis. We report a pilot study to determine whether their upper airways are prone to colonization with *Pseudomonas aeruginosa* (PA), which could constitute a source of infection to their children with CF.

Methods: Parents of children with CF (> 5 years of age) at our Center are eligible for this study. We plan to sample parents of 30 children, half of whom are colonized with PA (PA-positive). To increase the likelihood of PA detection, we will obtain 2 sets of nasal and oropharyngeal cultures from the parents, within a 3 month interval. Flocked swabs (COPAN Diagnostics Inc., Murrieta, CA) of throat and nares will be submitted in Eswab liquid transport media (COPAN). The transport tubes will be vortexed for 30 seconds and 100 µl of the homogenized specimen will be inoculated to MacConkey agar (Remel, Lenexa, KS). The agar plates will be incubated at 35°C in CO_2 and examined daily for 3 days. Also, we will administer a questionnaire to obtain data from the parents regarding their own history of CF-like disease. The study will include serotyping of any PA isolated from parents in comparison with that of PA isolated from their children. This study has been approved by our Institutional Review Board.

Results: At this juncture of the study, we have obtained one set of cultures from 16 parents of 12 children (6 M/6 F, age range 8-17 years). Six of these children are homozygous for ΔF508, 5 are compound heterozygote for ΔF508 and another CF mutation, while the remaining child has two other CF mutations. Half of the children were PA-positive. All of the parents' cultures were negative for PA. The health questionnaire of one of the parents revealed that she has a history of asthma, chronic sinusitis, bronchitis, and a positive sputum culture for PA in the past. Her sweat chloride concentration was 15 mmol/L and her stool elastase was normal. Seven out of the 16 parents reported smoking cigarettes. Of these, one parent suffered from chronic cough and 3 episodes of sinusitis over the past 2 years, and 2 noted coughing for more than 8 weeks over the past year. No other parents reported chronic respiratory symptoms.

Conclusions: These preliminary results indicate that it is uncommon for the airways of parents of children with CF to be chronically colonized with PA.

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NONTUBERCULOUS MYCOBACTERIA PREVALENCE AMONG PATIENTS WITH CYSTIC FIBROSIS: A SINGLE CENTER EXPERIENCE

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Background: Over the past 50 years, the median survival for CF patients has increased significantly (median 37 yrs). The improvement in survival has led to the emergence of drug resistant strains of traditional pathogens, such as *P. aeruginosa*, and new pathogens, such as nontuberculous mycobacteria (NTM). NTM infections are increasingly recognized as an emerging infection with studies reporting a prevalence ranging from 3-28% (1-3). The most common NTM species isolated are: *M. avium* complex followed by *M. abscessus*, but there is a wide geographical variation in overall prevalence and species distribution. Increase in NTM infection is multifactorial, due to increased survival, improved diagnostic methods and increasing clinician awareness.

Aims: Due to the evident knowledge gaps and the variability of the prevalence of NTM infections, the following retrospective study was conducted of all adult (>18years old) patients seen at the University of Miami Cystic Fibrosis Clinic, who had at least 1 sputum culture positive for NTM. For all eligible patients, demographic, clinical and microbiological data was collected from their medical records and from the PORT CF database.

Results: Overall, we had 92 adult patients followed in our cystic fibrosis center. The prevalence of NTM isolation among our CF population was 20.6% (19/92). According to the 2007 ATS/IDSA criteria, only 9.6% (9/92) met microbiological criteria of NTM disease (at least 2 positive sputum cultures), but only 6.5% (6/92) met clinical criteria also and were treated for NTM disease. The mycobacterial species identified were *M. abscessus* (13 patients 68%), *M. avium* complex (8 patients 42%) and *M. fortuitum* (1 patient 7.6%). We had 3 patients that had both *M. abscessus* and MAC.

Conclusions: NTM are important emerging pathogens in CF patients. Our South Florida population has a high prevalence and a different mycobacterial species distribution, as compared to those reported on a prior North American multicenter study (3). This is most likely multifactorial, considering our geography and heterogeneous patient population. Due to this ongoing trend of rising NTM prevalence, further study is warranted to help us clarify issues, such as discerning colonization from infection, its impact on lung function and the role of chronic macrolide therapy on antibiotic selection of NTM.

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CLINICAL FACTORS ASSOCIATED WITH NEPHROTOXICITY IN PEDIATRIC CYSTIC FIBROSIS PATIENTS RECEIVING AMINOGLYCOSIDES

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Rationale: Despite close monitoring of drug concentrations and renal function during inpatient admissions for CF pulmonary exacerbations,

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patients continue to develop nephrotoxicity while being treated with intravenous (IV) aminoglycosides.

Objectives: To identify clinical factors associated with greater risk of developing nephrotoxicity in pediatric patients with CF receiving IV aminoglycosides.

Methods: We performed a retrospective chart review of 9 CF patients $\leq$ 18 years followed at the James Whitcomb Riley Hospital for Children Cystic Fibrosis Center. These 9 patients received IV aminoglycosides co-administered with other IV antibiotics during inpatient admissions from December 2006 through January 2012. Data was collected regarding age, gender, BMI percentile, administration of IV antibiotics, aminoglycoside dosing interval every 8, 12, or 24 hours, non-steroidal use, IV fluids, IV contrast, baseline serum creatinine, maximum serum creatinine, presence of CF-related diabetes and drop in FEV1 from baseline to assess severity of exacerbation. Every 24 hour aminoglycoside dosing was implemented at our institution in 2008. Data were analyzed using Student's t-test and Fisher's exact test. Nephrotoxicity was defined as an increase in serum creatinine by 0.3 mg/dL or ≥ 1.5 times baseline (Acute Kidney Injury Network 2007 definition).

Results: Of the 9 patients reviewed, 3 had nephrotoxicity during one or more inpatient admission and 6 did not. Compared to those without nephrotoxicity, patients with nephrotoxicity had a statistically significantly higher average number of antibiotic courses over the reviewed time period (7.67 ± 0.58 vs 2.67 ± 1.86 , $p = 0.003$), a significantly lower BMI percentile (18.03 ± 13.87 vs 48.5 ± 19.6 , $p = 0.046$), were more likely to have received aminoglycosides dosed every 8 or 12 hours (52.17% vs 18.75% , $p = 0.049$) and were more likely to have had vancomycin co-administered with an aminoglycoside (100% vs 16.67% , $p = 0.048$). A trend toward significance was noted in the number of patients with nephrotoxicity who had CF-related diabetes compared to those without nephrotoxicity (67% vs 0% , $p = 0.08$). There was no difference found between groups in regard to average age, baseline serum creatinine, maximum serum creatinine, non-steroidal use, IV contrast administration, IV fluid administration or change in FEV1 from baseline.

Conclusions: This small pilot study demonstrates that higher number of IV antibiotic courses, lower BMI percentile, every 8 or every 12 hour aminoglycoside dosing interval and co-administration of vancomycin with aminoglycosides were associated with development of nephrotoxicity in pediatric CF patients during inpatient admissions at our institution. Further study in a larger number of patients needs to be carried out to validate these results.

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ERADICATION OF *PSEUDOMONAS AERUGINOSA* IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS: A COMPARISON OF PROTOCOLS

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Background: Cystic fibrosis (CF) causes impairment of mucociliary clearance leading to opportunistic infection with *Pseudomonas aeruginosa* (PsA). Chronic PsA infection is associated with accelerated decline in lung function and poor health related outcomes. The Children's of Alabama/UAB pediatric CF center (COA/UAB) previously reported an 82.9% rate of successful eradication (SE) utilizing an escalating dose and duration eradication protocol (EP) (Tier 1: 45% of patients achieving SE with inhaled tobramycin (TIS) alone, Tier 2: 29% achieving SE after a second course of TIS plus oral ciprofloxacin (cipro) and Tier 3: 26% achieving SE after a subsequent course of inhaled colistin (CIS) or IV antibiotics). Azithromycin (AZM) is an oral antibiotic that concentrates in white blood cells that may serve as a reservoir for viable PsA and is currently recommended for use in CF patients with chronic PsA infection. The objective of this study is to determine the effects of AZM when incorporated into a new PsA EP at COA/UAB.

Hypothesis: The addition of AZM to a PsA EP will improve efficacy of SE.

Methods: This retrospective chart review included 23 CF patients < 21 years of age with newly acquired PsA infection who have been treated using the EP that began in April 2010. The EP used TIS 300mg BID x 28 days and

AZM at approximately 7 mg/kg given thrice weekly after the first PsA positive culture (PC). Cipro was added to a second course of TIS and AZM or a third course of CIS and cipro if patients had subsequent PsA PC. SE of PsA was defined as 3 negative cultures over 6 months following the eradication attempt and while off antibiotic therapy. IRB approval was obtained from Samford University and UAB. Statistical analyses were performed using SPSS and GraphPad Prism. Data obtained included subject demographics, age at PsA acquisition, PsA quantity in sample, mucoid presence and quantity, strains of PsA, number of hospitalizations, azithromycin dose, tobramycin resistance, FEV1%, and FVC%.

Results: Twenty-three patients participated in the new EP that included AZM. Eighteen (78%) were SE and 5 (22%) were colonized after treatment failure. Six patients (26%) achieved SE using TIS and AZM alone (Tier 1), 5 patients (21%) required the addition of cipro (Tier 2), and 7 (30%) advanced to the addition of CIS (Tier 3) compared to 45%, 29%, and 26% using the previous EP. Comparing both protocols, there was no statistical significant change in FEV1% or FVC% pre- or post-EP.

Conclusions: The addition of AZM to our center's PsA EP did not significantly improve the rate of SE. However, both the previous and new EP maintained similar efficacy compared to historical data. While overall eradication rates were similar, a portion of our patients required the addition of systemic antibiotics to achieve SE. We are uncertain whether this tier escalation is due to an unintended adverse effect of AZM on PsA eradication or the result of selection bias. Limitations to this study include but are not limited to small sample size and perhaps inadequate dosing of AZM.

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METAGENOMIC ANALYSIS OF CYSTIC FIBROSIS AIRWAY MICROBIOME

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Antibiotics remain a cornerstone of therapy with standard microbiologic cultures of cystic fibrosis (CF) airway secretions and their antibiotic sensitivity testing guiding therapy. However, in many cases therapy remains largely empiric, particularly in advanced disease. Metagenomic analysis is a sequence-based analysis of the collective genomes and provides a more comprehensive and culture-independent assessment of the microbial and viral communities, yielding not only taxonomic but also critical functional information such as microbial virulence factors, antibiotic resistance, and metabolic profiles. We hypothesize that metagenomic analysis of CF airway sputum provides complementary information to standard microbiology assessments that may inform clinical decision-making.

In this study, 3 adult CF patients were recruited and followed through the course of a severe pulmonary exacerbation associated with a drop of $\geq 15\%$ in their FEV1. Spirometry, induced sputum samples, and patient reported outcomes (PROs including the CFQR and UCSD SOBQ) were obtained at presentation, and at the end of therapy. Viral and microbial metagenomes were obtained from induced sputum samples and were processed according to previously published procedures (Willner et al, PLoS One, 4(10), 2009). Bioinformatic analysis was used to obtain the microbial and viral taxonomy as well as functional annotations (Willner et al, PLoS One, 4(10), 2009).

Metagenomic analysis confirms similar microbial diversity demonstrated in previously published studies using 16S rRNA sequenced based taxonomy. Moreover, pathways associated with antibiotic resistance as well as heavy metal transport of cobalt (Czc resistance proteins), cadmium (cadmium transporting ATPase and resistance proteins), copper (Cus and Cut family proteins), and zinc (Czc family proteins) were identified, and in many cases, increased through the course of successful therapy. Genes associated with heavy metal transport and antibiotic resistance are frequently found on the same plasmid (Baker-Austin et al, Trends in Microbiology, 14(4), 2006) or share common regulatory pathways such as Czc operon.

This prospective study follows several patients through the successful treatment of a protocol-defined pulmonary exacerbation. The metagenomic signals associated with enrichment of heavy metal transport genes and antibiotic resistance positively correlate with improvement of lung function and patient reported outcomes. Metagenomic analysis provides complementary information to standard microbial assessments and has the potential to identify other genetic markers with functional significance. This pilot study

has the potential to inform a much larger study that could impact the evaluation and management of CF patients.

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DYNAMICS OF THE CYSTIC FIBROSIS LUNG MICROBIOME THROUGHOUT A CLINICAL EXACERBATION

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Cystic fibrosis (CF), caused by mutation in the cystic fibrosis transmembrane conductance regulator gene, results in a lung environment that is highly conducive to chronic polymicrobial infection. Pulmonary disease resulting from these chronic infections is the leading cause of morbidity and mortality in adult CF patients. Conventional culturing methods have identified several key pathogens associated with the cystic fibrosis lung. However, with the advent of deep sequencing technology, a more complete survey of all the bacteria in the CF lung, the CF lung microbiome, can now be made. We followed the dynamics of the CF lung microbiome in nine adult patients with CF throughout the course of a clinical exacerbation. Consistent with previous microbiome studies, we do not find a particular organism or set of organisms that predicts an exacerbation. Instead, we find that the bacterial communities are consistent within a patient and durable throughout the course of infection. Over 120 bacterial genera were detected in this study. Despite this large number of genera, we find that the bacterial communities of all nine patients can be described by relatively few genera. That is, a genus that is highly abundant in a single patient is also highly prevalent across patients. Although the mere presence or absence of a particular set of organisms is not enough to predict an exacerbation, given the correlation between abundance and prevalence, investigation into the molecular mechanisms of polymicrobial infections among a few highly abundant genera will likely yield important insights into respiratory infections in CF as a whole.

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NANOMOLAR-POTENCY SMALL-MOLECULE INHIBITORS OF APOPTOSIS INDUCED BY PSEUDOMONAS-DERIVED HOMOSERINE LACTONE

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A major cause of morbidity and mortality in cystic fibrosis (CF) is respiratory failure from *Pseudomonas aeruginosa*-associated chronic lung infection. Other conditions associated with *P. aeruginosa* infection include bronchiectasis, chronic obstructive pulmonary disease, urinary tract infections and chemotherapy-induced neutropenia. The pathogenicity of *P. aeruginosa* results, in part, from secreted effector and virulence factors that facilitate its growth, invasion and cytotoxicity. The expression of many *P. aeruginosa* effectors is coordinated by bacterial signalling molecules, including *N*-(3-oxo-dodecanoyl) homoserine lactone (C12). In addition, C12 activates multiple host responses including apoptosis, phosphorylation of p38 MAP kinase and eIF2 α , and c-jun transcription. Cell damage, including apoptotic cell death, has been reported to occur in the CF lung. We hypothesized that C12-induced apoptosis in CF lung epithelia enhances *Pseudomonas* pathogenicity. To test this hypothesis, we identified small-molecule inhibitors of C12-induced cellular responses using cell-based screening approaches. We identified triazoloquinoline (TQ) as inhibitors of C12-induced apoptosis in several cell types relevant to CF lung disease. In cultured macrophage-like (RAW) cells, the most potent TQ inhibited apoptosis following 25 μ M C12 with IC₅₀ ~150 nM, with near complete inhibition at higher TQ concentrations. C12-induced apoptosis was also strongly inhibited by TQ in primary cell cultures, including murine embryonic fibroblasts and bone marrow-derived macrophages. In all cells tested, TQ did not inhibit apoptosis caused by anisomycin or staurosporine, suggesting selectivity for C12-induced apoptosis. In addition to inhibiting apoptosis,

mechanistic studies indicated that TQ partially inhibited C12-induced phosphorylation of p38 MAPK and eIF2 α (~50% inhibition at 1000 nM), but did not affect C12-induced c-jun synthesis. These studies establish TQ compounds as inhibitors of C12-induced cellular responses involved in apoptosis that are of utility to investigate C12-induced host responses in the CF lung. TQs or related inhibitors of C12-induced apoptosis provide a new potential therapeutic approach against *P. aeruginosa* that is quite different from conventional antimicrobials.

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EVALUATION OF METHODOLOGIES FOR DNA EXTRACTION FROM MIXED MICROBIAL POPULATIONS

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With a recent surge in cystic fibrosis (CF) microbiome research comes debate and uncertainty associated with the selection of appropriate methods for the extraction of DNA. Culture-independent community profiling methods have been noted to occasionally miss species detected using culture based methods. To examine the extent of extraction-based species bias, we tested two popular DNA extraction kits with Gram positive and Gram negative bacterial controls, mixed-species communities of defined composition, and expectorated sputum samples from patients with CF. We evaluated DNA extraction kits effectiveness by terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA library construction. A mock community was constructed by mixing equal optical densities of eight overnight individual cultures of *Burkholderia cepacia*, *Agrobacterium tumefaciens*, *Erwinia amylovora*, *Staphylococcus aureus*, *Bacillus subtilis*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, and *Streptococcus mutans*. The manufacturer's protocols for the DNeasy® Blood & Tissue kit (Qiagen) and Mobio UltraClean® Soil DNA Isolation Kit with varying the condition of bead beating, presence/absence of lysozyme and/or sputolysin were tested. DNA from these extractions was used as template in amplifications with the universal 8F and 926R primers. The resulting PCR products were digested with *CfoI* and separated using the ABI system. The peaks generated were analysed to determine the species composition produced by each DNA extraction. Individual species TRFLP runs were included as references. Sputum samples from CF patients attending the Adult CF Clinic were also tested by sequencing of 16S rRNA libraries. Variability in purity and total DNA recovery was observed amongst all extraction methods tested. Most species in the mock community were detected in all extraction methods, but with differences in profiles noted between kits. Lysozyme pre-treatment resulted in improved detection of Gram positive species, both in the mock community and in sputum samples. Extraction using the Qiagen DNeasy kit with lysozyme pre-treatment resulted in broader diversity detected within sputum samples than the Mobio Soil DNA Isolation Kit, largely resulting from better detection of Gram positives. These results highlight the potential for introduction of biases in culture-independent characterization of mixed bacterial community samples based on the DNA extraction method used, and indicate the importance of optimizing DNA extraction protocols used in culture-independent studies of the CF microbiome.

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GENETIC VARIATION IN *P. AERUGINOSA* PROVIDES CLUES TO CF LUNG DISEASE HETEROGENEITY

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Damage to the lungs of cystic fibrosis (CF) patients occurs in a heterogeneous pattern with the apical regions generally becoming more damaged than the lower lungs. The etiology of this disease heterogeneity remains elusive. The strong association between *P. aeruginosa* infection and lung function declines raise the possibility that differences in bacterial functions could contribute to disease heterogeneity. Our lab and others have found that infecting *P. aeruginosa* strains evolve to produce a diverse population of genetic variants within the lungs of CF patients. We hypothesized that *P. aeruginosa* variants residing in highly damaged lung regions may have more injury potential than bacteria found in areas of mild disease. To address this hypothesis, we obtained secretions from lobar regions of explanted CF lungs and selected several hundred *P. aeruginosa* isolates from each region for phenotype testing. Using a combination of phenotype tests, we categorized the *P. aeruginosa* population into variant subpopulations. The bacteria were confirmed to be derived from a common ancestral strain by DNA fingerprinting. Surprisingly, different subpopulations dominated in the major anatomical lung regions of all four lungs we have studied. Sequence analysis, protein expression assays, and phenotypic tests of *P. aeruginosa* from one lung revealed that the predominant isolates in the upper lobe (the most injured region) were more capable of expressing type III secretion exotoxins as compared to isolates from areas of the lung with less injury. This phenotype caused enhanced cytotoxicity to eukaryotic cells in vitro, and increased virulence in a mouse lung infection model. Together, these data suggest that regional differences in CF airway *P. aeruginosa* populations could contribute to heterogeneous lung disease in CF patients.

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ACTIVATION OF THE EPITHELIAL SODIUM CHANNEL (ENAC) BY THE ALKALINE PROTEASE FROM *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen that significantly contributes to the decline in airway function and an increase in patient mortality in cystic fibrosis. Chronic airway infection by *Pseudomonas* induces sustained immune and inflammatory responses that result in damage to the airway. *Pseudomonas* ability to resist host defenses is aided by the secretion of virulence factors and the formation of biofilms. Secreted virulence factors act to modulate host immune and inflammatory responses. Biofilms encapsulate the bacteria, protecting them from host defenses and limiting therapeutic delivery. Among the virulence factors, a variety of proteases are secreted from *Pseudomonas*. Alkaline protease (AP), a zinc-metalloprotease, has been associated with *Pseudomonas* infection in the cystic fibrosis lung. Previous work suggests that protease secretion is correlated with bacterial exacerbation in the airway, though the mechanisms are unknown.

Recent studies suggest that misregulation of protease activities in the cystic fibrosis lung may alter water absorption and mucociliary clearance through proteolytic processing of ENaC. To evaluate the potential role of AP in altered ENaC activation, AP was purified and its folding, activity and ability to activate ENaC were assessed. Previously, we have shown that AP folding is coupled with calcium binding to a conserved Repeats-in-ToXin (RTX) domain. Here we show that AP folding was efficient under pH and calcium conditions thought to be in the airway surface liquid of normal and CF lungs. Short circuit measurements of ENaC in polarized monolayers indicated that AP activated ENaC in immortalized cell lines. This increase in ENaC

activity was kinetically distinct from that elicited by trypsin or elastase treatment and resulted in submaximal activation of ENaC. Heterologously expressed ENaC, with mutations in either the alpha or gamma subunit cleavage sites, demonstrated that the activation of ENaC was mediated by gamma subunit cleavage. Finally, post-transplant, primary human bronchial epithelial cells from both CF and non-CF patients showed ENaC activation after treatment with AP. Based on these data, patho-mechanisms associated with AP in the CF lung are proposed wherein secretion of AP leads to local, decreased airway surface liquid volume and a corresponding decrease in mucociliary clearance of pulmonary pathogens. Thus, local remodeling of the CF airway may be induced by secreted factors from *Pseudomonas aeruginosa* in order to favor pathogen adherence and colonization.

PULMONARY

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STRUCTURAL & FUNCTIONAL EVALUATION OF GAS-TRAPPING IN INFANTS WITH CF

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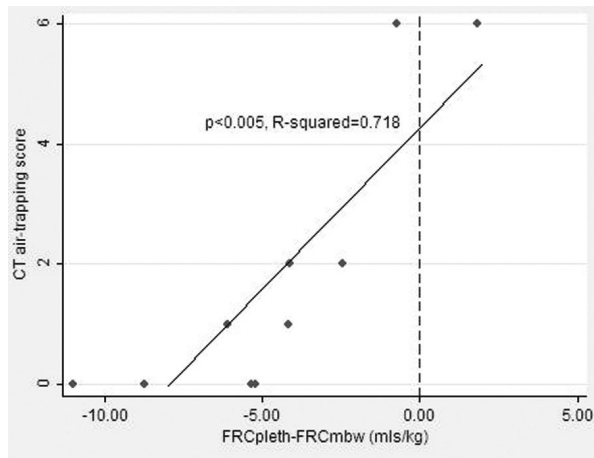
Background: Air-trapping is frequently identified in infants with cystic fibrosis (CF) on chest computed tomography (CT) scans.

Aims: To examine the association between the difference in functional residual capacity (FRCdiff) measured by plethysmography (FRCpleth) and multiple-breath washout (FRCmbw) and air-trapping scores from chest CT scans.

Methods: Infant lung function (ILF) was performed at 3 months, 1 year and 2 years of age under sedation with chloral hydrate. FRCpleth was measured using a Baby Bodyplethysmograph (Jaeger®, Germany) and FRCmbw using sulphur hexafluoride (Exhalyzer® D, Eco Medics, Switzerland). CT under general anaesthetic was carried out at 3 months and 1 year of age. Air-trapping was scored on expiratory images, defined as geographical regions of reduced density. The extent in each of 6 lung zones was defined as 0=absent, 1=affecting ≤50% of lung field and 2=affecting >50%, with maximum score of 12.

Results: Twenty-one infants of mean age 14.8 months (range 3.0-26.5 months) were tested. Ten infants had CT and ILF within the same month, 7 infants had CT an average of 11.6 months (range 9.4-13.6 months) prior to the ILF and 4 did not undergo CT. For all infants tested, n=21, mean (SD) FRCpleth was 18.4(3.99) mL/kg, coefficient of variation (CV) 1.97%, whereas mean (SD) FRCmbw was 20.8(3.6) mL/kg with CV of 4.7%. Mean FRCdiff (FRCpleth - FRCmbw) was -2.42 mL/kg (range -11.01 to 10.96 mL/kg). Linear regression (FRCdiff vs CT air-trapping score) was performed for n=10 infants with paired CT and ILF in the same month. FRCdiff was significantly associated with CT air-trapping score (see Figure). Neither FRCpleth (p=0.246) nor FRCmbw (p=0.114) alone were associated with CT air-trapping score.

Conclusion: FRCmbw was frequently larger than FRCpleth suggesting that measurements using the two different systems are not directly comparable. However, there was a strong correlation between FRCdiff and CT air-trapping scores assessed in the same month suggesting that FRCdiff may have potential as a surrogate for air-trapping which is one of the earliest and most common radiological findings in infants with CF.



Relationship between the FRCdiff and CT air-trapping scores. Each point (•) represents an infant with paired ILF and CT obtained during the same month.

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HIGH RESOLUTION COMPUTED TOMOGRAPHY (HRCT) IN ONE YEAR OLD CF NEWBORN SCREENED (NBS) INFANTS: NOT A USEFUL OUTCOME MEASURE
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Background: The use of a CF-specific CT scoring system has been advocated as a sensitive surrogate measure of early lung disease in CF. Although Brody-II CT scoring¹ has been validated and used to objectively quantify CF lung disease in children, its use has yet to be established in CF NBS infants.

Objectives: To score HRCT scans from CF NBS infants for lung abnormalities using Brody-II and to assess interobserver agreement.

Methods: As part of a prospective multicentre observational study of CF NBS infants from 2009-2011, HRCTs were performed under general anaesthesia in CF infants at a year of age across three tertiary respiratory centres using identical protocols.² Volumetric inspiratory (at 25 cmH₂O) and expiratory images were acquired. Scans were anonymised and scored independently using Brody-II by two experienced paediatric thoracic radiologists (Brody; Calder) after completion of the standard training session.

Results: Sixty scans were performed at a median age of 52 (43-64) weeks. Agreement between observers was generally only fair to moderate. Only air trapping score had substantial agreement. The number of scans with abnormalities described in the five components of the Brody-II and the interobserver agreement calculated using weighted Kappa statistics are summarised in the table. Overall, scores allocated were low suggestive of mild changes in this age group.

Conclusions: HRCT in CF NBS infants revealed far fewer structural abnormalities than reported by others.^{2,3} The majority of the scans were normal. When present, changes were mild which may account for the generally poor agreement between even experienced and trained scorers. Air trapping is the most reproducibly detectable abnormal finding in this age group. Better training might improve interobserver agreement and enable CT scoring to be a more useful measure. Currently HRCT is not a usable measure of mild CF lung disease and is not a suitable clinical trial endpoint in infants.

References: ¹Brody J Thorac Imag 2006; ²Stick J Pediatr 2009; ³Mott Thorax 2011

Acknowledgements: Children and parents involved in the study; Members of LCFC; Radiology and Anaesthetic Depts at GOSH, BLT, RBH; CF Trust, UK; GOSH Special Trustees; Collaborative London Research Network.

Number of scans with abnormalities described	Air trapping	Bronchial dilatation	Peribronchial wall thickening	Mucus plugging	Parenchymal changes
Radiologist A n (%)	20 (33%)	17 (28%)	16 (27%)	3 (5%)	47 (78%)
Radiologist B n (%)	15 (25%)	5 (8%)	3 (5%)	3 (5%)	24 (40%)
Kappa	0.7	0.2	0.5	0.4	0.4

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BRONCHIECTASIS AND TRAPPED AIR IN CYSTIC FIBROSIS LUNG DISEASE – EARLY PREDICTORS OF DISEASE SEVERITY

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Background: Progression of bronchiectasis (BE) and trapped air (TA) are important determinants of cystic fibrosis (CF) lung disease. BE and TA can be detected and monitored by chest computed tomography (CT). It is unclear what characteristics of school age children with CF predict progression of BE and TA.

Objective: Among school age children with CF, to identify predictors of severity of BE and TA 6 years later.

Methods: Single center longitudinal study. Inclusion: availability of two volumetric CTs (performed as part of routine clinical care) with a time interval between CTs of 6 years or more; age at first CT 6-13 years. CT₁ and CT₂ were anonymized and scored in a blinded fashion using the CF-CT BE and TA score, expressed as a percentage of a maximum score. In a univariate linear regression model, we tested the following predictors for severity of BE and TA at CT₂: socioeconomic status, FEV₁ at time CT₁, *Pseudomonas* ever prior to CT₁, respiratory tract exacerbations (RTE) defined as number of IV antibiotic courses in the year prior to CT₁, and BE and TA scores on CT₁. Significant predictors were tested in a stepwise multivariate model to identify independent predictors for severity of BE and TA at time of CT₂.

Results: Data were available from 27 patients with a median age at CT₁ of 9.2 years (range 6.9-12.9 years), male n=18, median time interval between CTs 6.5 years (range 5.8-7.9 years). Children with a history of *Pseudomonas* prior to CT₁ had a 7.91% higher score for BE on CT₂ (95% confidence interval (CI_{95%}) 2.26-13.58, p=0.008). A RTE in the year previous to CT₁ was associated with a 22.4% increase in TA score on CT₂ (CI_{95%} 1.64-43.2, p=0.036). A 1% higher BE score on CT₁ resulted in a 1.04% higher BE score on CT₂ (CI_{95%} 0.67-1.41, p<0.000) and a 1.57% higher TA score on CT₂ (CI_{95%} 0.06-3.08, p=0.042). In the multivariate analysis only BE score on CT₁ remained predictive of both BE (p<0.000) and TA on CT₂ (p=0.042).

Conclusion: Prior *Pseudomonas* infection and RTE in the year before baseline CT predicted BE and TA 6 years later. The effect of these predictors appeared to be mediated through BE on CT₁, which was highly predictive of both BE and TA severity 6 years later.

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HIGH RESOLUTION CT SCANS DEMONSTRATE GENDER-BASED DIFFERENCES IN PATTERN AND SEVERITY OF LUNG DISEASE IN ADULTS WITH CF

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Cystic fibrosis (CF) is defined by progressive pulmonary pathology typically first apparent in the upper lobes. Despite increasing life expectancy, many databases have shown females experience a more rapid decline in lung function, more severe pulmonary pathology and earlier mortality.

Poster Session Abstracts

Brody et al. demonstrated that a scoring system for pulmonary pathology on high resolution computed tomography (HRCT) predicts severity of lung disease and correlates with FEV₁ in children; however, this analysis has not been applied to adults to analyze gender-based differences in CF. We hypothesized that adult females demonstrate a different pattern of distribution of pulmonary pathology on HRCT.

Clinical HRCTs were analyzed from adult CF patients attending the Colorado CF Center. 14 females and 13 males with diagnosis in infancy had at least one HRCT scan. The subjects were age matched at the time of the scan (29.6 ± 8.1 years for females vs 31.6 ± 8.8 years for males, $p = \text{NS}$). The first scan for each subject was scored by a radiologist utilizing the Brody system. Scores for each pathologic modality were sorted by zone (upper, middle, lower). The cohorts demonstrated similar mean FEV₁ % predicted at the time of the scan (61.6 ± 24.3 for females vs 58.8 ± 17.0 for males, $p = \text{NS}$).

Despite lower age and greater FEV₁, the overall HRCT imaging score trended worse in female patients (53.9 ± 30.1 vs 39.9 ± 22.4 for males, $p = 0.18$). In the upper zones females have significantly greater total (12.4 ± 6.8 vs 9.7 ± 4.3 for males, $p < 0.05$), bronchiectasis (4.8 ± 2.8 vs 3.2 ± 2.2 for males, $p < 0.05$), and parenchymal scores (1.6 ± 1.2 vs 0.8 ± 0.7 for males, $p < 0.005$). In the middle zone, females trended towards worse total (8.1 ± 5.9 vs 5.9 ± 6.2 for males, $p = 0.09$), mucus plugging (0.8 ± 1.2 vs 0.4 ± 0.7 for males, $p = 0.06$), and parenchymal scores (1.1 ± 0.9 vs 0.7 ± 1.0 for males, $p = 0.08$). In the lower zone, females have increased total (6.5 ± 4.2 vs 4.4 ± 4.2 for males, $p < 0.05$), bronchiectasis (2.0 ± 1.7 vs 0.7 ± 1.1 for males, $p < 0.005$), and peribronchial thickening scores (1.9 ± 1.5 vs 1.3 ± 1.3 for males, $p < 0.05$).

Upper lobe total, bronchiectasis, and peribronchial thickening scores correlated well with FEV₁ in females ($R^2 = 0.40, 0.30$, and 0.35 , respectively, $p < 0.005$ for comparisons). Similar correlation to total, bronchiectasis, and peribronchial thickening scores were observed in middle ($R^2 = 0.28, 0.32$, and 0.34 , respectively, $p < 0.005$) and lower zones ($R^2 = 0.40, 0.20, 0.43$, respectively, $p < 0.005$) with correlation to the air trapping score in the lower zone ($R^2 = 0.56, p < 0.005$). There was no significant correlation between CT score and FEV₁ for males in any lobe.

This preliminary study demonstrates important differences in lung imaging scores between female and male CF adults not described in pediatric subjects. A stronger correlation between female subjects' CT scores and lung function suggests a pathophysiologic mechanism of female CF lung disease progression and calls into question the utility of HRCT scans as a marker of disease severity in adult CF males.

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REGIONAL MEASURES OF LUNG FUNCTION IN THE β -ENAC MOUSE OBTAINED USING X-RAY IMAGING VELOCIMETRY

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Introduction: X-ray velocimetry (XV) is a new imaging method that combines synchrotron-based phase contrast X-ray imaging with motion tracking methods used in fluid mechanics to detect the presence of lung disease. Structural changes that occur during lung disease alter the flow of air and the motion of the lungs, whether by obstruction increasing airway resistance, or by changes to lung parenchyma altering the mechanical properties of the tissue. Altered or abnormal motion of the lungs is thus also an indicator of disease. The XV technique is extremely sensitive to small changes in lung function as it is able to measure motion at thousands of points across the lungs in 4 dimensions (3 spatial dimensions over time), providing "pin-

point spirometry". As a demonstration of the technique's ability to characterize CF-like lung disease, we applied XV to adult β -ENaC mice.

Materials and Methods: 15 β -ENaC mice and 15 littermates (University of Heidelberg, Germany) were imaged under somnopenly anesthesia (i.p.) to acquire 4-dimensional data sets at the Spring-8 synchrotron in Japan. Imaging was synchronised to the respiratory cycle using our own custom pressure controlled small animal ventilator, and pulmonary function tests (Flexivent, SCIREQ), post mortem high-resolution computed tomography (HRCT), and histology were also performed.

Results: XV analysis yielded maps of lung expansion at high spatial and temporal resolution across the entire lung. Regional differences in total inspiratory expansion in the β -ENaC mice were observed relative to littermate controls. Local measures were integrated and validated against global measures from flexiVent lung function tests. HRCT also confirmed hyperinflation and emphysema in β -ENaC mice, with associated infiltration and irregular dilation of bronchi.

Discussion: For the first time, regional lung function has been measured across the entire β -ENaC lung throughout the respiratory cycle. The technique has been applied here to a mouse model that simulates some of the features of advanced human CF lung disease, but the sensitivity of the technique also shows promise for early disease detection, well before conventional measures show lung function deficits (Fouras et al., Ann. Biomed. Eng. 2012). Future studies utilising direct tracheal instillation of bacteria loaded agar beads will investigate the ability of the XV technique to detect the earliest effects of obstruction and remodelling related to infection and inflammation.

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IMPROVED AIR TRAPPING EVALUATION IN CHEST HIGH RESOLUTION COMPUTED TOMOGRAPHY IN CHILDREN WITH CYSTIC FIBROSIS USING REAL-TIME SPIROMETRIC MONITORING AND BIOFEEDBACK

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Rationale: Evaluation of cystic fibrosis (CF) lung disease by chest high resolution computer tomography (HRCT) is dependent upon sufficient and reproducible in- and expiratory breath hold during imaging. Traditionally, breath hold manoeuvres are unmonitored, but HRCT imaging might be improved by real-time spirometric monitoring and biofeedback software with implications on reproducibility and air trapping (AT) evaluation.

Objective: To evaluate the influence of spirometric breath hold monitoring and biofeedback software on lung density measures and AT scores compared to unmonitored breath hold manoeuvres in children with CF.

Methods: Two CF centres participated in this cross sectional group comparison study of chest HRCT. Comparison was made between HRCT examinations in CF children matched by age, gender and lung function. Imaging was performed with either spirometric monitoring and biofeedback software (Copenhagen (CPH)) or unmonitored breath hold manoeuvres (Gothenburg (GTB)). Images were anonymized and breath hold manoeuvres were concealed from the observer. Lung tissue attenuation was measured in Hounsfield Units (HU). Comparison of AT scores (modified Brody) between groups was calculated using Mann-Whitney non-parametric test. Attenuation comparisons were compared using unpaired parametric t-test. Influence on AT from attenuation, lung function or age was examined by multiple regression analysis.

Results: Eighty-four examinations were evaluated (42 in each group). Mean (95%CI) change in attenuation between in- and expiratory images was 436 HU (408 to 464) in CPH and 229 HU (188 to 269) in GTB ($p < 0.0001$) (Figure). Mean AT (95%CI) score was 6.9 (6.1 to 7.8) in CPH patients and 3.8 (2.9 to 4.7) in the GTB group ($p < 0.0001$). However, this difference in AT score disappeared ($p = 0.23$) after correction for change in tissue attenuation and age, suggesting that the groups were of comparable dis-

ease severity, and that the observed difference was due to inadequate exhalation during the GTB examination.

Conclusion: Spirometric breath hold monitoring and biofeedback imposed significant difference in lung attenuation and AT scores on chest HRCT compared to unmonitored breath hold manoeuvres in age matched CF children with comparable clinical disease severity. The method may be of additional value in group comparison studies and longitudinal assessments of CF changes on HRCT.

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AIRWAY INFLAMMATION IS PRESENT BY 4 MONTHS IN CF INFANTS DIAGNOSED ON NEWBORN SCREENING

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Introduction: Cystic fibrosis (CF) newborn screening (NBS) was introduced nationwide within the UK in 2007. NBS allows early introduction of treatment, often before any symptoms arise with the aim of reducing infection and inflammation in the airway.

Aims: The aim of this study was to establish the presence of infection and the degree of inflammation in airways in the NBS cohort. At our centre it is usual clinical practice to perform fiberoptic bronchoscopy (FOB) at the age of 3 months for all children with CF. Bronchoalveolar lavage fluid (BALF) was cultured and examined for cellular inflammation. We have previously reported the presence of bacterial infection in this cohort, even in asymptomatic infants. This further study examined the level of inflammation in both culture positive and negative infants with a comparison to children with established CF and healthy controls.

Results: Infants diagnosed by NBS undergoing routine FOB who had either BALF absolute cell count or differential cell count assessed were included in the study. Forty-four infants (48% female), median age (range) 15 (7-28) weeks met these criteria. The majority of these infants were symptom free, however 13 (29%) had bacterial isolates from their BALF. Data are also available for 71 children with established CF (median age 9.5 years; range 1.9-16.7 years) of whom 46 (65%) were culture positive on their BALF and for 6 healthy controls (median age 12.3 years, range 10.5-15.4 years), who underwent clinically indicated FOB during the same time period.

Cellular inflammation was present in the airways in infants diagnosed by NBS, both in those who were culture positive and culture negative in their BALF. Absolute BALF cell count and neutrophil differential count were significantly higher in both NBS and established CF patients compared with healthy controls ($p < 0.02$). Median absolute cell counts and neutrophil differentials can be seen in Table 1. For both NBS and established CF, in those who had bacteria isolated from their BALF, an increase in neutrophil differential was seen compared with those culture negative at the time of FOB although this did not reach significance in the NBS group (NBS CF $p = 0.05$, established CF $p < 0.01$).

Conclusion: Our results demonstrate that inflammation is already present by 4 months of age in asymptomatic infants diagnosed through newborn screening, although at a lower level than seen in older children with established CF. The results underscore the importance of early surveillance and lend support to the evolving focus on this age group for interventional trials.

	NBS CF micro +ve	NBS CF micro -ve	Established CF micro +ve	Established CF micro -ve	Healthy controls
n (absolute cell count)	10	22	33	15	6
Absolute cell count $\times 10^3$	418 (155.0-2060)	378 (75-1877)	1495 (140-48990)	555 (143-14780)	143 (70-270)
n (neutrophil differential)	13	24	22	15	6
Neutrophil differential (%)	17.2 (2.0-73.0)	8.4 (0.3-57.3)	53.2 (15.7-92.0)	19.3 (1.7-87.7)	1.4 (0.3-2.7)

Table 1: BALF cell counts $\times 10^3$. Figures given are medians (range)

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PROSPECTIVE EVALUATION OF RESPIRATORY EXACERBATIONS IN CHILDREN WITH CYSTIC FIBROSIS FROM NEWBORN SCREENING TO 5 YEARS OF AGE (FOR ACFBAL STUDY GROUP)

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Background: Respiratory exacerbations are an important clinical feature and a potential trial outcome measure especially in young children.

Aims: (i) To determine the rate, duration and clinical features in pre-school CF children; and (ii) to determine whether there were associations with growth, lung structure and function at age five.

Methods: Respiratory exacerbations were recorded prospectively in Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBAL) trial subjects from enrolment after newborn screening to age five, when all participants underwent clinical assessment, chest computed tomogram (CT) scans and spirometry.

Results: A total of 168 children (88 males) experienced 2080 exacerbations at an average rate of 3.66 exacerbations per person-years; 91.8% were community-managed and 8.2% required hospital-management. Exacerbation rate differed by site ($p < 0.001$) and was 26% lower (95%CI 12%, 38%) in children receiving 12 months prophylactic antibiotics. The rate of exacerbations in the first two years was associated with reduced FEV₁ (coef -0.20, 95%CI -0.36, -0.05), as was doctor reported tracheal tug/retractions during exacerbations (coef -0.59, 95% CI -1.00, -0.18). A history of hospital-managed exacerbations was associated with bronchiectasis (OR 2.74, 95%CI 1.42, 5.31) and with lower weight z-scores at age five (coef -0.30, 95%CI -0.57, -0.04). Parent reported wheeze during exacerbations was associated with air-trapping (OR 3.47, 95%CI 1.59, 7.56, $p = 0.002$) in chest CT scans at five years. There was a 12% increase (95%CI 2%, 21%) in the rate of exacerbations during years 4 and 5 per doubling of exacerbation rate in the first 2 years.

Conclusions: Respiratory exacerbations in young children are markers for progressive CF lung disease and are potential trial outcome measures for treatments in this age group.

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CHANGES IN LUNG CLEARANCE INDEX DURING PERIODS OF STABILITY AND PULMONARY EXACERBATION

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Introduction: Lung clearance index (LCI) has been previously reported to be a useful measure of lung function during pulmonary exacerbations (PEX) in children and adults with CF.

Objective: To assess the change in LCI and FEV₁ before, during and after a PEX.

Methods: Patients admitted to the adult and paediatric CF centres for treatment of a PEX were recruited. The Respiratory and Systemic Symptoms Questionnaire (RSSQ), multiple breath washout (MBW) test and spirometry were completed when patients were clinically stable (S1), at initiation (E1) and completion (E2) of intravenous (IV) antibiotic treatment and at ≥ 4 weeks post treatment when patients were clinically stable (S2). Participants completed 3 MBW tests, using 0.2% SF₆ and a modified Innocor™ device. LCI was reported as the mean of at least 2 acceptable tests. Spirometry was performed to ATS/ERS standards. Friedman test and Wilcoxon

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signed rank statistics were used to assess for significant differences between time points.

Results: Thirteen patients (7 male), mean age (SD) 31.2 (12.9), range 13-56 years, have been recruited. All patients had a PEX as defined by RSSQ (4/12 signs or symptoms on RSSQ) at E1. Data from n=10 pairs (E1-E2), n=7 pairs (E2-S2) and n=4 pairs (S1-S2) was analysed. Recruitment is ongoing.

The results of the Friedman test indicated that there was a statistically significant difference in LCI ($\chi^2=7.14$, $p=.03$) and FEV₁ ($\chi^2=8.86$, $p=.01$) across 3 time points (E1, E2, S2). Median values showed an improvement in both lung function measures from visit E1 to E2 and a decline from visit E2 to S2.

The Wilcoxon signed rank test showed a statistically significant improvement in LCI and FEV₁ from E1 to E2 following completion of IV antibiotics with an effect size of $r=.48$ (LCI) and $r=.60$ (FEV₁), respectively. A statistically significant decline was observed from E2 to S2 in LCI and FEV₁. S1 and S2 visits were not statistically different in either measure (Table 1).

Conclusions: Both measures show a peak in lung function following IV antibiotics and a return to stable values ≥ 4 weeks post IV's. These results show that LCI can be used to demonstrate changes in lung function during and post IV antibiotic treatment of a PEX, generating complementary but different results to FEV₁.

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Table 1: Wilcoxon signed rank test results between time points

z score and p value	E1 - E2	E2 - S2	S1 - S2
LCI	$z=-2.19$, $p=.03^*$	$z=-2.37$, $p=.02^*$	$z=-1.46$, $p=.14$
FEV1 (z-score)	$z=-2.70$, $p=.01^*$	$z=-2.37$, $p=.02^*$	$z=-7.30$, $p=.47$

* $p<.05$

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EFFECT OF PROTON PUMP INHIBITORS ON CYSTIC FIBROSIS (CF) PULMONARY EXACERBATIONS IN ADULTS

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Introduction: Gastroesophageal reflux (GER) is frequent in patients with CF and is often regarded as playing a role in the pathogenesis of respiratory disease. It is unknown whether medical treatment of GER has a beneficial effect on pulmonary exacerbation frequency and lung function. We conducted a double blind randomized pilot study comparing esomeprazole to placebo in individuals with CF and a history of frequent respiratory exacerbations. Primary outcome measure was time to first exacerbation. Secondary outcome measures included assessment of CF related quality of life (CFQ-R), gastroesophageal assessment score (GSAS), exacerbation rate and lung function.

Methods:

Inclusion Criteria:

-Age > 18

- > 1 respiratory exacerbation per year requiring oral or intravenous antibiotics for each of the two years prior to study entry, but < 5 exacerbations requiring intravenous or oral antibiotics during either of those years.

-Stable maintenance medical regimen during the preceding 6 weeks.

Exclusion Criteria:

-Previous anti-reflux surgery

-Reflux symptoms > twice per week (clinical indication for acid suppressor therapy)

-Use of a proton pump inhibitor (PPI) or acid suppressor agent within the previous 14 days

-Pulmonary exacerbation within the previous 14 days

-Use of medications that interact with PPIs

All subjects underwent 24-hour distal esophageal pH probe testing before randomization. Randomization was stratified based on study center and FEV1 decile, but NOT by presence or absence of GER on pH study.

Study duration was 24 weeks; patients were evaluated at the study center every 8 weeks.

Results: Seventeen subjects were enrolled and randomized, 13 had successful placement of the distal esophageal pH probe. Eight of the 13 (61%) demonstrated evidence of asymptomatic GER. There were no significant baseline differences between the esomeprazole and placebo groups. Subjects receiving esomeprazole experienced shorter time to first exacerbation compared with placebo, but difference did not meet statistical significance. Five of 8 subjects receiving esomeprazole compared with 2 of 6 subjects receiving placebo developed a pulmonary exacerbation during the 6 month follow up ($p=0.11$). Only subjects randomized to esomeprazole experienced a significant improvement in the GSAS ($p<0.001$). Neither group demonstrated a significant change in FEV1 or CFQ-R.

Conclusions:

-Asymptomatic GER was present in 61% of patients with CF.

-Treatment with esomeprazole led to trend of shorter time to pulmonary exacerbation and more frequent exacerbations compared with placebo.

-Esomeprazole led to significant improvement in GSAS compared with placebo despite absence of clinical GER symptoms in the study subjects.

-Esomeprazole did not lead to improvement in lung function or CFR-Q.

-A larger study is warranted to address potential risks of PPIs in CF.

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A RANDOMISED, OPEN LABEL PHASE 3 STUDY TO EVALUATE THE EFFICACY AND SAFETY OF A DRY POWDER FORMULATION OF COLISTIMETHATE SODIUM (COLOBREATH®) VERSUS TOBRAMYCIN NEBULISER SOLUTION (TNS) IN CYSTIC FIBROSIS SUBJECTS WITH CHRONIC PSEUDOMONAS AERUGINOSA LUNG INFECTION

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Objectives: To compare the efficacy and safety of a novel dry powder formulation of inhaled colistimethate sodium, Colobreathe®, versus tobramycin nebuliser solution (TNS) in an open label multi-center, randomised study.

Methods: Subjects (n=380, aged ≥ 6 years) from 66 CF centres in EU countries, Russia and Ukraine were randomized to Colobreathe® 125 mg twice daily or TNS 300 mg twice daily. The primary endpoint was non-inferiority of Colobreathe® for change in FEV1 % predicted after 24 weeks, pre-specified ≤ -3 %. Secondary endpoints were changes in antibiotic resistance and the safety and convenience of Colobreathe®, using a quality of life (QoL) questionnaire.

Results: After logarithmic transformation based on non-normal value distribution, the adjusted mean difference between Colobreathe® and TNS groups in the change in FEV1 % predicted at week 24 was -0.97% (95% CI: -2.74%, 0.86%) for the intent-to-treat (ITT) population (n=374), while it was -0.56% (95% CI: -2.71%, 1.70%) for the per-protocol (PP) population (n=261). In the Colobreathe® and TNS groups, the proportion of colistin-resistant isolates during the 24-week period was low ($\leq 1.1\%$) compared to 11.0-19.7% of tobramycin resistant isolates. At week 24, the QoL assessments were in favour of Colobreathe® in ITT and PP populations.

Conclusions: Colobreathe® demonstrated non-inferiority to TNS in lung function over 24 week treatment and CF subjects favoured Colobreathe® over TNS. We conclude that Colobreathe® is as effective and was similarly tolerated as TNS in the management of chronic *P. aeruginosa* infection in CF.

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HIGH DOSE IBUPROFEN SIGNIFICANTLY IMPROVES LONG-TERM CF SURVIVAL

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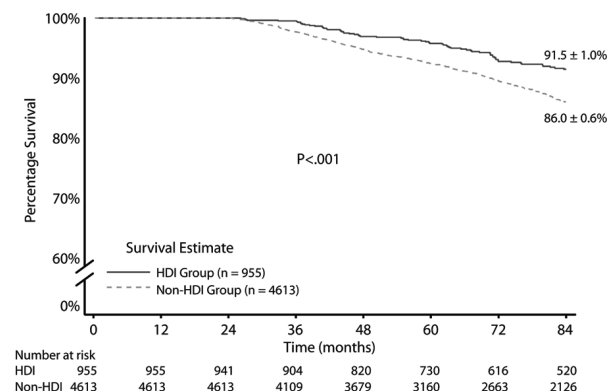
Background: Chronic administration of high dose ibuprofen (HDI) has been prospectively and retrospectively shown to significantly reduce FEV₁ decline rates, but is used in only a fraction of those who might benefit. Given that FEV₁% predicted has been suggested as a surrogate for survival in CF, it follows that administration of HDI over years should provide a CF survival advantage.

Methods: We analyzed treatment and demographic data from the Epidemiologic Study of CF (ESCF) supplemented with mortality data from the CFF Patient Registry from 2009 to compare 7-year survival for patients administered HDI for at least two consecutive years between 1994 and 2004 to patients not receiving regular HDI matched (up to 5:1) using propensity scores. Unadjusted time to all-cause mortality from baseline was evaluated using Kaplan-Meier survival curves. Patients who had not died were censored at the time of their last reported encounter. Relationships between HDI use and death were also evaluated with semi-parametric Cox proportional hazards models, with the HDI therapy variable unadjusted and then adjusted for a set of risk factors found to be associated with death in preliminary logistic regressions.

Results: We matched 926 HDI patients with 4,385 control patients. Mean $\pm$ SD age at baseline was 13.9 $\pm$ 7.6 yrs for HDI patients and 14.4 $\pm$ 7.5 yrs for matched controls. FEV₁ was 86.2 $\pm$ 21.6 % predicted for HDI patients versus 82.5 $\pm$ 24.3 for non-HDI controls. Seven-year Kaplan-Meier survival (95% CI) of HDI patients was 91.5% (89.4 to 93.5%) versus 85.6% (84.4 to 86.8%) for matched comparators (P<.001; Figure). Unadjusted fixed-effects Cox proportional hazard modeling showed HDI patients had a hazard ratio of death from all causes of 0.522 compared to matched controls (P<.001). When the Cox proportional hazards fixed-effects model was adjusted for 17 covariates, the hazard ratio associated with HDI use increased to 0.736, but remained statistically significant (P=.025).

Conclusions: Patients treated with HDI for at least 2 years experienced greater 7-year survival than matched patients chosen by propensity scoring when analyzed by Kaplan-Meier methods. Unadjusted and adjusted Cox proportional hazards models also showed statistically significant survival advantages. These data suggest that reduction in the rate of FEV₁ decline (the primary benefit of HDI) is associated with a significant survival benefit in CF.

This study was supported by Genentech, Inc.



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THE EFFECT OF INTRANASAL DORNASE ALFA ON CHRONIC SINUSITIS IN PATIENTS WITH CYSTIC FIBROSIS: A PILOT STUDY

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Background: Chronic paranasal sinusitis is a common complication in cystic fibrosis (CF). Radiographic abnormalities are seen in the majority of individuals due to viscous secretions, with subsequent mucosal thickening and polypoid obstruction. In many cases, chronic infection of the sinuses is associated with lower respiratory tract symptoms and lower quality of life scores. Functional endoscopic sinus surgery (FESS) is an effective procedure for the management of chronic symptomatic sinusitis in patients with CF, but recurrence of sinus disease is common. Dornase alfa (DNase) decreases the viscosity of airway secretions and has been shown to improve lung function in patients with CF. Currently DNase is indicated for use by oral inhalation to treat pulmonary disease in CF. We hypothesize that DNase may have a similar effect for CF sinus disease when administered by the intranasal route.

Specific aims: To examine the effects of intranasal DNase on post-operative sinus morbidity, recurrence of disease, symptoms and quality of life in patients with CF.

Methods: Fifteen patients who had undergone FESS within the previous week were enrolled in the study and randomized to either daily intranasal DNase or placebo in a double blind fashion for a period of 12 months. All patients had previously been prescribed orally inhaled DNase. A Sinusair device (PARI GmbH, Germany) with nasal adapter was used to nebulize study drug. Sinus CT scans were done preoperatively and at the end of the study period. The Chronic Sinusitis Survey (CSS) and Medical Outcome Study Short-form 36 item Health Survey (SF-36) were administered at the beginning of the study and at 6 and 12 months. Weekly visits were performed for the first month. Sinus cultures and endoscopic photographs were taken at 1, 2.5, 6, 9 and 12 month time points. Both CT scans and photographs were reviewed and blindly graded by two reviewers. To measure mucociliary clearance, a saccharin test was done at 6 and 12 months. Lung function, pulmonary exacerbations and courses of antibiotics directed against sinusitis were also recorded.

Results: Eleven subjects completed the 12 month study. Five of the subjects received DNase. Although CT and endoscopic scores were not different between groups, there was a significant decrease in CSS scores in the DNase group (p=0.003) with no change in the placebo group. There was no significant change in the SF-36 scores or FEV₁ in either group.

Conclusions: This pilot study suggests that the use of daily intranasal DNase may reduce sinus related symptom scores in patients with CF following sinus surgery.

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CHILDREN WITH CYSTIC FIBROSIS HAVE INCREASED AORTIC STIFFNESS AS MEASURED BY PHASE CONTRAST MAGNETIC RESONANCE IMAGING

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Improved care of cystic fibrosis (CF) patients has altered the prognosis to such an extent that almost all children reach adult life. As life expectancy increases, new clinical problems become evident. CF is a chronic inflammatory disease and the role of inflammation in the pathogenesis of atherosclerosis is well recognised, however it's believed that atherosclerosis is rare in CF. Increased arterial stiffness is an early feature of atherosclerosis and has been shown in adult CF patients. Arterial compliance is the inverse of stiffness and arterial distensibility is the change in vessel area for a given pressure change. Both decrease with atherosclerosis. We hypothesised that CF

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children would have altered aortic compliance and distensibility. Furthermore these differences would be exaggerated in the presence of CF diabetes and worsening lung function.

Methods: This was a case controlled study of children set in 2 London CF centres. 3 groups were recruited; children (<17 yrs) with FEV₁ <40%; children with FEV₁ >50% and age matched healthy controls. Patients were studied during a time of disease stability. Real time flow measurements were acquired from the ascending aorta and blood pressure was measured with a cuff based oscillometric device. The aorta was automatically traced on the magnitude images and a plug-in transferred the contours to the velocity-map image. Compliance was calculated using an optimization of the 2-element Windkessel model and distensibility was calculated from the following formula: (max area - min area)/(min area x pulse pressure).

Results: 30 CF children (12 FEV₁ <40%; 18 FEV₁ >50%) and 17 healthy controls participated. All children were a similar age, gender and body surface area. Within patient groups, BMI, CF diabetes incidence, chronic pseudomonas infection and genotypes were similar. Haemodynamic parameters are given in the Table. There was a significantly lower aortic compliance in all CF patients compared to healthy controls (p<0.0001), however there was no difference between the moderate and severe groups. Participants with more advanced lung disease (FEV₁ < 40%) had a significantly lower distensibility than the healthy controls (P<0.05). 8 (27%) children had CF diabetes. There was a suggestion of a difference between aortic distensibility between the 2 groups (p = 0.08), but compliance was similar.

Conclusions: For the first time we have shown that children with CF have decreased resting aortic stiffness. The longterm implication of this is unknown; however we need to consider cardiovascular health, especially as survival increases.

Table 1: Participant haemodynamics (median, interquartile range, Kruskal-Wallis)

	Controls	All Patients	FEV ₁ <40%	FEV ₁ >50%
Subjects	17	30	12	18
Pulse pressure (mmHg)	32 (27-37)	**43 (35-53)	**44 (42-52)	**43 (33-54)
Distensibility (mm Hg ⁻¹) x 10 ³	8.0 (5.0-10.4)	6.7 (3.1-9.5)	*4.5 (2.5-7.0)	8.1 (4.0-12.2)
Compliance	1.0 (0.93-1.3)	**0.76 (0.59-0.91)	**0.76 (0.59-0.91)	**0.76 (0.59-0.91)

* p<0.05 difference from controls

** p<0.0001 difference from controls

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DEATHS IN CHILDHOOD FROM CYSTIC FIBROSIS: 10-YEAR ANALYSIS FROM TWO LONDON SPECIALIST CENTRES

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Introduction: Death in childhood from cystic fibrosis (CF) is now an uncommon event in the UK. We wished to assess the circumstances surrounding deaths (and lung transplantation) in the modern era of CF care.

Methods: A retrospective review was carried out pooling data from two large paediatric specialist CF units in London for the 10 year period 2000-2009 inclusive.

Results: There were 11 deaths and 8 children who had a lung transplant out of 1022 children. Median age of death was 14.2 years and transplant 13.0 years; unusually one child died at 3.5 years and one at 9 years (both with significant comorbidity) but the rest were mostly teenagers. There was a female preponderance (82% deaths and 75% transplants). All were chronically infected with *Pseudomonas aeruginosa* and the majority with *Staphylococcus aureus* (68%); 4/11 who died had *Burkholderia cepacia* complex. Apart from one child (FEV₁ 69%), lung function indicated severe lung disease (median FEV₁ 33%, range 12-69%). Values 5 years prior to death were not predictive (median FEV₁ 62%, range 32-96%), and those 1 year prior were similar to the last recorded levels. Almost all (10/11) died in hospital and 5/11 (45%) were ventilated in the paediatric intensive care unit. Severe respiratory compromise and respiratory failure was the commonest mode of

death (64%), whilst 2 children (18%) died post-lung transplantation (one at 2 weeks post transplant, one 6 years later from obliterative bronchiolitis). One child died of multi-organ failure felt not to be related to the underlying CF, and one child died after cerebral infarction following bronchial artery embolisation for massive haemoptysis. Only 4 children (36%) were receiving palliative care, and in 6 (55%) cases a decision to withdraw care was made. None of the children who died were on a waiting list for lung transplantation.

Conclusions: The number of deaths in children with CF was small but often unpredictable, so active management was continued until late in the majority, reflected by the fact that almost all were in hospital, and more than half were ventilated. If death from respiratory failure follows a steady decline, palliative care should be instituted well in advance, followed by appropriate end of life care.

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PILOT STUDY OF INHALED DRY POWDER MANNITOL IN YOUNG PEOPLE WITH CF HOSPITALISED WITH PULMONARY EXACERBATION

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Inhaled dry powder mannitol (IDPM) is an osmotic agent which increases mucociliary clearance and improves lung function in CF when used over 12 months^[1]. The utility of IDPM in augmenting improvement during hospitalisation for acute pulmonary exacerbations in CF is unknown.

Aim: To assess the tolerability and efficacy of IDPM on lung function and exercise when used as an adjunct to intravenous antibiotics in young people with a pulmonary exacerbation.

Methods: A randomised double-blind placebo-controlled pilot study of IDPM (10x40mg capsules) twice daily for 12 days. Pulmonary exacerbation was defined by Fuchs criteria^[2]. Exclusion criteria: FEV₁ on admission <40%, oxygen requirement, oral corticosteroids and failure to tolerate a test dose of the randomised study drug. The study drug was administered prior to airway clearance treatments supervised by a physiotherapist. Spirometry (FEV₁ and FEF₂₅₋₇₅), plethysmography (RV and TLC), multiple breath washout (lung clearance index, LCI) and Bruce Protocol treadmill tests (VO₂) were measured on admission and Day 14. Changes from baseline were measured between groups using Student's t-tests and Mann Whitney.

Results: 23 participants (8-18 years) were recruited. IDPM was tolerated well with 22 participants (96%) completing the study. One participant was excluded due to vomiting after the test dose. Both groups had 11 participants. The placebo group had lower admission FEV₁ (58.0 (13.0)% vs 73.1 (15.6)% predicted, p<0.05) and FEF₂₅₋₇₅ (25.7 (20.1 - 71.8)% vs 44.1 (22.8 - 92.43)% predicted, p<0.05) than the IDPM group. All other parameters were equivalent on admission. Improvements in lung function and exercise between groups are described (Table 1). While there were differences noted, these did not reach statistical significance (ΔLCI p=0.051).

Conclusion: IDPM was well tolerated as an adjuvant therapy to intravenous antibiotics for acute exacerbation in young people with CF. While the sample size in this pilot study was too small to generate significant differences between groups, the greater magnitude of change in the IDPM group in peripheral lung function suggests that IDPM may be of benefit during inpatient admissions, and supports a large multi-center randomised control trial.

Acknowledgements: This study was funded by Pharmaxis Australia.

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Table 1. Efficacy of IDPM vs placebo between admission and Day 14

	IDPM	Placebo
ΔFEV1 (% predicted)	22.0 (8.0)	26.2 (24.4)
ΔFEF 25-75 (% predicted)	69.6 (45.2)	61.8 (55.1)
ΔLCI	-25.6 (13.3)	-12.8 (11.2)
ΔRV/TLC	-17.1 (10.5)	-5.1 (20.4)
ΔVO2 (ml/kg/min)	6.1 (18.0)	3.3 (13.0)

Data presented as percent change from admission. Mean (SD).

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FACTORS ASSOCIATED WITH INCONSISTENT DORNASE ALFA THERAPY IN PATIENTS WITH CYSTIC FIBROSIS

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Background: Consistency of chronic pulmonary medication prescription in cystic fibrosis (CF) is a factor in maintaining good adherence and health outcomes. Our objectives were to develop a metric for measuring medication consistency and to evaluate whether consistency of therapy as documented by clinicians is associated with patient-level factors.

Methods: Using data from the Epidemiologic Study of CF, we identified patients from 183 sites in 2003-2005 who had ≥3 encounters spanning 180 days. To mitigate the effect of sites with poor reporting, 11 sites were excluded due to inconsistent documentation of pancreatic enzyme therapy. We subsequently examined consistency of dornase alfa therapy at the patient level by identifying the number of recorded medication switches (on to off or off to on) during the period. Patients were included if they had at least 3 encounters with dornase alfa and did not appear to initiate or discontinue therapy during the period. Inconsistent therapy was defined as having ≥3 documented switches, or 2 switches with ≥2 consecutive encounters where dornase alfa was not indicated. In other words, consistent therapy was defined as always on dornase alfa or just a single encounter without dornase alfa. Multivariable logistic regression, stratified by age group, was used to identify patient factors associated with inconsistent documented dornase alfa therapy.

Results: We identified 8807 patients for the analytic cohort (15% aged <6, 30% aged 6-12, 22% aged 13-17, and 34% aged ≥18). Of these, 883 patients (10%) had inconsistent documented dornase alfa therapy over the 3 years. Inconsistent therapy was highest in the patients aged <6 (15%) and lower in children aged 6-12 (8%), adolescents (10%), and adults (11%). Five predictors of inconsistent dornase alfa therapy were significant (p<.05) or showed a trend (.05<p<.10) in a consistent direction: lower age (significant <6; trend 6-12), higher height-for-age percentile (significant <6 and ≥18), presence of crackles (significant 13-17), presence of wheezing (trend 13-17, significant ≥18), lower percentage of *Pseudomonas* cultures (significant 13-17, ≥18). For children aged <6, weight-for-age <10 percentile was associated with inconsistency. For children aged 13-17 and adults aged ≥18, higher age-specific FEV1 % predicted decile was associated with inconsistency.

Conclusion: In this large observational cohort of CF patients, inconsistent dornase alfa therapy as documented by clinicians is infrequent. Rates of inconsistent dornase alfa therapy, as well as patient-level predictors of inconsistency, vary by age. In adolescents and especially adults, inconsistency is generally associated with markers of better health.

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NASAL POTENTIAL DIFFERENCE (NPD) IS A USEFUL DIAGNOSTIC MODALITY FOR INDIVIDUALS WITH SOME FEATURES OF CLASSIC CF AND NON-DIAGNOSTIC SWEAT ELECTROLYTES OR CFTR MUTATION ANALYSIS

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Background: Atypical or non-classic cystic fibrosis (CF) often presents as a diagnostic dilemma. A patient typically presents with at least one organ phenotype that resembles CF, normal (<40 mEq/L) or borderline (40-60 mEq/L) sweat chloride (Cl⁻), and one or fewer mutations in CFTR. We tested the hypothesis that a nasal potential difference (NPD) could discriminate between non-CF, CF, and atypical CF in one or more of the following measures: baseline PD, response to amiloride, and/or response to low Cl⁻ combined with isoproterenol.

Methods: NPDs performed at our center were reviewed from 2001 through 2011. Data were excluded if the NPDs were part of an investigational drug study. The CF Foundation Therapeutics Development Network standard operating procedure was followed and data acquired by two different customized data acquisition software packages (BioBench then LabChart 6) by a certified NPD operator. Baseline NPD (mV), response to amiloride (change from end of Ringer's to end of amiloride superfusion), response to low Cl⁻ combined with isoproterenol (change from end of amiloride to the end of the isoproterenol superfusions) was calculated from the R and L inferior turbinate. Where data was available, sweat chlorides were included for analysis.

Results: Forty-six subjects 5-74 years old (yo), 35 Caucasian and 23 males were analyzed. Seven subjects had at least one CFTR mutation and 32 had sweat Cl⁻ results. Of those 32, 13 were in the normal range, 13 borderline and 6 were >60mEq/L. Interestingly, sweat Cl⁻ was often lower when performed at the Johns Hopkins Hospital. Five subjects had NPD consistent with classic CF; 20 subjects had NPD completely consistent with non-CF. Twenty-one subjects were considered to have atypical CF and of those, 13 were children less than 18 yo. A comparison was done using a nonparametric Kruskal-Wallis test. The median, interquartile range (IQR) and p values are shown in the Table.

Conclusions: NPD can be a useful diagnostic test when confronted with a potential diagnosis of CF. While sweat Cl⁻ testing, when combined with genotyping, is considered the gold standard for diagnosing classic CF, the NPD is shown to discriminate between CF, non-CF, and atypical CF when sweat Cl⁻ results are normal or borderline. There were no differences in sweat Cl⁻ results between the 3 groups, however there were significant differences in mean baseline PD, and changes in response to amiloride, to low Cl⁻ combined with isoproterenol and to ATP. Like sweat Cl⁻, NPD may need to be repeated over time to more fully understand the evolution of symptoms.

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Table 1

	Sweat Chloride mEq/L		Baseline PD mV		Change Baseline to Amiloride		Change Amiloride to Low Chloride + Isoproterenol		Change Isoproterenol to ATP	
	M	IQR	M	IQR	M	IQR	M	IQR	M	IQR
CF	46.5	42.5-52	-31.0	-34.5-27.5	18.5	14.5-19.5	-1.0	-2.5-1.5	-3.8	-8.8-2.3
Atypical CF	36.5	14-44.5	-20.9	-24.5-16.2	9.9	5-11	-10.0	-17.5-6.5	-16.8	-21.5-9
Non-CF	37	22-53.5	-20.6	-23.3-18.8	12.8	9.5-14.8	-13.3	-17.3-10.3	-13	-21.5-9
p value	0.475		0.008		0.002		0.004		0.05	

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MULTIPLE BREATH WASHOUT PARAMETERS FOR INTERVENTIONAL STUDIES IN CF: MOMENT RATIOS COMPARED TO LCI

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Background: The lung clearance index (LCI), measured by multiple breath washout (MBW), has been shown to be a sensitive maker of early CF lung disease (1) and we have recently demonstrated that it can be used as an outcome measure in clinical trials (2). LCI, however, is affected by changes in breathing pattern which may impact on test variability especially in infants and young children. Moment analysis of MBW parameters assesses the shape (skewness) of the washout curve and has been shown to be less affected by variability in breathing frequency or tidal volume. Moment analysis is performed by setting the starting gas concentration at 100% and by plotting end-tidal gas concentrations against the number of lung turnovers (FRCs). Moment 0 is the area under this curve while moments 1 and 2 represent single and double multiplications with the number of lung turnovers. Results are then expressed as moment ratios (μ_1/μ_0 and μ_2/μ_0) with higher moment ratios being more reflective of ventilation inhomogeneity (VI) in the periphery of the lung.

Methods: We used two data sets to assess the potential of moment ratio analysis to quantify VI in CF patients. First, we assessed the sensitivity of moment ratios compared to LCI to differentiate health and disease in a cross-sectional validation study of LCI equipment in both healthy and CF children. Subsequently, we re-analysed data from a recently conducted interventional trial (2) to investigate whether moment ratios were able to detect treatment effects of hypertonic saline similar to LCI. MBW was determined by both mass spectrometry (AMIS 2000; Innovision A/S, Odense, Denmark) using 4% SF₆ as tracer gases and by nitrogen washout (Exhalyzer D, Eco Medics AG, Switzerland). We used data from healthy subjects to standardize all outcomes to z-scores for comparison.

Results: Forty-three healthy children and 38 children with CF between the ages of 6 – 18 years were included into the cross-sectional study. With the mass spectrometry system on average, LCI was 7.72 z-scores (-9.5; -6.0) higher in CF compared with healthy children, the first moment ratio was also 7.2 z-scores higher in CF (-9.0; -5.3), whereas the second moment ratio was 12.2 z-scores higher (-15.7; -8.7), however the variability was nearly twice as high. The magnitude and variability of the differences were very similar for the nitrogen washout system. In the interventional cross-over trial on hypertonic saline in CF children of similar age (n=19), treatment effects were observed for both μ_1/μ_0 ($\beta=-0.23$ (95%CI -0.36; 0.09)) and μ_2/μ_0 ($\beta=-1.9$ 95%CI -3.4; 0.4)).

Conclusions: These findings suggest that moment ratios may provide a complementary outcome to the LCI in clinical trials.

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CF-RELATED CONSTRICTIVE BRONCHIOLITIS IN ADOLESCENTS WITH REFRACTORY PULMONARY DECLINE

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Background: Accelerated loss of lung function during adolescence is a common, yet poorly explained finding in CF. This decline commonly is

believed to represent the culmination of several factors, including increased airway secretions, refractory chronic infections, and/or reduced adherence to prescribed therapies. Despite the incorporation of numerous therapies targeting different aspects of the CF pathogenic chain, the slope of median FEV₁ decline during adolescence has changed minimally over the past two decades. We reviewed clinically-indicated lung biopsy specimens obtained during adolescent deterioration to gain insight into underappreciated pathologic contributors of pulmonary decline.

Methods: Five CF adolescents with rapid pulmonary decline over 12-24 months that was refractory to aggressive intervention (e.g. frequent inpatient treatment of pulmonary exacerbations; maximized outpatient therapies with cycled antibiotics; mucolytics and anti-inflammatories; bronchoscopy to identify unusual pathogens; evaluation for ABPA etc) underwent CT imaging and ultimately lung biopsy to rule out alternative diagnoses. Biopsy specimens were reviewed by a single pathologist with expertise in lung pathology in CF and non-CF pediatric lung disorders.

Results: The mean age of patients at the time of biopsy was 16.3 years (range 11.38 - 19.97 years), including two males and three females. The rate of lung function decline (change in absolute FEV₁/year) for the 24 months preceding biopsy was -13.98 (range -24.14 to -8.84). This rate of decline was >2 SD beyond the mean of children age 6-17 years at our CF care center. All CT scans demonstrated a mix of bronchiectasis and hyperinflation that was variable across lung regions and within patients. Lung pathology included a predominant constrictive bronchiolitis pattern in all subjects, with variable bronchiectasis and mucus impaction. Two subjects additionally demonstrated a bronchiolitis obliterans organizing pneumonia (BOOP) pattern in parallel with constrictive bronchiolitis. Perivascular fibrosis was also observed, and varied from mild to severe across the five subjects.

Conclusions: Lung pathology in five CF adolescents with rapid and refractory pulmonary decline demonstrated a common pattern of constrictive bronchiolitis. The role of small airway fibrosis in non-endstage CF lung disease has not been commonly described, and represents a novel aspect of lung pathology shared with other CF-affected organs (pancreas, liver). Investigating CF-relevant mechanisms of tissue remodeling and fibrosis may offer novel insight into the pathophysiology of CF respiratory decline refractory to conventional therapy.

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DIFFERENCES IN DISEASE EXPRESSION BETWEEN CF WITH PANCREATIC INSUFFICIENCY, PANCREATIC SUFFICIENCY AND PRIMARY CILIARY DYSKINESIA

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Background: Pulmonary disease in both CF and primary ciliary dyskinesia (PCD) is caused by impaired mucociliary clearance (MCC) through different molecular mechanisms. Patients with CF and pancreatic insufficiency (CF-PI) have malabsorption with worse nutritional status, are diagnosed earlier and have worse survival compared to patients with CF and pancreatic sufficiency (CF-PS). Patients with PCD do not have gastrointestinal disease and malnutrition and therefore might have disease similar to CF-PS.

Aim: To compare lung disease expression between patients with CF-PI, CF-PS and PCD.

Patients and Methods: Patients (n=164) followed at Hadassah Medical Center were included: 88 with CF-PI, 42 with CF-PS (130 CF) and 34 with PCD. Clinical status, microbiology, FEV₁ and high resolution CT- total Brody Score (CT-TBS) were analyzed.

Results: CF-PI and PCD patients were similar in current age and both were significantly younger than the CF-PS group. CF-PI patients were diagnosed earlier than CF-PS and PCD patients (p<0.001). Mean FEV₁ was similar in the three groups however, FEV₁ had a strong negative correlation with age among CF patients as a group (r=-0.349, p<0.001) and in both CF subgroups: CF-PI (r=-0.348, p=0.018) and CF-PS (r=-0.474, p=0.022). Among PCD patients the correlation was not statistically significant (r=-0.358, p=0.061). Interestingly, patients with CF as a group (p<0.001) and CF-PI and CF-PS as subgroups (p=0.006 and 0.001, respectively) had a signifi-

cantly higher body mass index (BMI) than patients with PCD. A strong correlation between BMI and FEV₁ was found in the CF-PI group ($r=0.338$, $p=0.007$) and, to a lesser extent, in the PCD group ($r=0.438$, $p=0.06$). No correlation was found between BMI and FEV₁ in the CF-PS group ($r=-0.05$, $p=0.77$), who maintained stable and good BMI values at all ages. CT-TBS was highest (worse structural disease) in the CF-PI group and lower and very similar in the CF-PS and PCD groups. In the CF-PI group, most of the pathology was found in the upper lobes, in PCD in the middle and lower lobes and no consistent lobar pattern was seen in the CF-PS group. In both CF groups CT-TBS had a strong negative correlation with FEV₁; however, in PCD there was no correlation between CT-TBS and FEV₁. *P.aeruginosa* was the most prevalent bacterial infection in CF, with highest rates in the CF-PI group, whereas in PCD the most prevalent bacteria in sputum was *H. influenzae*. Colonization rates by methicillin-sensitive *S. aureus* (MSSA) were significantly higher in both CF groups and methicillin-resistant *S. aureus* (MRSA) colonization was similar in all 3 groups.

Conclusion: Although CF-PI, CF-PS and PCD result from impaired MCC, the patients have different lung disease expression. When comparing disease expression in patients with CF to other diseases, CF-PI and CF-PS should be analyzed separately. Furthermore, in contrast to CF, FEV₁ is not a reliable predictor of lung disease severity in PCD, and other parameters such as HRCT should be used when evaluating disease severity and progression.

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INTER-HOSPITAL VARIATION IN THE FREQUENCY OF SINUS SURGERY IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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Background: Many individuals with cystic fibrosis (CF) suffer from chronic rhinosinusitis and nasal polyposis. Chronic rhinosinusitis can significantly impact a patient's quality of life. CF Foundation (CFF) registry data indicates that 2-3% of pediatric patients per year have sinus disease requiring surgery. Little is known about the optimal treatment, indications for surgery, and outcomes of treatment for CF rhinosinusitis, leading to wide variation in practice patterns. The purpose of this study is to evaluate the frequency of sinus surgery in patients less than 21 years of age across multiple pediatric hospitals.

Methods: The Pediatric Health Information System (PHIS) compiles inpatient administrative data from 42 free-standing pediatric hospitals in the United States. We conducted a retrospective analysis of PHIS for the period January 1, 2008-January 1, 2011 to evaluate the frequency of sinus surgery at each hospital. We identified CF patients and sinus surgery during inpatient encounters using ICD-9 diagnosis code 277.0 and ICD-9 procedure code 22. Our search algorithm was verified using de-identified data from Children's Hospital Boston. Additionally, we collected demographic data and data for each hospital on hospital size, number of pediatric otolaryngologists, average FEV1, and percentage of patients meeting minimum care guidelines (based on publically-reported 2009 CFF care center data). Of the 42 participating hospitals 30 hospitals had complete data. Our main outcome variable was percentage of patients undergoing sinus surgery. Centers were divided into tertiles by center size, number of patients, and performance using FEV1. For the variables percentage of patients meeting CFF guideline care and number of otolaryngologists analysis was carried out by grouping centers above and below the mean. The MEANS procedure was used for statistical analysis.

Results: We identified 5482 CF patients among 30 hospitals; 903 patients underwent 1425 sinus operations over the 3 years. The total number of CF patients at each hospital ranged from 39-364 (average age 10, average age undergoing surgery 12, 50% male) and the total number of sinus surgeries ranged from 4-205. There was no difference between male to female ratio, average age, and average age at time of sinus surgery among the included hospitals. However, there was a 15-fold variation in the frequency of per-patient sinus surgery over a 3 year period by hospital (range 3-47%). There was no difference in the frequency of sinus surgery when comparing hospitals by average FEV1 ($p=0.65$), and number of otolaryngologists ($p=0.68$). There was an association between higher frequency of sinus surgery and hospitals with larger size ($p=0.05$), higher number of CF patients ($p=0.016$), and hospitals associated with CF care centers that have a higher percentage of patients receiving CFF guideline care ($p=0.03$).

Conclusions: There is large variation in the incidence of endoscopic sinus surgery in 30 of the largest pediatric hospitals. Sinus surgery is performed more frequently in larger centers and in those centers that adhere more closely to CFF guidelines. This study clearly highlights the need for further research into the indications and benefits of sinus surgery in pediatric patients with CF.

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COMPARISON OF SF₆ AND N₂ MULTIPLE BREATH INERT GAS WASHOUT IN CHILDREN WITH CF

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Background: Lung clearance index (LCI) derived from multiple breath inert gas washout (MBW) has been shown to be very sensitive in detecting peripheral airway dysfunction in children with cystic fibrosis (CF) (1). MBW with SF₆ gas washout has so far been the gold standard, but a new system measuring N₂ gas washout has recently been introduced (Exhalyzer D, EcoMedics AG, Duernten, Switzerland). The aim of this study was to assess the agreement of LCI and FRC, respectively, obtained from SF₆ MBW and N₂ MBW.

Methods: Cross-sectional study in CF patients who undertook triplets of SF₆ MBW using a mass spectrometer for gas analysis (Innovision A/S, Odense, Denmark) and N₂ MBW using indirect N₂ measurements (Exhalyzer D) on the same occasion. Intra-test variability (%CV) was calculated for FRC and LCI, and limits of agreement of LCI between the two methods were assessed using Bland-Altman plot. Paired t-test was used in comparison of test results. Results are presented as mean (SD).

Results: 28 CF patients aged 6.3 to 16.7 yrs (median 10.6 yrs) were included. Both LCI (N₂: 9.4 (1.6) vs SF₆: 8.6 (1.6), $p<0.0001$) and FRC (N₂: 1.6 L (0.8) vs SF₆: 1.4 L (0.6), $p=0.0001$) were significantly higher in N₂ MBW than in SF₆ MBW. Variability of LCI within triplets was less variable in N₂ MBW (N₂: 3.0% (2.3) vs SF₆: 5.6% (2.7), $p<0.001$), while FRC variability was higher (N₂: 5.3% (3.1) vs SF₆: 3.6% (2.5), $p=0.03$). Bland-Altman plot showed acceptable agreement between the two methods (Fig. 1) with mean LCI difference = 0.7 and limits of agreement (+/- 1.96 SD) between the two methods ranging from -0.9 to 2.3.

Conclusion: In children with CF we found acceptable agreement between FRC and LCI obtained from two different MBW methods (N₂ and SF₆ MBW). Both FRC and LCI were significantly higher with the new N₂ MBW system and as the difference might be clinically relevant, results from the two methods cannot be used interchangeably. The intra-test variability was acceptable with both techniques.

(1) Robinson et al. Inert gas washout: theoretical background and clinical utility in respiratory disease. *Respiration* 2009;78:339-55.

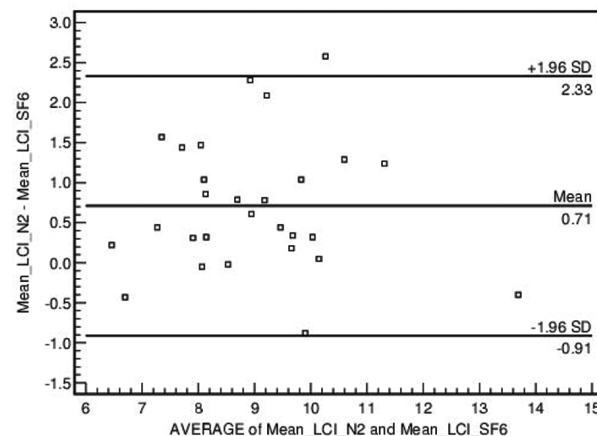


Fig. 1. Bland-Altman plot of LCI N₂ vs. LCI SF₆

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COMPARISON OF THE NORMALISED CONCENTRATION OF NITROGEN AT TURNOVER 6 OF MULTIPLE BREATH NITROGEN WASHOUT MEASUREMENT WITH LUNG CLEARANCE INDEX
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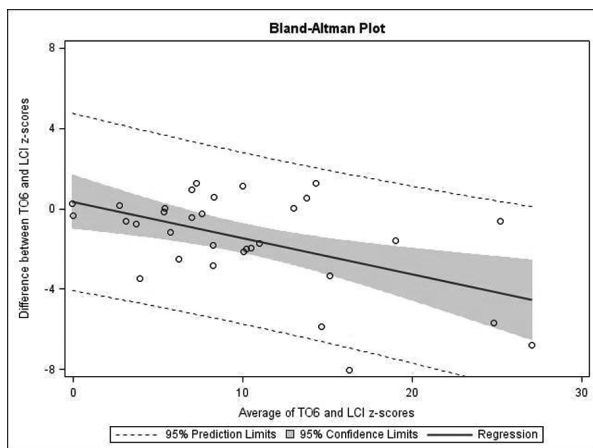
Background: The multiple breath washout technique (MBW) is a measure of peripheral airway dysfunction. MBW is a highly sensitive method and might be used in monitoring early disease activity in patients with cystic fibrosis (CF). The lung clearance index (LCI), which is reached when the normalised concentration of inert gas (Cn) is below 2.5% with an upper limit of normal at 7.1 turnovers, is the best evaluated outcome from the MBW test (1). LCI is however time consuming to reach in patients with severe lung disease and a new parameter is warranted to shorten test duration. Our aim was to assess level of agreement between Cn at 6 turnovers (TO6) and LCI as outcome parameters of MBW.

Methods: A cross-sectional study including patients with CF performing triplets of MBW on a nitrogen (N₂) washout based system (Exhalyzer D, Eco Medics AG, Duernten, Switzerland). Mean LCI and mean Cn_{N₂} at TO6 of the triplet measurements (both in z-scores) were used for analysis. Z-scores were calculated using Swedish and Danish normative values (B. Houlitz, in submission). Pearson's test was used to assess correlation between the two parameters and paired t-test to compare the means. Limits of agreement were analysed using a Bland-Altman plot.

Results: Thirty-two clinically stable children with CF, mean age (range) 10.7 years (6.3-17.1) were included. Mean (range) LCI and TO6 z-scores were significantly different 10.9 (-0.2 - 30.5) and 9.4 (-0.2 - 24.9) (p-value = 0.0012) respectively, but were significantly correlated (r = 0.96, p-value < 0.0001). The Bland-Altman plot (Fig. 1) is showing lack of agreement between LCI and TO6 due to an increasing difference with increasing LCI z-scores.

Conclusion: The normalised concentration of N₂ at TO6 is strongly correlated with LCI, but due to an increasing difference with increasing LCI values, TO6 and LCI cannot be used interchangeably to monitor disease severity and at the moment TO6 is not a recommended parameter to shorten test duration.

(1) Robinson et al. Inert gas washout: Theoretical background and clinical utility in respiratory disease. *Respiration* 2009; 78(3):339-355.



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REFERENCE PERCENTILES FOR FEV₁ AND BMI IN EUROPEAN CHILDREN AND ADULTS WITH CYSTIC FIBROSIS

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Background: The clinical course of cystic fibrosis (CF) is usually measured using the percent predicted FEV₁ and BMI Z-score referenced against a healthy population as achieving normality is the ultimate goal of CF care. Referencing against age and sex matched CF peers however adds valuable information for comparing CF populations and better illustrates variability between patients. Here, we used a large database of European CF patients to compute CF specific reference equations for FEV₁ and BMI, derived CF-specific percentile charts and compared these European quantiles to their nearest international equivalents.

Methods: 34859 FEV₁ and 40947 BMI observations were used to compute European CF specific percentiles. Quantile regression was applied to raw measurements as a function of age and height. Results were compared with the North American equivalent for FEV₁ and with the WHO 2007 normative values for BMI.

Results: FEV₁ and BMI percentiles illustrated the large variability between CF patients receiving the best current care. The European CF specific percentiles for FEV₁ were significantly different from those in the USA from an earlier era, with higher lung function in Europe. The CF specific percentiles for BMI declined relative to the WHO standard in older children. Lung function and BMI were similar in the two largest contributing European countries (France and Germany).

Conclusion: The CF reference percentile approach for FEV₁ and BMI provides additional information for peer to peer and population comparisons within CF patients. These references also provide tools for computing quantitative traits for use in genome wide analyses. It is a first step towards the possibility to a fair comparison of European CF patients and health system performance.

The authors present this abstract on behalf of the French CF Modifier Gene Study Investigators and the European CF Registry Working Group.

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PATIENTS DIAGNOSED WITH CF IN ADULTHOOD; CLINICAL COURSE FOLLOWING DIAGNOSIS AND TREATMENT AT A REGIONAL UK CENTRE

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Introduction: The proportion and number of patients with CF diagnosed in adulthood is increasing. The UK PORT CF data registry 2010 reported 301 new diagnoses of which 33 (10.9%) were diagnosed in adulthood. Adult diagnosed patients represent a unique population that differ in their clinical, genetic and prognostic characteristics compared to those diagnosed in childhood. With a different spectrum of CFTR mutations, infec-

tions and organ involvement their response to standard CF treatments is unknown.

Aim: To determine the clinical status of CF patients diagnosed in adulthood and assess their response to CF centre treatment over a four year period.

Methods: All patients attending the Regional CF Centre in Leeds and diagnosed after 16 yrs of age between 2005 and 2011 were included. Clinical data at diagnosis were collected from electronic patient records (EMIS®) and details of treatment commenced documented. Best FEV₁, weight and BMI for each subsequent 12 month interval were recorded.

Results: Twenty patients (12 male, 75% pancreatic sufficient (PS)) were included in the analysis. Median (range) age at diagnosis 30(17-60) yrs, BMI 21.8(16.7-31.9), FEV₁ 66(43-101) and FVC 87(62-106)% predicted. Ten patients were diagnosed due to bronchiectasis and 10 had a non-respiratory cause (infertility (6), family history (3) and malabsorption (1)). Median sweat chloride level was 64nmol/L; 75% had level >60nmol/L and 25% indeterminate/normal level. The diagnosis of CF was made in 19/20 (95%) using a combination of sweat test and genotype. Late diagnosed patients had a significantly lower prevalence of DF508 mutation (0% homozygous, 60% heterozygous and 40% no DF508 mutation). Median BMI at diagnosis was greater in those with PS vs. PI (22.0 vs.19.2, p<0.01). BMI increased from 21.8 at diagnosis to 24.6 at 4 years, (p<0.001). Median weight gain over 4yrs was 6.25kg (5kg PS vs.12.3kg PI, p<0.005). FEV₁ was greater at diagnosis in those with a non-respiratory diagnosis (93 vs.60% predicted, p<0.005). FEV₁ increased from 66% at diagnosis to 78% at 4 years. In patients with bronchiectasis FEV₁ increased from 60 to 75% predicted at 1 yr; this increase was maintained at 4 yrs. There was no increase in FEV₁ in those with a non-respiratory diagnosis. Eleven of 20 patients were prescribed Pulmozyme®; FEV₁ increased from 61% at diagnosis to 74% at year 1. No increase in lung function occurred in those who were not prescribed Pulmozyme®.

Conclusions: Patients presenting with CF in adulthood are more likely to have lower sweat chloride levels, lower prevalence DF508 mutation, pancreatic sufficiency with less gastrointestinal symptoms and milder pulmonary involvement. In response to CF centre treatment and care they do demonstrate significant improvements in both nutritional and respiratory status, especially those with bronchiectasis and pancreatic sufficiency. Improved prognosis highlights the importance of diagnostic testing for CF in patients with unexplained bronchiectasis with repeat sweat tests and extended genetic mutation analysis.

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STUDY OF THE EFFICACY OF LONG-TERM TREATMENT WITH HIGH VOLUME COMPARED TO STANDARD VOLUME OF HYPERTONIC SALINE ON PULMONARY EXACERBATIONS IN PATIENTS WITH CYSTIC FIBROSIS

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Background and Aims: Beneficial effects of nebulized hypertonic saline (HS) in cystic fibrosis (CF) patients are well documented. Long term use of HS increases mucociliary clearance and lung function and decreases pulmonary exacerbations. The aim of this study was to determine if the inhalation of a higher volume (10 mL) of HS increases the time without pulmonary exacerbations in CF patients compared with the inhalation of a standard volume (5 mL).

Methods: In this multicenter, open label, randomized controlled, parallel-group clinical trial, 71 patients with stable CF who were at least six years old and with ≥30% of forced expiratory volume in one second (FEV₁) were randomized to inhaled 5 or 10 mL of 7% HS twice daily for 48 weeks. All other standard therapies were continued during the trial. Clinical data, number of exacerbations, antibiotics requirements, lung function, changes in

sputum colonization and quality of life were recorded at each of the seven visits included in the study. Induced sputum was collected at each visit to determine inflammatory biomarkers: cytokines (IL8, IL6, IL10, IL1β, IL12, TNF), neutrophil elastase, calprotectin, lactate and triggering receptor expressed on myeloid cells-1 (TREM-1). Cytokines and TREM-1 were also measured in blood samples.

Preliminary Results: The mean cumulative function of number of exacerbations per patient-year in the 5mL group was 2.07 as compared with 1.87 in the 10mL group (difference, 0.2; 95%CI, 0.77 to 1.58; p=0.582). There was less risk of exacerbation in the 10 mL group with no statistical significance (HR=0.887; 95%CI, 0.627 to 1.255; p=0.563). The two groups did not differ significantly in the free survival rate of exacerbations (log-rank p=0.566) and the percentage of free time of use of antibiotics per exacerbation (difference, 0.06; 95%CI, -4.11 to 4.24; p=0.976). There was no difference in inflammatory biomarkers in both groups. The rest of the results and conclusions will be presented at the North American Cystic Fibrosis Congress 2012.

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DESIGN AND AEROSOL DISPERSION PERFORMANCE OF MULTIFUNCTIONAL MICROPARTICULATE/NANOPARTICULATE AEROSOL FORMULATIONS FOR TARGETED PULMONARY DELIVERY AS DRY POWDER INHALERS FOR CYSTIC FIBROSIS TREATMENT

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Purpose: To rationally design and comprehensively characterize novel microparticulate/nanoparticulate multifunctional dry powder inhaler (DPI) aerosol formulations (antibiotic drug, lung surfactant-mimic phospholipids, and mucolytic agent) with essential particle properties for targeted dry powder aerosol delivery for cystic fibrosis (CF) treatment. Additionally, the effects of lung surfactant-mimic phospholipids and mucolytic agent on aerosol dispersion performance and sustained-release behavior were systematically examined.

Methods: Two first-line pulmonary antibiotic drugs (tobramycin and azithromycin) were selected. Synthetic dipalmitoylphosphatidylcholine (DPPC) and sodium dipalmitoylphosphatidylglycerol (DPPG) were used in their lung surfactant-mimic composition. D-mannitol was chosen as a mucolytic agent. Co-spray drying from organic solution was under optimized spray-drying conditions. The physicochemical properties of spray dried (SD) aerosol formulations were comprehensively characterized by scanning electron microscopy (SEM), laser light diffraction particle sizing, differential scanning calorimetry (DSC), hot-stage microscopy (HSM), powder X-ray diffraction (PXRD), Karl Fischer coulometric titration (KF), gravimetric water vapor sorption (GVS), confocal Raman microspectroscopy chemical imaging (CRM), and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy. The *in vitro* sustained-release behavior was conducted at 37°C and modeled. The aerosol dispersion performance was conducted using the Next Generation Impactor® (NGI®) with the Handihaler® DPI device at an airflow rate (Q) of 60L/min.

Results: SD spherical particles had size in the range of ~500nm to 2.0µm with low water content. The incorporation of lung surfactant-mimic phospholipids provided sustained antibiotic drug release over several hours for the co-spray dried systems which fit the Korsmeyer-Peppas model with high correlation. The emitted dose % and fine particle fraction % were ~90% and ~65%, respectively. In the two-component aerosol formulations, the incorporation of mannitol enhanced lower stage deposition on stages 4 (1.67µm) and 5 (0.95µm) each with ~10-12% deposition compared to ~5-7% from the pure drug formulations. The co-spray dried phospholipids aerosol formulations had increased lower stage deposition in the range of ~23-32%.

Conclusion: Novel microparticulate/nanoparticulate SD multifunctional lung surfactant-mimic aerosol formulations with essential particle properties for targeted pulmonary delivery of antibiotics for CF treatment were successfully designed and produced by organic solution advanced co-spray

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drying. The sustained-release behavior and aerosol dispersion performance could be tailored and optimized by incorporation of lung surfactant-mimic phospholipids and mannitol.

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OUTCOMES OF LOBECTOMY IN CYSTIC FIBROSIS

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Background: Patients with cystic fibrosis (CF) have progressive lung disease. A small proportion of CF patients develop localized destruction of the lung and some may develop recurrent hemoptysis or pneumothorax. Resection of the involved lung is considered an option to limit spread of disease and/or to stop life threatening hemoptysis or air leak.

Objective: To evaluate the efficacy of lobectomy in patients with CF and either worsening localized lung disease or recurrent hemoptysis/pneumothorax. Improvement, if any, was quantified by comparing changes in lung function and pulmonary disease activity within two years before and after surgery.

Methods: We retrospectively reviewed charts of all patients with CF followed at the CF Center at Nationwide Children's Hospital, Columbus, OH, who underwent lobectomy over the last 15 years (1995-2010). Patients who were followed for at least one year before and after their surgery were included in this study (n=15). We compared number of hospital admissions, emergency room visits, pulmonary exacerbations requiring intravenous antibiotics and/or oral/nebulized antibiotics, weight, BMI and PFT. PFT measurements done within one month before and/or after pulmonary exacerbations were not included in the study and % predicted values were compared. Statistical Analysis: Outcomes were compared using standard statistical software and a p-value of < 0.05 was considered significant.

Results: Among our cohort of CF patients (n=520), 15 patients with CF underwent lobectomy in the last 15 years and were included in the study. Median age at the time of surgery was 20 years (range 1-41); adult 10; children 5; male 6; female 9. Two patients died during the post-operative period and one patient died within 2 years after the surgery. Fourteen had chronic *Pseudomonas aeruginosa* airway infection. One patient required lung transplantation several years after lobectomy. The indication for surgery in 12 out of 15 (80%) was recurrent pneumothorax/hemoptysis and among them 6 had emergency surgery. Thirteen of 15 (87%) had the right upper lobe removed. Median hospital stay was 15 days (range 8-85), median ICU stay was 6 days (range 2-70), median days with chest tubes were 7 (range 4-40). There was no significant improvement in mean weight, BMI %, number of CF exacerbations requiring either nebulized or oral intravenous antibiotics, number of hospital admissions or PFTs (mean% predicted FEV1 and FVC) at one year and two years post lobectomy when compared to one year or two years before lobectomy. There was no significant improvement in any other measures.

Conclusion: In patients with CF who underwent lobectomy, there was a significant mortality associated with the procedure. Among those who survived, there was no improvement in clinical parameters during the two year follow up period. Lobectomy should be reserved for CF patients with severe, recurrent and life-hemoptysis/pneumothorax when all other options fail.

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PERFORMANCE OF MULTIPLE BREATH WASHOUT TESTING POST-SPIROMETRY OPTIMIZES MEASUREMENT IN CHILDREN WITH OBSTRUCTIVE DISEASE

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Rationale: Lung clearance index (LCI) derived from multiple breath washout (MBW) testing is a sensitive measure of airways obstruction, and shows promise as a clinical and research outcome measure. LCI may be affected by mucus stasis/plugging in airways and hence could improve following spirometry. Our objective was to determine change in LCI after spirometry in children with obstructive airways disease and healthy controls using N₂ washout MBW testing.

Methods: Subjects aged 5-21 with obstructive airways disease (CF, asthma, or primary ciliary dyskinesia (PCD)) or healthy controls were recruited at baseline state of health. N₂ MBW testing was performed before and after spirometry (no bronchodilator given). Outcomes recorded for each subject included mean LCI pre- and post-spirometry (for all acceptable MBW maneuvers), intra-subject LCI coefficient of variation (CoV), and best FVC, FEV₁ and FEF₂₅₋₇₅ percent predicted. Analysis included Wilcoxon rank-sum and sign-rank testing comparing pre- and post-spirometry LCI and CoV in subjects with obstructive disease vs. healthy controls.

Results: Nineteen subjects have enrolled to date – 8 control, 11 with obstructive disease (6 CF, 4 asthma, 1 PCD) – at mean age 13.5 years (range 7-21). An average of 3 MBW maneuvers were performed pre- and post-spirometry. Spirometric measures for control vs. obstructive groups included mean FEV₁ 104±7% vs. 92±25% predicted (p=0.2) and mean FEF₂₅₋₇₅ 103±15% vs. 74±37% predicted (p=0.04).

Mean pre-spirometry LCI was 6.53(±0.6) vs. 10.09(±4.04) for control vs. obstructive disease (p=0.006); post-spirometry LCI was 6.61(±0.72) vs. 9.72(±4.01) (p=0.01). Mean LCI decreased post-spirometry in 43% of controls and 81% of the obstructive group; in the remainder of subjects LCIs either increased or was unchanged. Change in mean LCI from pre- to post-spirometry was not statistically significant in either group (p=0.3 control, p=0.06 obstructive); however, subjects with obstructive disease had a significantly larger percent change in LCI after spirometry compared to controls (mean 3.7%(±4.4) decrease vs. 1.7%(±3.2) increase in controls; p=0.03).

Mean pre-spirometry LCI CoV was 5.0(±4) vs. 6.6(±3.4) for control vs. obstructive disease, respectively (p=0.3); post-spirometry CoVs were 3.7(±1.9) and 3.6(±2.9) (p=0.7). Following spirometry, CoV decreased in 57% of controls and 81% of obstructive group. Change in CoV post-spirometry was statistically significant in subjects with obstructive disease (p=0.04), but not controls (p=0.5); percent change in CoV was not significantly different between groups.

Conclusions: In our comparison of N₂ washout MBW testing pre- and post-spirometry, CoV of LCI improved post-spirometry in subjects with obstructive disease, without significant change in LCI itself. While subjects with obstructive disease had significantly larger percent change in LCI post-spirometry than healthy controls, difference in LCI between the groups remained significant following performance of spirometry, with higher mean LCI in the obstructive group. Our findings suggest MBW testing performed following spirometry may have improved accuracy, particularly in subjects with obstructive disease. Enrollment is ongoing.

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LONG-TERM SAFETY AND EFFICACY OF TOBRAMYCIN INHALATION POWDER IN CYSTIC FIBROSIS PATIENTS 6-21 YEARS OF AGE : RESULTS FROM A PHASE III EXTENSION STUDY

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Background: Tobramycin Inhalation Powder (TIP) delivered via the T-326 Inhaler is a novel drug/device combination designed to improve tobramycin delivery to the lungs. The Establish tobramycin Dry powder efficacy (EDIT) Trial was designed to confirm the efficacy and safety of TIP in cystic fibrosis (CF) patients with chronic *Pseudomonas aeruginosa* (Pa) infection over a single treatment cycle. An extension to this core study was designed to further evaluate the long-term safety and efficacy of TIP for the treatment of Pa infections in CF patients.

Methods: Patients who completed the core study [Cycle 1 - 28 days on/28 days off treatment with twice-daily TIP 112 mg or placebo (PBO)] were invited to participate in this multicenter, single arm, open-label, uncontrolled study. After the core, patients received twice-daily TIP 112 mg for three additional treatment cycles (Cycles 2, 3 and 4). The primary objective was to evaluate the safety profile of TIP over these three treatment cycles. Efficacy assessments included relative change in FEV1 (% predicted); decrease in Pa sputum density; new anti-pseudomonal antibiotic use and days of hospitalization due to respiratory events.

Results: Of all patients enrolled, 94.5% (52/55) completed the extension study (mean age: 12.9 y; males: 36.4%; mean FEV1 % predicted: 59.9%). Treatment-emergent adverse events (AEs) were reported by less than one-half of patients (26/55, 47.3%). During Cycles 2, 3 and 4, 16 (29.1%), 13 (23.6%) and 12 (23.1%) patients reported AEs respectively. The most commonly reported AEs were cough (9.1%), respiratory tract

infection (9.1%), dysphonia (5.5%) and hypoacusis (5.5%). AEs were mostly mild or moderate in severity. One patient discontinued the study due to AEs (hypoacusis, middle ear effusion and sinus polyp). Three (5.5%) patients reported serious adverse events (aspergillosis, lung infection and pneumonia; none were suspected to be related to study medication. No deaths were reported. The relative change from baseline in FEV1 % predicted was statistically significantly higher at all post-baseline time points with a highest mean increase of 17.3% ($p<0.001$) at the end of dosing Cycle 4 (Day 29). The mean decrease in sputum density of Pa biotypes ($\log_{10}$ CFU/g) from baseline was statistically significant at the end of each treatment period, the maximum being -3.9 in Cycle 4. Use of anti-pseudomonal antibiotic was low; 5 patients (9.1%) reported new anti-pseudomonal antibiotic use either by oral (3 patients) or IV (3 patients) administration, with median duration of 15 days for both the routes. Hospitalizations due to respiratory SAEs were reported in 2 (3.6%) patients of the PBO group with median duration of 13 days.

Conclusion: Treatment with additional 3 cycles of TIP shows an acceptable safety and tolerability profile in CF patients, with no new safety signals identified. The data from this extension study provide evidence of sustained efficacy and bacterial suppression over 3 additional treatment periods.

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DIFFUSION CAPACITY FOR NO (DLNO) IS SUPERIOR TO DIFFUSION CAPACITY FOR CO (DLCO) AND COMPARABLE TO FEV1 IN LONGTERM MONITORING OF CYSTIC FIBROSIS PATIENTS

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Background: Due to large-scale destruction, changes in membrane diffusion (Dm) may occur in cystic fibrosis (CF), in correspondence to alterations observed by computed tomography (CT). Dm can be easily quantified via the diffusing capacity for nitric oxide (DLNO), as opposed to the conventional diffusing capacity for carbon monoxide (DLCO). As shown in a previous study, DLNO and DLCO reflected CT-morphological alterations of the lung better than other measures. In the current study, we aim to assess changes of DLNO/CO over time compared to standard lung function tests.

Methods: All consecutive patients of the CF outpatient clinic in Munich were asked to participate in the study. In addition to standard lung function (spirometry, body plethysmography), DLNO/CO - measurements are recorded during the outpatient visit, together with symptoms, current medication and standard lab results.

Results: From a total of 227, 52% are follow-up measurements. Overall, there is a high correlation of FEV1 with DLCO (Pearson correlation coefficient =0.80, $p<0.01$) and higher with DLNO (0.86, $p<0.01$). The correlations remain stable over time and reflect the subject's individual course. As shown in a previous study, there is also a high correlation of DLNO with CT-scan scores.

Conclusion: We propose the use of DLNO as an alternative method to monitor the lung function in cystic fibrosis patients. The combined use of DLNO and DLCO might offer additional information about lung inflammation and is independent from patient's cooperation.

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UTILIZATION OF BODE INDEX FOR PULMONARY EXACERBATION RATES IN PATIENTS WITH CYSTIC FIBROSIS

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Background: CF is a multisystem disease, but a single physiologic variable (FEV1) is commonly used to grade the severity of disease. Unfortunately, FEV1 alone may not be predictive of disease outcomes in all cases. Other functional parameters monitored for CF patients (pts) include the exercise capacity (6MWD), BMI and degree of functional breathlessness. The BODE index has been shown to predict hospitalizations for pts with COPD. Our aim was to assess the utility of BODE index for pulmonary exacerbation (PE) rates in CF pts.

Methods: Between July 2010 and May 2011, pts were recruited from the adult CF clinic at Keck Hospital of USC. IRB approval was obtained for this study. All CF pts in the study were clinically stable and receiving appropriate therapy at enrollment. Those receiving oxygen therapy were included but were required to remain on a stable dose for at least 2 months. Pts were excluded from the study if they were too ill to perform the PFT or 6MWD. Data was obtained during clinic visits, including the BMI, FEV1, 6 MWD and CFQ-R respiratory domain. All data points were collected within a maximal one month period for each pt. Together, they comprise the BODE index. For the FEV1, 6MWD and CFQ-R, pts received points from a range of 0 (min value) to 3 (max value) and for BMI, a value of 0 or 1 (Table). Quartile 1 was defined as a score of 0-2, quartile 2 by a score of 3-4, quartile 3 by a score of 5-6 and quartile 4 by a score >7. Pts were followed for 24 months after initial scoring to assess PE rates in order to compare annual PE rate between BODE index versus FEV1.

Results: Seventy-three out of 200 CF pts were included in the study. The median age was 29.1 years and 40% were women. Median baseline values for all pts were: BMI of 30kg/cm², FEV1 63.2% pred, 6 MWD of 465m and CFQ-R Respiratory 67.6. PE rates were higher when using the BODE index compared to FEV1 alone (Table).

Conclusion: BODE index appears to be more discriminative with PE rates than FEV1 alone.

Variable	Points			
	0	1	2	3
BMI (kg/m ²)	>22	<22		
FEV1, %predicted	>70	40-69	<40	
CFQ-R Respiratory Domain	75-100	50-74	25-49	0-24
6MWD (m)	>350	250-349	150-249	<149
BODE quartile	PE rate in 2010		PE rate in 2011	
1	0.48		0.74	
2	1.7		2.1	
3	2.1		3.1	
4	2		3	
FEV1, %predicted				
>70 (median 91.5%; 75-104%)	1.7		2.1	
40-69 (median 51%; 41-69%)	2.1		3.1	
<40 (median 31%; 19-38%)	2		3	

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RELATIONSHIP BETWEEN N₂ MULTIPLE BREATH WASHOUT (MBW) INDICES OF GLOBAL AND PERIPHERAL AIRWAY INHOMOGENEITY AND FEV1 IN CHILDREN WITH CF

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Abnormal ventilation distribution measured by MBW tests is a more common finding than abnormal FEV1 in CF(1).

Aim: To assess the relationship between CF lung disease severity as determined by ventilatory capacity (FEV1) and global ventilation inhomogeneity (LCI; lung clearance index) and indices of inhomogeneity in the conducting (Scond) and acinar airway zones (Sacin) (1).

Method: Spirometry (Jaeger Masterscreen) and N₂ MBW (Exhalyzer D, Eco Medics AG Switzerland) were performed in 26 children with CF (17 boys, median age 12.3(7.5, 18.1)) and 44 healthy controls aged 7-18 years. LCI, Scond and Sacin were also expressed as z-scores based on results in the control group and FEV1 was related to established reference values (2). Indices were related using linear regression. The data are given in median (min, max) if not stated otherwise.

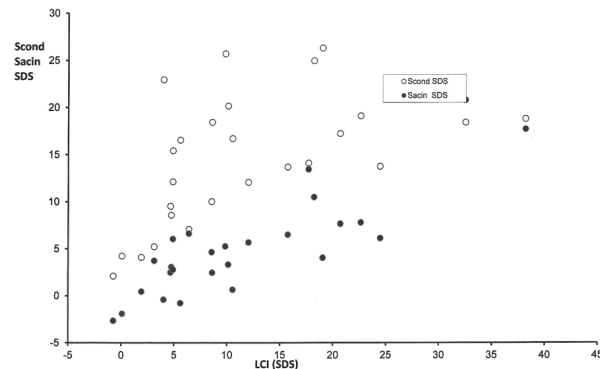
Results: In controls LCI was 6.54 (0.28) (mean (SD)), Scond 0.021 (0.004) and Sacin 0.051 (0.012). In CF, FEV1 was 91(43, 113)% and LCI 9.12 (6.33, 17.24). Expressed as SDS, LCI was 9.2(-0.7, 38.2)SDS, Scond 14.8 (2.1, 26.3)SDS and Sacin 4.3 (-2.6, 20.8)SDS. LCI was normal in 3 patients. Sacin was normal in 5 patients including 2 of the patients with normal LCI but none had Scond within normal limits. LCI and Sacin correlated significantly with FEV1 (r_2 0.70 and r_2 0.56, $p<0.001$) while Scond did not (r_2 0.14, $p=0.06$). Sacin and Scond did not correlate (r_2 0.12, $p=0.085$) while the correlation between Scond and Sacin vs LCI is shown in the fig-

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ure. Scnd increased early in the progression of disease up to LCI of 10 SDS but not above while Sacin increased in parallel with an increase of LCI.

Conclusion: LCI and Sacin are related to overall global disease severity and progression. Scnd seems to be an early marker of peripheral lung involvement but does not reflect later disease progression.

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THE IMPORTANCE OF APPROPRIATE REFERENCE SOURCES FOR SPIROMETRY: LESSONS LEARNED FROM THE UK CYSTIC FIBROSIS GENE THERAPY "RUN-IN" STUDY

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Introduction: The "Run-In" was a longitudinal observational study of cystic fibrosis (CF) patients designed to assess optimal patients and outcome measures for our current multi-dose gene therapy trial. Spirometry was performed at each Run-In study visit and volumes converted to % predicted values according to published reference sources; historically these were separate for adults and children. Here, we describe the issues arising from this approach, and highlight the benefit of using a reference source which bridges the transition from child to adulthood.

Methods: CF subjects (≥ 10 years; $FEV_1 \geq 40\%$ predicted) were recruited from three sites in London and Edinburgh. Visits were undertaken during periods of stability every 3-6 months; data presented here are from the first 4 visits. At each visit, a series of laboratory, physiological and clinical tests were performed, one of which was spirometry using an Easyone spirometer. Volumes were converted to % predicted values according to Rosenthal¹ (<18 years) and Quanjer² (≥ 18 years) reference equations. The FEV_1 raw data has been subsequently re-analysed using Stanojevic look-up tables³ which became available during the course of the trial. These span all age ranges.

Results: A total of 191 patients attended visit 1 (mean age 22.7 years, 55% male; 91 patients <18 years). Rosenthal and Quanjer FEV_1 % predicted values were both significantly higher than the Stanojevic values: mean differences 2.8 (95%CI 1.9-3.7) for children with Rosenthal equations ($p < 0.0001$), and 2.4 (95%CI 2.1-2.8) for adults using Quanjer equations ($p < 0.0001$).

Ten patients transitioned between paediatric and adult reference ranges during the study period. The mean absolute drop in FEV_1 % predicted values across the study visits spanning a patient's 18th birthday was 9.7 with Rosenthal to Quanjer equations in comparison to 4.1 when Stanojevic reference values were used. The slope representing decline in FEV_1 % predicted values over study visits 1-4 was significantly greater with Rosenthal/Quanjer than with Stanojevic ($p = 0.001$), largely due to an artefactual drop when switching from Rosenthal to Quanjer values at age 18. As an example, a female patient aged 17.8 years at visit 1 had a drop in absolute FEV_1 % predicted between visits 1 and 2 of 11% when Rosenthal/Quanjer were used but only 3% with Stanojevic reference values.

Conclusions: Our results highlight issues raised when separate adult and paediatric spirometry reference sources are used in longitudinal study. They make interpretation of data in terms of change over time impossible and beyond the research setting have implications for clinical decision making. The UK CF Gene Therapy Consortium has converted to using the Stanojevic reference source for longitudinal spirometry analysis in its recently commenced multidose trial of nebulised non-viral CF gene therapy.

Supported by the UK CF Trust.

References:

1. Rosenthal et al. Thorax 1993;48:794-802
2. Quanjer et al. Eur Respir J 1993; 6 suppl. 16: 5-40
3. Stanojevic et al. AJRCCM 2008; 177:253-260

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DISCORDANCE IN PREDICTED SPIROMETRIC VALUES IN HISPANIC PATIENTS WITH CYSTIC FIBROSIS

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Rationale: Since ethnic differences in pulmonary function are known to occur, evaluating lung function values require application of the appropriate reference equation for a given patient population. Failure in choosing the adequate equation could lead to errors in the diagnosis and in classification impairment. Predicted values were usually derived from cross-sectional studies in healthy subjects and are subject to cohort effect. The American Thoracic Society recommends race-specific spirometric reference values from the National Health and Nutrition Survey (NHANES) III for clinical evaluation of pulmonary function in whites, African-Americans, and Mexican-Americans in the United States. However, the recommended race-specific references values may not apply to other sizable proportions of the US population, Hispanics of non-Mexican origin. In Florida, Hispanic population corresponds to 22.5% and has diverse origins. The Cystic Fibrosis registry (Port CF) uses Hankinson: Mexican-American (for male age 18+ and females age 16+) reference equations as a predicted value for spirometry in patients with cystic fibrosis, regardless their Hispanic origins.

Objective: To evaluate the discordance of different equations on spirometric FEV_1 values for the Hispanic patients with cystic fibrosis in our site.

Methods: Spirometry values collected on Port CF for patients (male age 18+ and females age 16+) were reviewed. Forty-one participants were self-identified as Hispanic. Reference equations published by Crapo, Knudson and Hankinson-Mexican American were used to calculate FEV_1 predicted and percentage of predicted values on this population. Also, a reported race/ethnic adjustment factor of 0.88 (LLN= 88%) was applied to Hankinson equation (HankinsonC).

Results: The FEV_1 predicted values (in liters) were similar when using Crapo or Hankinson: Mexican-American equations. However, values tend to be lower when Knudson and HankinsonC equations were used. In our patients, mean FEV_1 (percentage of predicted) was 55.7 ± 3.7 , 59.2 ± 4.0 , 55.8 ± 3.7 and 63.5 ± 4.3 for Crapo, Knudson, Hankinson Mexican-American and HankinsonC respectively. A mean difference of 7.7% was observed between Hankinson: Mexican-American and HankinsonC (values range from 3 to 17%) and 4.2% between HankinsonC and Knudson (values range from -9.8 to 11%) and 8.2% between HankinsonC and Crapo (range -2.8 to 20.7%).

Conclusions. These results suggest that there is a large degree of discordance observed across all comparison equation in patients with cystic fibrosis. Depending on the change in FVC, these differences may not have resulted in discordant PFT interpretation; however, they could potentially impact the interpretation of severity of obstruction. The effect of race on the discordance between various reference equations requires further studies.

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VITAMIN D STATUS IS ASSOCIATED WITH PULMONARY EXACERBATIONS IN CHILDREN WITH CYSTIC FIBROSIS

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Background: In the lung, vitamin D plays an important role in pulmonary development, inflammation, host defense, and repair. Vitamin D insufficiency (< 30 ng/mL) in children is associated with severe asthma exacerbations. An investigation of vitamin D status and pulmonary exacerbations in children with CF is needed.

Methods: Data was used for patients ages 6-18 years during the years 2000-2010 from our local CF database. Models were created to investigate the association of vitamin D 25-OH levels and IV exacerbation frequency. Vitamin D 25-OH levels were introduced in different models as either continuous or categorical variables (> 30 vs. <30). The probability (log-odds) of a pulmonary exacerbation within one year after vitamin D measurement was modeled using repeated measures logistic regression with covariates of age, sex, age at CF diagnosis, pancreatic status, BMI z-score, and chronic infection status for *Pseudomonas* and MRSA. Results are reported as odds-ratios and 95% confidence intervals.

Results: The number of vitamin D measurements included was 958. The number of unique patients included was 212. Covariates associated with pulmonary exacerbations requiring IV therapy were female sex, older age, lower BMI z-score, chronic *Pseudomonas* infection, and vitamin D 25-OH status. For every 1 point decrease in Vitamin D 25-OH level, the odds of having a subsequent pulmonary IV exacerbation within 1 year increased by 1.019 times (95% CI 1.001-1.037; p 0.03). With vitamin D insufficiency (< 30 ng/mL), the odds of having a subsequent pulmonary IV exacerbation within 1 year increased by 1.98 times (95% CI 1.31-3.00; p 0.03).

Conclusions: Vitamin D insufficiency is associated with pulmonary exacerbations requiring IV therapy in children with cystic fibrosis.

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TOLERABILITY AND ACCEPTABILITY IN PATIENTS WITH CYSTIC FIBROSIS (CF) OF TWO FORMULATIONS OF 7% HYPERTONIC SALINE: A PROSPECTIVE MULTICENTER CLINICAL STUDY

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Background: Airway surface liquid (ASL) height is compromised in CF lung pathogenesis. Inhaled hypertonic saline (HS), osmotically drawing water from the interstitium to the ASL, has been shown to decrease the number and severity of exacerbations, reducing lung disease progression rate. HS, though desirable, convenient and safe, is associated with bronchoconstriction and irritation of the mucosa of the higher airways which can reduce adherence. Hyaluronic acid (HA), known for its hydrating properties, has recently been studied for its barrier role in the matrix. The combination of HS and HA has been employed as a single administration in CF patients (pts) in 3 trials.

Aim: The prospective, middle-term evaluation of the combination product in pts who already showed poor tolerance to HS.

Materials and Methods: 40 CF pts of both sexes aged >8 years with stable disease in the last 30 days who previously showed intolerance to HS were recruited from 4 Italian CF centers and were double-blindly randomized to 2 groups of treatment which resulted in homogeneous groups for baseline characteristics:

- standard therapy with 5mL 7% HS: 20 pts (mean age 26.4±11.9, 15 females, mean FEV1 75.4%pred±22.3, mean BMI 20.58 kg/m² ±2.03)
- 5mL 7% HS and 0.1% HA: 20 pts (mean age 21.7±10.8, 9 females, mean FEV1 80.3%pred±18.2, mean BMI 20.53 kg/m² ±3.68).

The treatment was given twice daily for 28 days. The outcomes were judgments about cough, throat irritation, salty taste (assessed on a scale of 4

grades: absent, mild, moderate, severe) and global pleasantness (expressed on a 1-10 scale) assigned by pts at the first inhalation and then weekly.

Another endpoint was the effect on FEV1, measured in basal conditions, after short-acting β₂ and after the randomized solution both at the beginning and at the end of the treatment period.

Results: At the first inhalation the severity of cough (p<0.0001), throat irritation (p=0.0050) and saltiness (p=0.0007) were greater in pts treated with HS. Symptoms were milder in HS+HA group. This trend was confirmed in the analysis of the whole treatment period (p<0.0001 for the 3 symptoms).

Judgments of global pleasantness were consistently higher in pts treated with HS+HA since the first administration (p=0.0130), and in the overall analysis of the monthly data (p<0.0001).

FEV1 did not significantly decrease after the first inhalation in both treatment groups with no differences between groups (treatment difference of % Change in FEV1 -0.2 [95% I.C. -3.0/2.5]) and after the treatment period (treatment difference of % Change in FEV1 -1.6 [95% I.C. -4.8/1.5]).

Five pts interrupted the treatment, 4 because of poor tolerability (2 in HS group and 2 in HS+HA group), and 1 due to a respiratory exacerbation.

Conclusion: The addition of HA to HS reduces the prevalence and severity of cough, throat irritation and saltiness and may improve adherence to HS. Pulmonary function did not change in the 2 groups. Longer-term studies may further assess the benefit of chronic treatment.

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CYSTIC FIBROSIS: COMPARING DISEASE OUTCOMES IN AFRICAN AMERICANS AND CAUCASIANS

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Cystic fibrosis (CF) affects 30,000 people in the United States, including 1 in 3200 Caucasians and 1 in 15,000 African Americans (AA). First reports of the disease in persons of African origin date back to 1954.¹ Kulczycki et al described CF in African Americans in 1964.² We studied the spectrum and severity of CF among African Americans and Caucasians. We analyzed the clinical features, genotype, and severity of pulmonary and extra-pulmonary involvement in African American patients as compared to Caucasians.

Study design: Retrospective case control study on CF patients followed at the University of Tennessee Cystic Fibrosis Care and Research Center at Le Bonheur Children's Hospital. AA patients constituted study subjects. Gender and age (+/- 12 months) matched Caucasian patients served as controls. Race was classified based on self identification by patients or families. Medical records were reviewed for demographic data, CF diagnostic criteria, pulmonary function testing, sputum microbiology and presence of pulmonary complications. Parameters of extra pulmonary disease analyzed included presence of meconium aspiration or distal intestinal obstruction syndrome, growth parameters (weight, height and body mass index (BMI) percentiles), serum levels of fat soluble vitamins and presence of significant liver disease. We also analyzed the number of clinic visits per year and average annual hospitalizations in the preceding 5 years. Statistical analysis was performed using SAS analysis software.

Results: 20 AA subjects and 20 controls (11 males, 9 females) were studied. Both groups were similar for age at diagnosis and for sweat chloride levels. None of 20 subjects and 12/20 controls (60%) were homozygous for c.1521_1523delCTT (delta F508; p =0.0002). 35% of alleles in subjects were c.1521_1523delCTT as compared to 75% for controls (p < 0.0001). One subject, who is heterozygous for c.1521_1523delCTT, has a novel CFTR mutation : c.438_440delTCA (p.His147del). Pulmonary disease severity including pulmonary function and sputum microbiology was similar among subjects and controls as was the annual hospitalization rate and number of clinic visits. Vitamin D deficiency (p=0.03) was significantly more prevalent in African American patients as compared to Caucasian controls. Five of the 20 African Americans (25%) had liver disease, two of whom were heterozygous for c.1521_1523delCTT. None of the Caucasian patients had CF liver disease (p=0.047).

Discussion: We report in-depth analyses on diagnostic testing as well as measures of pulmonary and extra-pulmonary disease severity in cystic fibro-

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sis among African Americans. Higher prevalence of vitamin D deficiency in this population is previously unreported as is the more common occurrence of CF liver disease. We also report a novel CFTR gene mutation.

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FATTY ACID LEVELS AND INVOLVEMENT OF SMALL AIRWAYS IN PATIENTS WITH MILD CYSTIC FIBROSIS

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Background: Cystic fibrosis is associated with altered plasma fatty acid levels. A deficiency of essential fatty acids like linoleic acid (LA) and docosahexaenoic acid (DHA) and increased levels of arachidonic acid (AA) have been described in patients with cystic fibrosis. Supplementation of high doses of omega-3 fatty acids was effective in terms of correction of the lipid imbalance but data on improvement in lung function and other clinical aspects are not conclusive. Maybe altered metabolism of AA and DHA to pro-resolving lipid mediators like lipoxins, protectins and resolvins play a major role on clinical impact of supplementation therapy. Therefore, in our study we focused on mild cystic fibrosis patients where endogenous pro-inflammatory signals are supposed to be dampened by pro-resolving control mechanisms.

Methods: In our study we examined 32 patients (6- 55 years of age) with mild CF (Forced Vital Capacity (FVC) >75 %predicted) and 22 healthy control subjects (7-23 years of age). Patients were assigned to two groups according to involvement of small airways. Small airways disease (SAD) in our mild CF patients was diagnosed as maximal expiratory flow (MEF), when 25% of FVC remains to be expired (MEF25) was less than 50% predicted. CF patients without (n=19) and with (n=13) SAD were examined by body plethysmography and induced sputum was analyzed for differential cell count and sputum inflammatory biomarkers were measured by RT-PCR and cytometric bead array (CBA). In addition blood plasma levels of polyunsaturated fatty acids (PUFAs) were determined by LC-MS/MS (liquid chromatography- tandem mass spectrometry)-based lipidomic analysis.

Results: As expected, in terms of lung function, the ratio of residual volume (RV) to total lung capacity (TLC) was significantly increased in patients with SAD (RV/TLC: CF without SAD 100.2 (%predicted) vs. CF with SAD 156.0 (%predicted), mean; (p<0.001)). Differential cell count of sputum showed increased neutrophils (PMNs) in our CF patients in comparison to controls (control group 5.0% vs. CF patients 36.5%, mean; (p<0.001)), but no significant differences between the SAD and non-SAD group. DHA levels were significantly lower in CF patients in comparison to healthy controls (DHA: control 54.6 mg/L vs. CF 36.4 mg/L, mean; (p< 0.05)), but no significant differences were confirmed between patient groups (DHA: SAD: 42.8 mg/L vs. non-SAD 32.3 mg/L, mean; (n.s.)).

Conclusions: Our study showed that neutrophilic inflammation was significantly increased and DHA plasma levels were significantly reduced in mild CF in comparison to healthy control subjects. However, CF patients with and without SAD did not differ significantly in terms of PUFA levels suggesting limited inflammation in mild CF disease. It is possible that these mild CF patients benefit from intervention with omega-3 fatty acids to a greater extent than patients with moderate or severe CF lung disease.

PHYSICAL & RESPIRATORY THERAPY

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USE OF PEP IN COMBINATION WITH HYPERTONIC SALINE INHALATION IN CHILDREN AND ADOLESCENTS WITH CYSTIC FIBROSIS

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Background: Positive expiratory pressure (PEP) therapy and hypertonic saline are two well established therapies used to aid airway clearance in cystic fibrosis (CF). Poor ventilation and obstruction begin in the peripheral airways in patients with CF, therefore methods of targeting deposition of medications to these regions may be beneficial. It is well known that patients with CF have a high treatment burden, and high levels of non-adherence to airway clearance techniques (ACT), especially in adolescence. This is a major challenge for those caring for patients with CF. Combining PEP with the inhalation of an aerosol has been described in the literature in adult populations, showing generation of smaller aerosol particle size and a proportional redistribution of aerosol to the peripheral airways. This therapy is used in clinics around Australia, however no published data exists on patient acceptability, clinical effectiveness, or effects on adherence.

Aim: This retrospective pilot study assessed patient acceptability, perceived effectiveness and self reported adherence, of combining PEP and hypertonic saline, compared to conventional use of hypertonic saline followed by PEP.

Methods: Seventeen patients (8 boys), with a mean age of 13.1yrs (10-17) and mean FEV1% of 60.5 (20.9 – 87.4), who had self-reported poor adherence were included in the study. Patients were followed up 6 months after commencing the combined PEP and hypertonic saline therapy. Outcomes included visual analogue scales (VAS) of perceived ease of expectoration, treatment time, treatment adherence and overall effectiveness; the Chest Physiotherapy Satisfaction Survey (CPSS), ranging from 1-5; and change in FEV1% from baseline to 6 months. Twelve of the participants were outpatients at time of initiation and 5 were inpatients (baseline FEV1% was post-admission for the inpatients).

Results: Combined therapy was perceived to improve ease of expectoration and overall effectiveness in 100% of patients, amount of sputum expectorated in 94%, treatment time in 82% and adherence to physiotherapy in 77%. Mean combined therapy CPSS score (SD) for the outpatients and inpatients at initiation was 4.1 (0.4) and 4.2 (0.3) respectively. These scores are comparable to other well established ACT reported in the literature. Mean change in FEV1% (SD) at 6 months was 4.2% (6.8) in those initiated as outpatients, and - 4.0% (9.6) in those initiated as inpatients.

Conclusion: In adolescents struggling with treatment adherence this study found combined therapy was perceived to be more effective, less time consuming and beneficial for adherence compared to performing therapies separately; and satisfactory as an airway clearance technique. The initiation of combined therapy had no detrimental effect on FEV1% over the 6 month trial. All patients chose to continue combined therapy at the end of the trial. The results from this preliminary study are encouraging and we believe that further investigation into this combined therapy is warranted.

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THE SHORT TERM EFFECT OF THE "COUGH ASSIST" AND "AUTOGENIC DRAINAGE" PHYSIOTHERAPY ON LUNG FUNCTION IN PATIENTS WITH CYSTIC FIBROSIS

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Background: Excessive production of viscid mucus may result in airway obstruction, recurrent infection and diffuse inflammation in cystic fibrosis (CF) patients. It has been shown that physiotherapy may reduce sputum retention, atelectasis and the duration of exposure to proteolytic agents. Airway clearance using cough assist may offer a useful tool to pro-

pel mucus from the peripheral to the central airways to be expectorated subsequently.

Our aim was to study the effect of airway clearance using the cough assist on lung function in the short term in cystic fibrosis (CF) patients.

Methods: Thirty-four CF patients (age 9-39 yrs) were randomized to receive, on alternate routine visits to our pulmonary unit, autogenic drainage physiotherapy (AD) for 30 min (28 patients), or airway clearance using two models of cough assist (CA) the "Pegaso cough" by "Dima italia" or the "Emerson CoughAssist™ Mechanical In-Exsufflator" (MI-E) by "Philips Respironics" (24 patients).

The cough assist was operated manually by an experienced physiotherapist. The level of insufflation and exsufflation was +25 cmH₂O and -35 cmH₂O respectively. The breathing cycles continued until mucus was transported from the peripheral to the central airways, and was followed by spontaneous cough, the entire procedure continued 30 min.

Pulmonary function test (PFT) was performed before and 15 minutes after treatment. A change of at least 10% from baseline values of FVC or FEV1 was considered significant.

Results: The mean improvement in FVC and FEV1 for the CA group was 8.41%±8.7 and 8.81%±5.8 respectively (p<0.001). Twelve patients (50%) improved PFT at least 10% above baseline values. In contrast the mean improvement in FVC and FEV1 for the AD group was 2.47%±5.8 and 1.49%±5.4 respectively. Three patients (10%) improved PFT at least 10% above baseline values.

Conclusions: Airway clearance using the cough assists was superior to autogenic drainage in improving short-term lung function in a group of 34 CF patients. The integration of mechanical cough assist might give a new tool for CF patients to clear secretions from the lungs.

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URINARY INCONTINENCE IN CHILDREN AND ADOLESCENTS WITH CYSTIC FIBROSIS

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Urinary incontinence (UI) is common in female adolescents and women with cystic fibrosis (CF). By trying to avoid urinary leakage, people with CF may reduce the effectiveness of cough, airway clearance techniques (ACT) and exercise which may lead to decline in respiratory status and affect quality of life (QOL). Stress UI (SUI) is rare in children, however in adolescent girls with CF it is reportedly the most common type of UI. As risk factors of UI in children are different from adults, SUI should not be assumed in children with CF without careful assessment. In children UI is treatable with urotherapy. In adult women pelvic floor muscle training (PFMT) has been shown to be effective, however its effectiveness and adherence has not been studied in children.

Aims:

1. Develop a UI screening tool to identify type and causes of incontinence
2. Treat accordingly
3. Assess prevalence and relationship between onset of UI and severity of lung disease, age, menarche and CF-related diabetes
4. Assess impact of UI on airway clearance, exercise and QOL
5. Assess treatment effectiveness and adherence

Method: A screening tool based on validated questionnaires was developed and administered by physiotherapists to children >5 yrs at CF clinic annual review. Treatment was provided accordingly. Follow-up data was collected on subsequent clinic visits and compared to baseline.

Results: 111 patients have been screened (64 female), mean age 12.7yrs (SD 3.8); 80% mild lung disease, 16% moderate and 4% severe. 25 (23%) patients (18 female) reported UI, 24 with day UI and 3 with nocturnal enuresis (NE). Prevalence in females and males was 28% and 15% respectively. Causes of UI were SUI 17 (68%, 16 female); void postponement (VP) 8 (32%, 7 female); overactive bladder (OAB) 3 (12%, 1 female) and constipation 7 (28%, 6 female). Some had more than 1 contributing factor. Frequency of UI ranged from <once/month to >once/day. 68% reported small amounts, however 32% reported seepage to outer clothing. Of those with SUI the most common activities causing UI were cough (100%), laugh (76%) and exercise (65%).

Treatment was standardised based on an appendix developed to guide diagnosis and treatment. Those with SUI received education, taught PFMT

and optimal posture for ACT. Urotherapy was given to those with VP and constipation. Those with OAB and NE were referred to a continence specialist. To date, we have follow-up data (≥3 months) on 17 children. 6 reported nil UI, 5 reported UI <1/month and 3 had significant improvement. 2 reported they did not follow advice given and had nil change.

We found no relationship between onset of UI and severity of lung disease, age, menarche, or CFRD. Of those with SUI, UI was reported to impact effectiveness of cough in 35%, exercise in 24% and QOL in 28%.

Conclusion: This screening tool is suitable for use in a busy clinic setting to identify UI and guide treatment. UI can impact effectiveness of cough, affect exercise and QOL. Urotherapy and PFMT are effective and well adhered to in this population who already have a high treatment burden. We plan to continue screening our clinic and follow this cohort to understand more about UI in this population.

Acknowledgments: Alison Elliott is funded by Whites Group.

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THE USE OF HIGH FREQUENCY CHEST WALL OSCILLATION DURING AN ACUTE INFECTIVE PULMONARY EXACERBATION OF CYSTIC FIBROSIS

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Background: Cystic fibrosis (CF) patients hospitalised for an acute infective pulmonary exacerbation require increased airway clearance. Specialist physiotherapists may be a limited resource. We investigated the use of high frequency chest wall oscillation (HFCWO), in addition to "usual" airway clearance techniques (ACTs).

Objective: The aim was to assess the utility of HFCWO (The Vest[®] Airway Clearance System by Hill-Rom) as a self administered therapy compared to European ACTs in facilitating recovery from an acute infective pulmonary exacerbation in people with CF when used in addition to supervised physiotherapy.

Method: A non-blinded randomised, controlled design was used. Patients who met inclusion criteria were randomised to control or HFCWO groups. All patients received four daily sessions, two supervised by a specialist CF physiotherapist and two carried out independently. The control group carried out their usual ACTs, the study group used HFCWO with pauses to huff and cough. The primary outcome measurement was change in FEV₁.

Results: Thirty-six patients (64% male) participated. Data was analysed using the Wilcoxon Rank sum test.

Conclusion: Change in FEV₁ was not significantly different between groups, however a significant improvement in FVC was demonstrated. Despite a trend towards a shorter LOS in the HFCWO group this did not reach significance. There was no significant difference in total sputum weight. Data on treatment times and adherence may prove interesting areas for further analysis. HFCWO should be further explored as an adjunct in treatment of infective pulmonary exacerbations of CF.

	Control	HFCWO	P Value
Age Mean (SD) (years)	29.8 (± 11.7)	25.8 (± 7.3)	NS
Baseline FEV ₁ Mean (SD) (mL)	1490 (± 900)	1570 (± 540)	NS
Change in FEV ₁ Median (IQR) (mL)	120 (50, 260)	240 (80, 360)	0.18
Change in FVC Median (IQR) (mL)	70 (-170, 370)	370 (90, 620)	0.05
Length of Stay (LOS) Median (IQR) (days)	14 (13, 15)	13 (10, 14)	0.08
Total Sputum Weight Median (IQR) (g)	195 (29, 377)	213 (87, 332)	0.41

Poster Session Abstracts

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CANADIAN NATIONAL AIRWAY CLEARANCE STUDY: PATIENT SATISFACTION WITH POSITIVE EXPIRATORY PRESSURE VERSUS HIGH FREQUENCY CHEST WALL OSCILLATION

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Airway clearance therapy (ACT) is an important component of treatment in cystic fibrosis with various methods being used throughout the world. In Canada where positive expiratory pressure (PEP mask) is the most commonly used technique, patients were interested in learning how high frequency chest wall oscillation (HFCWO) would compare to PEP mask. In particular, is each technique as efficacious as the other, do both require the same treatment time length, can both be performed independently, is each technique easy to perform and flexible as to where they can be performed. Thus we designed the following study.

Objective: To evaluate patient satisfaction, quality of life and adherence with HFCWO as compared to PEP mask therapy in the treatment of CF.

Methods: This was a randomized controlled study involving 12 CF centres in Canada. After a 2 month washout period where subjects performed an ACT other than PEP or HFCWO, subjects were randomized to perform either PEP or HFCWO for a period of one year. Subjects were assessed at 3 monthly intervals. Respiratory exacerbation requiring oral, inhaled and/or intravenous antibiotic therapy was our primary outcome. Secondary outcomes included treatment time length, ease of performance, flexibility of treatment and adherence to treatment. These were measured at 3 monthly intervals using a satisfaction questionnaire which rated comfort, independence and flexibility on a Likert scale of 1 to 5, with 5 being the most comfortable, requiring the least assistance and the most flexible. The CF quality of life questionnaire (CFQ) was also completed at 3 monthly intervals and subjects kept a daily adherence diary.

Results: 107 subjects from 12 CF centres were enrolled in the study. 51 subjects were randomized to PEP and 56 to HFCWO; 88 subjects completed the study. There were 16 dropouts during the washout period and 3 further after randomization. There were significantly more respiratory exacerbations in the HFCWO group compared to the PEP group ($P=0.007$), (Mann Whitney test). Treatment time was significantly longer in the HFCWO group. Subjects felt both treatments were equally comfortable and offered the same degree of independence. However PEP was a more flexible ACT with subjects finding the HFCWO device too cumbersome to take outside the home. Number of treatments per day and reported number of misses per week were equivalent. CFQ data are still being analysed.

Conclusions: Over a one year period, PEP mask therapy was associated with less pulmonary exacerbations than HFCWO and required a shorter treatment time and more flexibility.

Supported by a grant from Cystic Fibrosis Canada.

Results

	PEP	HFCWO	SIG
Treatment time minutes	19±7.93	29±9.73	$P<0.001$
Comfort 1-5	4.46±0.69	4.39±0.78	
Independence 1-5	3.84±1.27	3.94±1.2	
Flexibility 1-5	4.44±0.94	3.75±1.38	$p<0.001$
mean number times per day	1.75	1.76	
Misses per week	0.5	0.5	

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CANADIAN NATIONAL AIRWAY CLEARANCE STUDY: HOW PHYSICALLY ACTIVE ARE CF PATIENTS?

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Background: Physical activity and exercise have been shown to be factors that can contribute to the health of CF patients. Research has shown an association between physical activity and higher aerobic capacity in CF

patients. The Habitual Activity Estimation Scale (HAES) questionnaire is a reliable and valid instrument that can be used to assess activities of varying intensities in CF patients (Wells et al, *Pediatr Pulmonol* 2008;43:345).

Objectives: To report on baseline activity levels of cystic fibrosis patients across Canada participating in the High Frequency Chest Wall Oscillation (HFCWO) study.

Methods: This was a randomized controlled study involving patients at least 6 years of age from 12 CF Centers in Canada. At screening, patients were administered the HAES questionnaire. The questionnaire divides the day into time segments between meals and from awake time until bedtime. The percentage of time spent inactive, somewhat inactive (eg, watching TV), somewhat active (eg, walking), or very active (eg, running, swimming) for each time epoch were reported. Patients were asked to report on a typical midweek day in the previous 2 weeks and a typical Saturday within the previous 2 weeks. Weekday and weekend days are reported separately. Results are reported as the percentage of awake time spent active (somewhat active+very active) and percentage of awake time spent very active.

Results: In all, 106 (57 female, 49 male) subjects successfully completed screening (mean age 13.7 years, range 6-46 years) from 12 CF centers across Canada. For 6-12 year olds, there was no difference between males and females in any of these parameters for weekday or weekend days (table). Similar results were found for those older than 12 years of age. Comparing younger (6-12 yo) and older males (>12 yo), young patients spent a greater percentage of weekday time being very active, however there was no significant difference in the percentage of weekday total time spent active. No significant differences were found for weekend time spent in activity. Comparing younger (6-12 yo) and older females (>12 yo), young females spent a higher percentage of time being very active both on weekday and weekend days, compared to older females. There was no difference in the percentage of time spent in total activity.

Conclusion: Younger (6-12 yo) males and females appear to spend a higher percentage of time being very active. This difference is seen on weekdays only in younger males and on both weekday and weekend days in younger females. Values for older patients were similar to those reported by Wells et al.

Supported by a grant by CF Canada.

	Males		Females	
	6-12 yo	>12yo	6-12yo	>12yo
n	29	20	35	22
Weekday Active (% awake time)	43.7±16.13	39.1±24.74	45.3±21.14	45.9±22.28
Weekday Very Active (% awake time)	19.6±13.54	8.6±11.62*	20.3±17.14	5.2±6.83*
Weekend Day Active (% awake time)	52.5±17.30	48.9±21.80	56.1±14.76	50.2±19.80
Weekend Day Very Active (% awake time)	20.0±15.32	13.2±13.01	22.0±18.04	11.7±15.67*

* $p<0.05$ within gender age group differences

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CARDIORESPIRATORY RESPONSES TO NINTENDO WII IN CHILDREN WITH CYSTIC FIBROSIS: A PILOT STUDY

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Background: Exercise can play a vital role in the management of cystic fibrosis (CF) in children. Research has shown an association between physical activity and higher aerobic capacity in CF patients. Active video games may provide an engaging medium for children to exercise in. An adequate training stimulus is considered as 3 METS (3 x resting oxygen consumption) for moderate activity and 6 METS for vigorous activity (ACSM/CSEP guidelines).

Objectives: 1) To investigate the cardiovascular response of children with CF while playing Wii Active on the Nintendo Wii system, 2) to compare exercise responses between playing Wii Active Boxing and Wii Active Running.

Methods: Four children (1 female, 3 male, age 14-15 years), with mild to moderate CF (FEV1 83.0±20.9 %) from the Montreal Children's Hospital Cystic Fibrosis Clinic have been studied to date. Following a maximal progressive exercise test, participants were randomly assigned to perform two consecutive 12-minute exercise tasks, Boxing and Running, created on the Nintendo Wii with the EA Sports Active game. Subjects had a 30-minute

rest period between tasks. Subjects were asked to report their level of dyspnea using the modified 10-point Borg scale (Borg, 1998), once during resting breathing, at minute intervals during the Wii exercise, and following completion of each exercise period.

Results: The mean time spent exercising in the boxing and running game was 11m16s ($\pm$ 1m27s) and 11m59s ($\pm$ 1m48s), respectively. There were nine rest periods during the boxing task, amounting to 2m53s ($\pm$ 0m32s) of rest. The running task had five rest periods, amounting to 2m42s ($\pm$ 0m42s) of rest. Within the boxing task, a significant increase in the average heart rate of 145% and VO_2 of 147% were noted between the rest and exercise periods (93.69bpm–135.99bpm, $p = 0.04$; 0.36L/min–0.94L/min, $p = 0.02$). Participants maintained on average 43.7% of total exercise time within a 70–85% HRmax range. Within the Running task, a similar significant increase in the average heart rate of 261% and VO_2 of 445% were noted between the rest and gaming periods (96.43bpm–142.46bpm $p = 0.01$; 0.33L/min–1.47L/min, $p < 0.01$). Participants maintained on average 60% of exercise time within a 70–85% HRmax range. Half enjoyed one of the two activities over the other.

Conclusion: Exercise with Wii active games appears to be feasible and safe for patients with cystic fibrosis. Moderate intensities were obtained with the running game, but not with the boxing game. These results are consistent with the few reports in healthy individuals.

	Boxing		Running	
	Rest	Exercise	Rest	Exercise
HR (%max)	51.30%	74.41%	61.61%	77.95%
VO_2 (%max)	15.30%	39.56%	14.09%	62.10%
RR (%max)	46.80%	78.26%	45.05%	94.87%
VE (%max)	18.8%	43.74%	17.74%	62.33%
BORG		2.43 $\pm$ 0.94		3.30 $\pm$ 1.10

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HABITUAL PHYSICAL ACTIVITY IN ADULTS WITH CYSTIC FIBROSIS

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Habitual physical activity is believed to contribute to overall fitness, which has been associated with survival in CF. Previous studies have focussed on children and adolescents.

Aim: To evaluate habitual physical activity (HPA) among adults with CF compared to age matched controls.

Methods: A prospective sample of adult patients were recruited from the CF Outpatient Department together with a control group who completed the long-form 7-day recall International Physical Activity Questionnaire (IPAQ). Criterion validity of the IPAQ in a sample ($n=30$) of our CF cohort was evaluated against the ActiGraph GT1M accelerometer with a significant correlation in weekly energy expenditure ($r=0.46$, $p=0.010$). Physical activity was compared between the CF and control groups for overall IPAQ physical activity scores and the domains of work, transport, domestic chores and leisure measured in MET.Minutes/Week (Mm/w).

Results: There were 101 participants with CF and 35 control participants; 45% females in the CF group vs 70% in the controls. The groups were compared [mean(sd)] and were similar for age: CF 29(9)yrs, control 32(10)yrs, $p>0.05$; and BMI: CF 22(4)kg/m², control 23(3), $p>0.05$. FEV1 % predicted for CF was 60(23) vs 101(13). The two groups reported similar levels of physical activity accumulated for moderate and vigorous physical activity: CF moderate activity 1256 (1805) vs control 1645 (3223) Mm/w, [mean(sd)] $p=0.648$; vigorous activity 2170(3560) vs 2787(4242) $p=0.110$. The CF group did less physical activity than controls for IPAQ total score 5309 (6277) vs 7808 (5493), $p=0.01$; work-related physical activity 1887(4285) vs 3707 (5292), $p=0.003$ and transport-related physical activity 613 (1018) vs 1315 (1123) $p< 0.001$. Physical activity time related to leisure and domestic activities did not differ between groups: CF 1269(1607) vs 1565(2134), $p=0.376$. A greater percentage of the CF participants did not work or their work-related physical activities lasted less than 10 minutes at a time (58% CF vs 21% control). Correlation statistics for pooled data showed higher levels of physical activity were significantly associated with better respiratory function (Pearson R from 0.18 to 0.23,

$p<0.05$). Physical activity was also inversely correlated with resting heart rate (-0.10 to -0.27). In females with CF, lower levels of HPA were associated with low lung function. This association was not found in males with CF or controls. The majority of participants in each group had overall physical activity intensity scores which classified them in the high intensity category (55% of subjects with CF vs 69% controls) followed by moderate physical intensity (37% vs 26%) and low intensity (8% vs 6%). The 8% of the CF group in the low intensity category did not differ significantly from the rest of the CF group in terms of age, BMI and lung function.

Conclusion: Adults with CF engage in less habitual physical activity vs controls, especially in relation to work and transport. Assessment of vocational physical activity should be considered when determining physical activity treatment goals and prescription.

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EVALUATION OF MUSCULOSKELETAL AND POSTURAL FUNCTION IN CYSTIC FIBROSIS USING A PHYSIOTHERAPY SCREENING TOOL

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Postural changes have been reported in patients with CF as they age. There is concern that over time significant postural changes may cause pain and limitation of normal chest movements which in turn may impact negatively on lung function.

Aims: To measure musculoskeletal and postural function in adults with CF; to identify problems related to disease progression and to further develop a clinically appropriate musculoskeletal assessment screening tool.

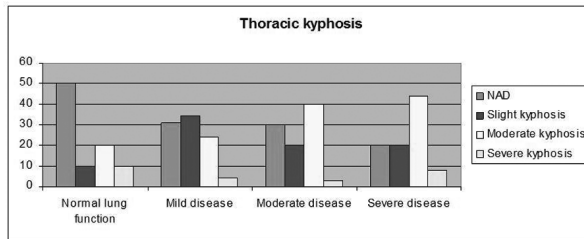
Method: A physiotherapy screening tool was developed to assess musculoskeletal function in the inpatient and outpatient settings when patients were at baseline function. Spinal curves, head, shoulder and scapulae positions, thoracic extension and shoulder flexion, thoracic lateral flexion and rotation, hamstring, calf and quads muscle length were assessed.

Results: Ninety-four patients were screened aged 18 to 50 years with 11% with normal lung function (FEV1 % predicted greater or equal to 80%); 31% with mild lung disease (FEV1 60–79%); 32% with moderate lung disease (FEV1 41–59%) and 26% with severe lung disease (less than 41%). The following postural changes were found in this sample: cervical changes 33%; thoracic kyphosis 42%; increased lumbar lordosis 35%; anterior shoulder protraction 51%; elevated shoulders 34%; abducted or winged scapulae 49%; decreased thoracic lateral flexion 31%; restricted thoracic rotation 31%; tight hamstrings 52%; tight calf muscles 26%. Quadriceps length was normal in all subjects. Thoracic kyphosis, scapulae abduction/winging and anterior shoulder protraction appeared to be related to disease severity.

Discussion: Clothing hid many of these changes. Using this screening tool, we were able to identify postural changes that required specific prescription of strengthening, stretching and mobility exercises with the aim of correcting and preventing further changes.

Conclusions: In summary, tight hamstrings and calf muscles were found across the disease spectrum. Thoracic kyphosis, abducted/winged scapulae and protracted shoulders in this sample were related to disease severity. The results of postural screening in these subjects highlight the importance of regular review of musculoskeletal function in adults with CF in order to prevent problems developing and to manage problems as they are diagnosed. The screening tool has been further developed to incorporate more objective measures. Musculoskeletal screening should be considered as part of the annual review in CF.

Poster Session Abstracts



Degree of thoracic kyphosis relative to lung disease

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EXERCISE: PERCEPTIONS OF PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Exercise training is beneficial in children with cystic fibrosis (CF), yet children with CF are less active compared to healthy peers. Individually-tailored exercise programs that address patient-specific perceptions of physical activity (PA) may help to increase exercise participation in this population. We aimed to systematically assess PA perceptions in an outpatient pediatric CF clinic with the goal of improving exercise education and prescribing individualized exercise programs.

Methods: Consecutive patients with CF eight years and older were recruited to complete an electronic questionnaire assessing PA perceptions during outpatient clinic visits between May 2011 – February 2012. The questionnaire consisted of a total of 25 items modified from the Exercise Benefits and Barriers Scale (EBBS) and CF-specific questions from previous publications by Boas (1999) and Swisher (2008).

Results: One hundred twenty patients 8-21 years (51% male) completed the questionnaire. One hundred thirteen (94%) respondents agreed that children with CF should exercise. Perceived benefits of exercise include: increased longevity (96%), improved body image (96%), improved mucus clearance from lungs (95%), enjoyment of exercise (89%), general sense of well-being (89%), and improved sleep quality (81%). One hundred (85%) respondents felt that children with CF can exercise as well as other children and 112 (94%) felt that children with CF can run marathons. Ninety (75%) respondents felt that exercise results in coughing and 61 (51%) believe that exercise results in weight loss.

Seventy-seven (64%) respondents expressed an interest in increasing their participation in exercise while 17 (14%) respondents were unsure if they wished to increase their exercise participation. Twenty-six (22%) respondents were not interested in increasing their exercise participation. Possible barriers to participation include: fatigue (60%), shortness of breath (54%), having to exercise alone (23%), lack of enjoyment (21%), lack of support from friends (20%), lack of time (19%), lack of support from family (14%), risk of getting hurt (7%), financial constraints (4%), and embarrassment regarding exercise (3%).

Conclusion: Our data demonstrate that the majority of children and young adults with CF appreciate the importance of exercise and do not perceive the diagnosis as a barrier to exercise. Our data also suggest that weight loss and respiratory symptoms associated with exercise may be a concern of many patients with CF. Exercise education may be warranted to address these concerns and other barriers to exercise participation.

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INFLAMMATION AND OXIDATIVE STRESS DURING EXERCISE IN PATIENTS WITH CYSTIC FIBROSIS

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Exercise capacity is an independent predictor of mortality in patients with cystic fibrosis (CF), even after adjusting for lung function. In fact, improvements in exercise capacity are associated with longevity in patients with CF; however, exercise elicits an inflammatory and oxidative stress response that may differ among patients and apparently healthy individuals.

Purpose: This study sought to explore the inflammatory and oxidative stress response during moderate intensity exercise in young patients with CF and healthy controls.

Methods: Fourteen young patients with CF and 14 demographically matched controls participated in this study. Subjects performed submaximal exercise on a cycle ergometer at a workload equal to 20%, 40%, and 60% of maximal exercise capacity. Each stage lasted 5 minutes. Blood samples were taken at baseline and during the last minute of the test. Biomarkers of inflammation and oxidative stress were evaluated. A two-way (group x time) mixed factorial ANOVA was performed on all biomarkers using SPSS software.

Results: Although lung function was lower in patients with CF (% predicted FEV1 = 88±5% vs. 105±3% respectively), exercise capacity was similar between groups (1.5±0.2 vs. 1.9±0.2 L/min, respectively). Inflammatory biomarkers (Eotaxin, MCP-1, MCP-2, and RANTES) were elevated (p<0.05) at rest in patients with CF; however, biomarkers of oxidative stress (AOC, ascorbyl free radical, 8-iso, FRAP, and LH) were similar (p>0.05) between groups. During exercise, controls exhibited a slight decrease in Eotaxin, MCP-1, MCP-2, and RANTES, whereas increases were observed in patients with CF (interaction ranges p=0.07 – 0.161). Interestingly, no group by time interactions were observed for biomarkers of oxidative stress; however, when collapsing across groups (time main effect) near significant increases in FRAP (p=0.06), 8-iso (p=0.09), and the ascorbyl free radical (p=0.08) were observed.

Conclusion: In contrast to healthy counterparts, these data suggest that patients with CF have an exaggerated inflammatory response during submaximal intensity exercise. Additionally, both groups exhibited slight elevations in oxidative stress during exercise. Future studies in patients with CF should examine the effects of exercise training on inflammation during both exercise and unstressed states. This study was supported in part by the Georgia Health Sciences University Child Health Discovery Institute (RAH) and the American Heart Association (RAH).

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DYNAMIC HYPERINFLATION DURING CARDIOPULMONARY EXERCISE TESTING IN CYSTIC FIBROSIS

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Background: Dynamic hyperinflation during cardiopulmonary exercise testing (CPET) in cystic fibrosis (CF) has not been well studied, and little is known regarding its prevalence, risk factors, and clinical associations.

Methods: Using clinical data from the Toronto CF database from 2002 to 2008, the present study sought to characterize and determine the prevalence of dynamic hyperinflation during CPET in a large population of adults with mild-to-moderate CF (n=109); and evaluate the impact of dynamic hyperinflation on lung function and exercise tolerance, clinical symptoms, and patient prognosis over a two-year period.

Results: In all, 58% of patients responded to CPET with dynamic hyperinflation. Patients with evidence of dynamic hyperinflation during

CPET had significantly lower lung function (FEV_1 66 ± 19 vs 79 ± 18 %pred., $p < 0.01$) and exercise tolerance (peak oxygen uptake 28.7 ± 8.1 vs 32.9 ± 6.1 mL.kg⁻¹.min⁻¹, $p = 0.02$), and experienced greater shortness of breath at peak exercise (7 ± 3 vs 5 ± 2 Modified Borg scale, $p = 0.04$) compared to patients who responded without dynamic hyperinflation. However, responding to CPET with or without dynamic hyperinflation did not significantly predict FEV_1 at 2 years ($p = 0.06$), or increase the likelihood of experiencing a pulmonary exacerbation over a two-year period ($p = 0.24$). A significant, but, weak relationship was shown between FEV_1 (%pred.) and dynamic hyperinflation ($r = 0.37$, $p < 0.01$) (Figure 1).

Conclusions: There is a high prevalence of dynamic hyperinflation during exercise in adult patients with mild-to-moderate CF, and it is associated with reduced lung function and exercise tolerance and increased exertional dyspnea. However, identifying dynamic hyperinflation during CPET had limited prognostic value for lung function and pulmonary exacerbation. Dynamic hyperinflation is difficult to predict from FEV_1 , therefore, CPET is a useful tool to directly determine its presence.

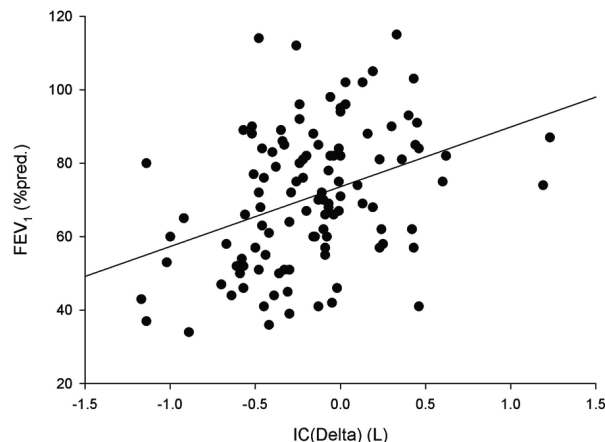


Figure 1: Relationship between FEV_1 and dynamic hyperinflation ($r = 0.37$, $p < 0.01$). N.B. A negative inspiratory capacity (IC) delta (i.e., change from rest to peak exercise during CPET) indicates dynamic hyperinflation; whilst a positive IC delta indicates no dynamic hyperinflation.

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OUTCOMES EVALUATION OF PULMONARY REHABILITATION FOR PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Pulmonary rehabilitation (PR) is an individualized program aimed at helping patients manage and cope with their symptoms. Comprehensive PR includes education regarding pulmonary disorders, exercise training, nutrition classes, and psychosocial counseling. Traditional PR programs for patients with chronic respiratory diseases such as COPD have shown positive outcomes. In addition, physical activity has been shown to benefit cystic fibrosis (CF) patients. As a result, PR may be a beneficial intervention for CF. However, few centers have designed PR programs for CF patients and there are no current studies describing the outcomes of PR programs for CF patients. The purpose of this research was to conduct an outcomes evaluation of an AACVPR-accredited PR program for CF patients.

Methods: The study examined clinical and psychosocial data of all CF patients who have participated in the program including pre- and post-PR 6 minute walk tests (6MW), pulmonary function tests (PFT), body mass index (BMI), and health-related quality of life (HRQOL). It also explored differences based on age, severity of disease, program compliance, and gender.

Results: There was a positive, significant difference in pre- and post-PR 6MW results ($p = .001$), and a positive, significant difference in pre- and post-PR BMI values ($p = .002$). A significant difference between pre- and

post-PR 6MW distances for the varying levels of disease severity ($F(5,27) = 2.838$, $p = 0.035$) suggests PR may be more beneficial for CF patients with mild, moderate, or moderately-severe lung impairment. Female patients had a greater improvement in BMI from pre- to post-program than male patients ($F(1,34) = 4.160$, $p = 0.049$). There was no significant difference noted in pre- and post-PR FEV_1 and FEV_1/FVC PFT values. Analysis of other variables was not performed due to incomplete records.

Conclusions: All analyzed variables either showed improvement or did not decline from pre- to post-PR, indicating that CF patients likely benefit from PR much like others with chronic respiratory diseases.

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SIX-MINUTE WALK TESTING DURING HOSPITALIZATION FOR CF PULMONARY EXACERBATION

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Background: Studies of six-minute walk testing (6-MWT) in cystic fibrosis (CF) do not clearly demonstrate a correlation between lung function, expressed as percent-predicted FEV_1 ($FEV_1\%$), and six-minute walk distance (6-MWD). Data from Gruet et al. [1] suggest that the maximum heart rate after 6-MWT approximates the heart rate at anaerobic threshold during formal cardiopulmonary exercise testing (CPET) in CF patients, which could allow for greater refinement of exercise prescription. We sought to identify predictive factors for 6-MWD during hospital admission for CF pulmonary exacerbation (CFPE).

Methods: Twenty-eight subjects underwent 6-MWT according to American Thoracic Society standards during inpatient treatment for CFPE. Ratings of perceived exertion (RPE) and heart rate were measured before, during, and after walking. Recovery time was defined as the time necessary for the heart rate measured shortly after completing 6-MWT to return to a level at or below that which was measured before 6-MWT. Spirometric and anthropometric data were obtained. Parameters were compared using Spearman rank correlation (ρ). The Kruskal-Wallis test was employed to describe differences in data between subgroups. $P < 0.05$ was significant for all relationships.

Results: Data are presented as median and (range). Subject age was 24 (12-69) years. Length of stay was 9 (4-19) days. The 6-MWT was performed within 48 hours of spirometry in 71% of cases and within 48 hours of discharge in 64% of cases. The 6-MWD and activity-related pulse increase were directly correlated ($\rho = 0.38$) for all subjects. The 6-MWD was longer (562.4 m vs. 433.7 m) for 5 subjects in whom resting heart rate was < 100 beats per minute and an activity-related increase of ≥ 42 beats per minute was observed. $FEV_1\%$, age, weight, RPE, and recovery time were not correlated with 6-MWD. Seven subjects with resting heart rates ≥ 100 beats per minute had lower median $FEV_1\%$ (29% vs. 56%) than 21 subjects with slower baseline pulses. Nonetheless, these groups were similar in terms of age, 6-MWD, recovery time, and RPE, and they performed spirometry after comparable treatment intervals. Compared to subjects who were ≥ 22 years old, those who were younger took longer to recover and weighed less despite similar $FEV_1\%$.

Conclusions: During hospitalization for CF pulmonary exacerbation, heart rate before and after 6-MWT is a useful indicator of functional impairment. Resting tachycardia is commonly observed in patients with severely-reduced lung function, but $FEV_1\%$ alone does not predict 6-MWD. Patients with lower baseline heart rates and cardiac reserve are likely to walk farther, probably due to better overall conditioning. That older patients recovered faster and weighed more than younger patients likely reflects a bias toward longevity in those with milder CF phenotypes.

Reference: [1] Gruet M, Brisswalter J, Mely L, Vallier JM. Arch Phys Med Rehabil 2010; 91: 602-7.

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DO REDUCED LEVELS OF PHYSIOTHERAPY STAFF AT WEEKENDS INFLUENCE CLINICAL OUTCOME DURING HOSPITAL ADMISSION?

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Introduction: The UK economic climate has brought an increased requirement to justify physiotherapy staffing levels. The in-patient physiotherapy workforce is reduced at our adult CF centre during weekend days compared to weekdays; interventions are provided by a less concentrated and specialist work force during weekend days. Routine objective physiotherapy patient assessment includes measurement of daily wet sputum weight and spirometry (admission and then twice weekly). This provides opportunity to evaluate the effect of the differing work force on these outcomes.

Methods: A retrospective review of clinical physiotherapy records of all patients admitted over a 3 month period. Exclusion criteria: spirometry contraindicated or not possible, a stay of < 10 days, haemoptysis, missing records. The average daily weight of sputum expectorated was compared for weekdays and weekend days. Spirometry response was compared from admission to first repeat measurement.

Results: Thirty-six records were analysed. Median (range) average daily sputum 11.8g (0 – 101), FEV₁ 1.3L (0.5–3.3), FEV₁% pred 37.6% (18.6 – 91.0). Mean (SD) FVC 2.7L (0.9) FVC% pred 64.6% (18.4).

A significantly greater daily weight of sputum was expectorated on weekdays compared to weekend days median 12.6g (0 to 114.5) vs. 9.7g (0 – 87.5g) $p = 0.012$.

The % predicted FEV₁ and FVC improved significantly from admission to repeat measure 1; FEV₁% pred median difference (range) 4.6% (-8.3 to 29.7) $p < 0.001$, FVC% pred median difference 7.9% (CI 5.6-10.2) $p < 0.001$.

There was no significant difference in the spirometric changes between the weekday (n=12) and weekend day (n=24) groups. Median change in the % predicted FEV₁ weekday 5.7% (-4.0 to 18.3) vs. weekend day 3.3% (-8.3 to 29.7) $p = 0.68$; Mean change in the % predicted FVC 9.1% (6.9) vs. weekend day FVC% 7.4 (6.9) mean difference 1.7 (95%CI – 3.3 to 6.6) $p = 0.49$.

Conclusion: Whilst the differences in average daily sputum expectorated are small this data provides an indication that decreasing physiotherapy provision may adversely affect outcome.

The lack of data on those unable to perform spirometry means that those patients likely to require most assistance with their physiotherapy were excluded from this retrospective review. Many factors contribute to spirometric improvement during an acute in-patient admission making it unlikely that this outcome would be able to detect a change in physiotherapy provision alone.

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MAXIMAL EXERCISE TEST IN PATIENTS WITH CYSTIC FIBROSIS

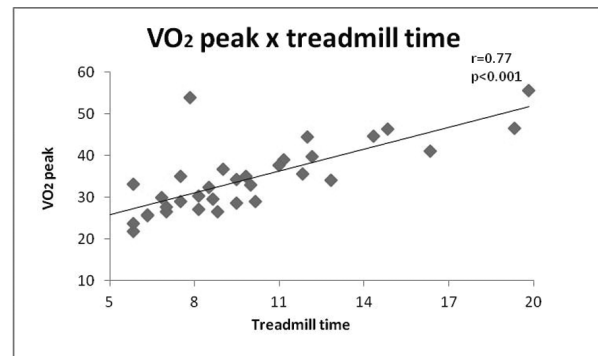
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Objectives: Physical activity and physical exercise tolerance can be used to estimate functional limitation, as well as to quantify the impact of cardiopulmonary diseases on daily activities and quality of life. The aim of this study was to describe peak oxygen uptake of the cardiopulmonary test in children and adolescents with cystic fibrosis (CF) and to correlate this parameter with pulmonary function tests, anthropometric variables and domain of quality of life.

Methods: This cross-sectional prospective study was conducted with CF patients aged 7 to 20 years. Parameters measured were peak oxygen uptake (VO₂peak) by maximal exercise test, percent predicted forced expiratory flow at one second (FEV₁%) by spirometry, anthropometric variables like skin fold thickness (TSF), arm muscle circumference (AMC), body mass index (BMI) and quality of life.

Results: Thirty-four CF patients were evaluated. Twenty were males (58.8%). Mean age was 13.08±3.07, mean FEV₁% was 95.11±18.15% and mean VO₂peak was 34.12±8.47. There was a significant correlation of the VO₂peak with time on treadmill ($r=0.77$; $p<0.001$) and with dyspnea of modified Borg scale ($r=0.42$; $p=0.014$). There was also a significant negative correlation between VO₂peak and triceps skinfold (DCT) ($r=-0.35$; $p=0.038$) and between VO₂peak and body mass index z-score (BMIZ) score. There was no significant correlation between FEV₁ and physiologic parameters of the maximal exercise test. There was a significant correlation between the quality of life domain and time ($r=0.73$; $p=0.010$) and speed on treadmill ($r=0.76$; $p=0.007$).

Conclusion: The maximal exercise test gives objective information on exercise capacity and limitation, not correlated with other evaluated parameters. The maximal exercise test identifies different physiologic phenomena, since VO₂peak represents a more comprehensive expression of cardiopulmonary compromise.



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IMPORTANCE OF 6 MINUTE WALK TEST IN THE EVALUATION OF ROMANIAN TEENAGERS WITH CYSTIC FIBROSIS

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Measurement of lung function is the central part of the patients with cystic fibrosis assessment, but we consider that exercise testing should become an important tool in the continuous evaluation and care of these patients. The 6 minute walk test (6 MWT) is used to assess the individual's response to exercise. The 6 MWT is easy to carry out and reflects very well the activities of daily living.

The aim of the study is to design an exercise training program, based on 6 MWT results, and to evaluate the effects of this complex protocol including incentive therapy, individualized supervised training program: swimming, trampoline, walking, jogging, aerobics, cycling 3 times per week and airway clearance techniques.

Physical performance is dependent on skeletal muscle mass (SMM), pulmonary capacity and inspiratory muscle force. In order to improve physical performance of our patients we include in the program: aerobic and anaerobic training, with the purpose of pulmonary capacity improvement, incentive therapy for inspiratory muscle force, and strength training in order to increase skeletal muscle mass.

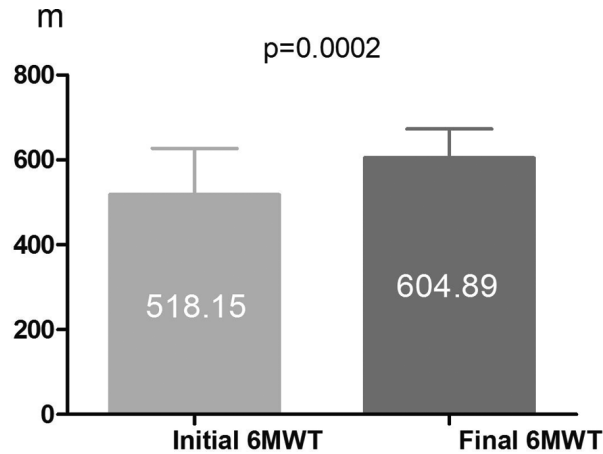
Materials and Methods: We conducted a 6 month study on 20 patients from the Romanian National Cystic Fibrosis Center, age between 12-18 years. Inclusion criteria were: FEV₁ or FVC lower than 60% of predicted, SaO₂ lower than 94% at rest.

Results and Discussion: The initial assessment showed limitations of exercise due to poor skeletal muscle mass, pulmonary status and respiratory muscle strength. After combining airway clearance techniques, incentive therapy and individualized physical trainings we observed significant improvements regarding pulmonary function, ease of breathing and increased fitness (6 minute walking distance (m) increased from 518.2±108.9 to 604.9±68.00, see Figure).

Conclusions: A positive correlation was observed between SMM and 6MWT performance, at the end of the study. This kind of protocol creates

pleasure and joy during physiotherapy which enhanced the patients' adherence to the programme.

Figure. 6MWT evolution after 6 months of intervention
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THE EFFECTS OF A 10 WEEK HOME-BASED SUPERVISED EXERCISE TRAINING PROGRAM IN INDIVIDUALS WITH CYSTIC FIBROSIS RELATED DIABETES

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Background: Regular exercise provides important benefits to individuals with cystic fibrosis (CF); however the effects of regular exercise in individuals with cystic fibrosis related diabetes (CFRD) are not well understood. We aimed to examine the effects of supervised home-based aerobic and resistance exercise training on glycaemic control, aerobic fitness, muscular strength, body composition and health-related quality of life (HRQoL) in individuals with CFRD.

Methods: Five individuals with CFRD were recruited for this study (n=3 male, aged 28±9, FEV1 66±20%pred, BMI 21.8±2.8, baseline HbA1c 9.8±2.1). Participants completed a 10 week exercise intervention, consisting of two sessions per week of supervised, home-based resistance and aerobic exercise training. Glycated haemoglobin (HbA1c), 3 day Continuous Glucose Monitoring System (CGMS), Dual Energy X-ray Absorptiometry (DEXA), Modified Shuttle Walk Test (MSWT), Six Repetition Maximum (6RM) and Cystic Fibrosis Questionnaire-Revised (CFQ-R) were assessed pre- and post-intervention.

Results: Adherence to exercise was excellent, with participants completing all of the scheduled exercise sessions. Exercise training improved aerobic fitness, with increases in the total distance covered (pre 750±319m, post 1098±318m, p=0.042) and maximum speed (pre 7.2±1.5km/hr, post 8.8±1.3km/hr, p=0.04) in the MSWT. Muscular strength improved for all resistance exercises (p<0.05), and lean mass tended to increase (p=0.089). Following the intervention, there was a mean reduction in HbA1c (0.88±1.15), although this was not significant (p=0.082). Four out of the five (80%) participants had a post-intervention HbA1c below their pre-exercise HbA1c, yet with the small sample size, this was also not significant (p=0.371). There were no significant changes in CGMS or HRQoL.

Conclusion: Regular aerobic and resistance exercise training is beneficial for individuals with CFRD, with improvements demonstrated in aerobic fitness and strength. It is encouraging that regular exercise training may also

improve glycaemic control, however studies with a larger sample size are required to validate this.

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SPINAL DEFORMITIES IN YOUNG PATIENT WITH CYSTIC FIBROSIS (CF): PROPOSAL OF A SCREENING AND FOLLOW UP PROTOCOL

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Background: Increased survival in patients (pts) with CF showed the importance of assessing extrapulmonary complications, as scoliosis (S); the prevalence of S in North America was 20 times greater in CF children than in the general population.

S is an evolutive spinal deformity which causes functional disorders on the spinal structures and leads to a restrictive pulmonary disease. Skeletal CF abnormalities (malnutrition, delayed bone mineralization and pubertal development, muscular dysfunctions, osteoporosis) may increase the risk of S.

The conservative treatment with brace is often difficult in CF pts because of ventilatory problems.

Aims: 1) create a screening protocol to determine prevalence of S in a group of CF pts; 2) assess the reliability of S screening by CF respiratory therapist (RT); 3) create a flow-chart to monitor S.

Materials and Methods: Pts with CF diagnosis (confirmed by sweat test or genotyping), 6-19 years old (yrs), regular follow-up in Treviso CF Centre and ability to perform the evaluation maneuver were included. The study is divided into 4 parts:

1) Creation of a protocol to detect spinal deformities: evaluation of posture and measurement of hump by quantification of the angle of trunk rotation (ATR) using Bunnell's scoliometer in the Adams forward bending test position;

2) RT evaluation of all pts;

3) Blind specialist (sp) evaluation of all pts with ATR≥4° and some with ATR 0°-3°;

4) Definition of a flow chart to monitor S evolution in all pts.

Results: In all, 55 pts (26 males) with mean age 12.91 yrs (6.17-19.22), mean BMI 17.86 kg/cm², mean FEV1 91.42%pred and mean FVC 96.36%pred performed the RT's evaluation. Male and female groups are homogeneous for age, BMI and indexes of respiratory function. Based on ATR values, pts are divided in two groups: 1) negative group (N) with ATR 0°-3°; 2) positive group (P) with ATR>4°. Between N and P there was a statistical difference for FVC (p>0.001).

Twenty thoracic (74.07%), 5 lumbar (18.52%) and 2 thoracolumbar (7.41%) curves were found; leg dysmetria >1 cm was evidenced in 66.66% of P.

All pts of P and some of N were also blindly evaluated by a sp physician and measured ATR was compared to RT's values. There was a significant correlation between the 2 evaluations (R2=0.857; p<0.0001).

Seven spine radiographs were indicated by specialist's assessment; S (>10° Cobb) was diagnosed in 3 pts (5.45%), 2 males, with the indications of brace.

Conclusions: The higher prevalence of S in CF pts (5.45%) showed the importance of assessing this aspect. This simple screening protocol with ATR measurement is a feasible and reliable way to assess spinal deformities in CF pts and to identify the need for sp evaluation. Considering the frequency of reevaluation in the healthy population and the high prevalence of risk factors in young CF pts, a flow chart was created: all pts have to be reassessed by the rt every 6 months (pts with fast statural growth should be evaluated more frequently) and pts with ATR>4° should be sent to the sp.

Further studies will be necessary to establish S prevalence in adult CF pts. Reassessment of these pts and follow-up of pts with brace prescription will be necessary.

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OVER AGE 40 YEARS WITH CYSTIC FIBROSIS? A SURVEY OF ATTITUDES, BELIEFS AND BARRIERS TO EXERCISE

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Background: The life expectancy of people with CF is increasing with many now reaching their 40s. It is unknown whether exercise capacity affects prognosis in this older age group.

Objectives: To explore the attitudes, beliefs and barriers to exercise in the maintenance of health and well-being amongst older people with CF and to ascertain the frequency, intensity and duration of reported exercise levels.

Method: Postal questionnaire to our centre's cohort of CF patients aged ≥ 40 years ($n=112$). Most recent annual review demographic data were identified from the patient registry. Data analysed by Stata version 12.1 (Stata-corp, Texas, USA).

Results: 57 patients responded (51% response rate; M=35, F=22); two thirds (66% M, 64% F) reported exercising daily or 4-5x/week - this included: housework (86% M=F); stairs for exercise (31% M, 50% F); stretches/postural exercises (31% M, 27% F), other popular choices included home/gym-based exercise machines, running, cycling, swimming and walking. The median reported Borg score for the most frequently reported weekly exercise was 4/10; a weak correlation was seen between the minutes of the most frequently reported exercise and Borg score ($p=0.054$). At least 150 minutes of this moderately intense exercise/week in 30 minute sessions was achieved by 66% M, 59% F. Men appeared to like exercise while women were more ambivalent; however both felt the most important reasons to exercise were to keep fit and healthy, to make them feel good, and to help clear their chests, while improving bowel function was seen as least important. Few perceived any significant barriers to exercise except lack of motivation and having no time (reported more by women than men, $p=0.002$ and 0.021 respectively). Overall exercise was felt to be more important to people now compared with when they were 16-18yrs old (65% M, 59% F) although over half felt their level of exercise had decreased as they had aged (53% M, 59% F).

Conclusion: The responders to this survey were in relatively good health at the median age of 48yrs. They participate in a large variety of aerobic and anaerobic exercise, with around 60% achieving the UK Government's recommendations for 150 minutes of moderately intense exercise in 30-minute sessions/week. Few barriers were identified to exercise; moreover the benefits were clearly appreciated, and its importance was felt to grow with age. Survival differences may relate, in part, to exercise intensity at different stages of life; prospective studies are necessary to elucidate this further.

Variable	Male	Female	p value
Age (median (IQR))	48 (45, 50)	48 (42, 50)	0.69
$\Delta F508/\Delta F508$ (n (%))	15 (43)	7 (32)	0.36
≤ 1 Admission in last 12 months (n (%))	29 (83)	76 (17)	0.18
BMI (mean (SD))	24.0 (2.41)	22.4 (2.99)	0.03
Pancreatic insufficiency (n (%))	25 (71)	14 (64)	0.54
FEV ₁ %pred (mean (SD))	59.0 (24.4)	57.5 (23.6)	0.81
FVC %pred (mean (SD))	83.2 (26.1)	82.4 (18.8)	0.90
%SpO ₂ (median (IQR))	96 (95, 97)	96.5 (95, 97)	0.48
CF-related diabetes (n (%))	11 (31)	6 (27)	0.31

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THE RELATIONSHIPS BETWEEN HYPERINFLATION DURING EXERCISE AND SYMPTOMS IN ADULTS WITH CYSTIC FIBROSIS

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Exercise tolerance is reduced in patients with cystic fibrosis (CF). Ventilatory limitation, peripheral skeletal muscle weakness and poor nutritional status may contribute to exercise intolerance. The mechanisms of exertional dyspnoea are less understood, but it seems that dynamic hyperinflation may play a role. So we investigated the role of exercise dynamic

hyperinflation on breathlessness (DYS) and leg fatigue (LEG) in CF patients. Seventeen stable CF patients (32 ± 8 SD yrs; FEV₁ 2.66 ± 0.7 L; $68 \pm 16\%$ pred; inspiratory capacity (IC) 3.4 ± 1.0 L), during constant load cycle ergometry at 80% VO₂ max were studied. Intensity of breathlessness and leg fatigue, by Borg scale, and IC were recorded every 2 minutes. The individual slopes of the change in IC vs DYS and IC vs LEG were also computed.

Results: In the vast majority of patients we did not observe a correlation between changes in IC during exercise vs either DYS ($r^2 = 0.30 \pm 0.28$) or vs LEG ($r^2 = 0.35 \pm 0.26$). In addition, resting IC did not predict the rate of change in DYS or LEG. Conversely, we found a close relationship between the rate of increase in DYS per unit change in IC and the rate of increase in LEG per unit change in IC ($r^2 = 0.85$ $p < 0.0001$). Importantly, we found a good relationship between the baseline IC and tolerance limit (Tlim) ($r^2 = 0.44$ $p < 0.005$), but not between baseline FEV₁ and Tlim.

Conclusions: CF patients show considerable variation in the rate at which symptoms develop during exercise, suggesting that different physiological processes underline these symptoms. Baseline IC strongly predicts the duration of the endurance tolerance, while the degree of resting hyperinflation is poorly predictive of exercise induced changes in DYS and LEG.

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PHYSICAL ACTIVITY AND INFLAMMATION IN CYSTIC FIBROSIS

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Introduction: Physical activity is associated with acute changes in the levels of inflammatory mediators. The effect chronic physical activity plays on markers of inflammation is less defined. We sought to understand this better by measuring time spent in moderate-vigorous physical activity (MVPA) and correlating to interleukin-1 β (IL-1 β), interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumor necrosis factor- α (TNF α) in persons with cystic fibrosis and controls.

Methods: CF subjects who had not had a pulmonary exacerbation in the preceding two months and healthy controls were recruited. MVPA was measured by using an Actigraph® accelerometer over a one week period after enrollment. Serum and sputum were collected from persons with CF and serum was collected from controls. IL-1 β , IL-1ra, IL-6, IL-8, IL-10, and TNF α were measured in the serum of persons with CF and controls by multiplex assay. Viscosity of the sputum prevented analysis of sputum by multiplex assay. Findings of the serum analysis are presented in this abstract. Pearson Product Moment Correlations (PPMC) were used to assess the relationship of MVPA to inflammatory mediators.

Findings: Seven persons with CF and 9 controls completed the study and had acceptable accelerometry for analysis. Age, BMI and MVPA were not significantly different between persons with CF and controls. FEV₁ was lower in persons with CF (67% versus 89%, $p=0.002$). Healthy controls had no significant correlation between MVPA and serum cytokine levels (PPMCs -0.16 to 0.19). In persons with CF, the PPMC of IL-6 to MVPA was 0.57 while all other PPMCs were less than 0.42 and not felt to be significant. No significant differences were found in serum IL-1 β , TNF α , IL-6, IL-10 and IL-1ra between persons with CF and controls. Serum IL-8 was higher in persons with CF than in healthy controls (10.5 pg/mL versus 4.6 pg/mL, $p=0.03$). Post-hoc analysis in persons with CF showed serum IL-8 to be negatively correlated with FEV₁ %predicted with a PPMC of -0.56.

Discussion: Other studies have shown that acute exercise increases IL-6 and TNF- α but not IL-1 β and IL-1ra. IL-6 also is a key regulator of other cytokines by stimulating IL-10 and IL-1ra production and inhibiting TNF- α and IL-1 β production. Multiple studies have shown significant increases in IL-6 during exercise so the positive correlation between IL-6 and MVPA was not surprising. The reason the correlation was found in persons with CF but not controls is unknown. Physical activity was measured in the week after serum was drawn so the IL-6 may have been reflective of recent physical activity rather than chronic physical activity. This may be supported by the fact that IL-6 was higher in persons with CF than controls (10.6 pg/mL versus 4.1 pg/mL) but this difference did not meet significance ($p=0.08$). Serum IL-8 was found to be higher in persons with CF than controls. We also found serum IL-8 to be correlated with FEV₁. This correlation has also been found by other investigators but our PPMC was higher (-0.56 versus -

0.37). This is likely explained by our smaller sample size. Further studies are needed to better understand the correlation physical activity may have on inflammation in persons with CF.

TRANSPLANTATION

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SOCIOECONOMIC STATUS AND ACCESS TO LUNG TRANSPLANTATION FOR ADULT CYSTIC FIBROSIS PATIENTS IN THE UNITED STATES

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Background: Patients with Medicaid experience persistent health disparities for a wide range of diseases and treatments in the United States compared to those without Medicaid, but disparities with respect to access to lung transplantation for cystic fibrosis (CF) patients are largely unknown.

Objective: To determine whether access to lung transplantation for CF patients with end-stage lung disease differs according to Medicaid status.

Study Design: Observational study of 2,167 adult CF patients with end-stage lung disease who underwent initial lung transplant evaluation between 2001 to 2009 identified through the United States CF Foundation Patient Registry including 1009 (47%) receiving Medicaid and 1158 (53%) without Medicaid. The main outcome measure was acceptance for lung transplant following evaluation.

Results: In all, 1,349 (62%) of 2,167 adult CF patients who underwent initial lung transplant evaluation were accepted. In a multivariate logistic model accounting for demographic characteristics, disease severity, potential contraindications to lung transplant, and pre-/post-use of the lung allocation score, Medicaid recipients were 54% [95% confidence interval (CI):25%-88%] more likely to be not accepted for lung transplant compared to patients without Medicaid. This association was independent of other SES indicators including race, education, zip code median household income, and driving time from residence to closest transplant center (OR=1.37, 95% CI:1.10-1.72). Patients not completing high school (OR=2.37, 95% CI:1.49-3.79) and those residing in the lowest (vs. highest) zip code median household income category (OR=1.39, 95% CI:1.01-1.93) were also more likely not to be accepted for lung transplant.

Conclusions: In this nationally representative study of adult CF patients, multiple indicators of a lower socioeconomic position, including receipt of Medicaid, were associated with higher likelihoods of not being accepted for lung transplant. A more complete understanding of what specific aspects of socioeconomic position influence acceptance for lung transplantation is needed in order to ensure more equitable allocation of this treatment.

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LUNG TRANSPLANT AND CYSTIC FIBROSIS: IMPLICATIONS OF THE LUNG ALLOCATION SCORE

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Introduction: Cystic fibrosis is the third most common lung transplant diagnosis in the United States. The top five in order from most common to least common are COPD, IPF, CF, idiopathic pulmonary hypertension, and sarcoidosis. Outcomes for patients on the waiting list with CF have not been recently analyzed. The purpose of this study was to compare waiting list outcomes of those with CF to other diagnoses with the aim of identifying areas of improvement for referral, transplant criteria, and transplant outcomes.

Methods: UNOS waiting list data was analyzed for the transplant years 1988 to 2010. For each of the common transplant diagnoses, the total number of transplant removals off of the waiting list was analyzed for the reason for removal. Specifically, percentages of those patients on the waiting list actually transplanted, died, and too sick to transplant were calculated for

each diagnosis. This data was further analyzed for CF patients before (years 2000-2004) and after (years 2006-2010) implementation of the Lung Allocation Score in May of 2005 to determine the impact of the new scoring system on this subset of patients.

Results: As seen in the Table, compared to the other common transplant diagnoses, cystic fibrosis patients on the waiting list between the years 1988 to 2010 had average rates of transplantation (60%) while having similar rates of dying on the transplant list (24%) and being too sick to transplant (2.3%). When analyzed before and after implementation of the Lung Allocation Score, the percentage of those patients receiving a transplant increased (61% to 80%), while the percentage of patients dying on the waiting list decreased (35% to 14.8%). The number of those too sick to transplant increased (1.9% to 4.7%).

Conclusion: In cystic fibrosis patients, rates of transplant, death, and too sick to transplant are comparable to those of other diagnoses. However, with the implementation of the Lung Allocation Score in 2005 there has been a significant increase in the percentage of patients receiving transplants and a significant decrease in those dying on the waiting list. This would indicate that in the first five years since implementation, the Lung Allocation score has improved waiting list outcomes for the majority of cystic fibrosis patients. The data showing more patients too sick to transplant may reflect an increase in late stage referrals, and earlier discussion of transplant options or referral for transplant evaluation may be indicated for certain patients.

Table- Waiting List Properties by Diagnosis 1988-2010

	Received Tx%	Died%	Too Sick%
CF	60	24	2.3
IPF	62	23	4.1
COPD	70	13	3.6
Sarcoidosis	50	27	4.5
IPAH	31	32	3.8

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ADOLESCENT CF LUNG TRANSPLANT RECIPIENTS HAVE DECREASED SURVIVAL WHEN COMPARED TO CF LUNG TRANSPLANT RECIPIENTS IN OTHER AGE GROUPS

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Bilateral lung transplant is a treatment for end-stage cystic fibrosis (CF) lung disease when there is no further treatment available. The aim of lung transplant is to improve patient life expectancy. CF is the leading indication for lung transplant in adolescents (12-17) accounting for 72% of all lung transplants in this age group. Survival for pediatric lung transplant is similar to that of the adult population. The half-life of adult recipients was 5.4 years compared with 5.5 years in pediatric recipients. We hypothesize that there is a difference in survival of patients with CF based on age at the time of bilateral lung transplantation.

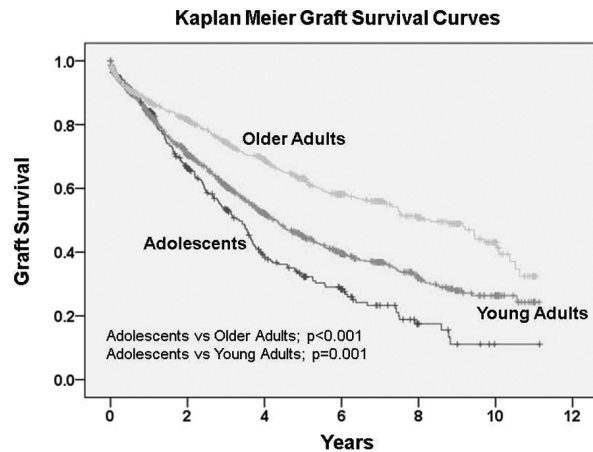
This study was a retrospective analysis of the United Network of Organ Sharing (UNOS) Database for all lung transplant recipients with CF performed in the United States from October 1999 to June 2011. Patients were grouped on the basis of age into 3 groups [adolescents (age 13-18), young adults (age 19-30), and older adults (age 31-50)]. The primary outcome of interest was graft survival.

There were 2,129 lung transplants performed in CF patients between the ages of 13-50 years including 303 (14%) in adolescents, 1063 (50%) in young adults, and 763 (36%) in older adults with follow-up data available for 99%. Adolescents were more likely to be female, have an increased donor weight to recipient weight ratio, have a lower creatinine level, have a shorter wait list time and donor ischemic time compared to young adults and older adults ($p < 0.05$ for all). Adolescents had significantly lower graft survival (median survival 3.3 years, 95% CI 2.8-3.8), compared to young adults (median graft survival 4.3 years, 95% CI 3.8-4.8) and older adults (median graft survival 8.3 years, 95% CI 4.5-5.4) ($p < 0.05$ for both). On multivariable analysis, controlling for donor and recipient characteristics, adolescent age was independently associated with worse graft survival compared to young adults (HR 1.3, 95% CI 1.1-1.6) and older adults (HR 2.2, 95% CI 1.8-2.7).

Adolescents comprise a minority of CF patients that undergo lung transplant in the United States and have the lowest overall survival. Older adults

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have graft survival that is over twice as long as adolescents. The decreased survival observed in adolescents is independent of some donor and recipient characteristics. Further study is needed to improve outcomes in this high risk group of patients.



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NUTRITIONAL SUPPORT AND SURVIVAL FOLLOWING LUNG TRANSPLANTATION IN CF PATIENTS

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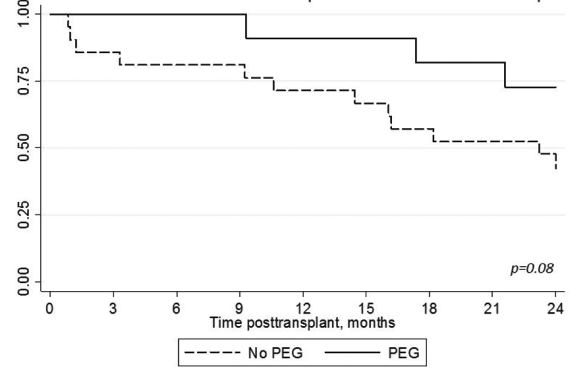
Background: Optimal nutritional support is essential in maintaining good outcomes in cystic fibrosis. The ideal approach to optimal nutritional support prior to lung transplantation in patients with cystic fibrosis remains debatable. Enteral nutritional support through initiation of a percutaneous endoscopic gastrostomy (PEG) tube is a known method to increase BMI. Our aim was to investigate if the presence of aggressive nutritional support as signified by the presence of a feeding tube was associated with decreases in mortality after lung transplantation.

Method: A single center retrospective analysis was performed on all patients with cystic fibrosis from approximately 9 different midwestern CF care centers who underwent lung transplantation at Loyola University Medical Center. BMI at the time of transplant, feeding tube prior to transplant, pancreatic sufficiency, and post-transplant survival were analyzed using Kaplan-Meier survival estimates and assessed with a log-rank value.

Result: Fifty CF patients who underwent lung transplantation were included in the cohort. Sixty-four percent (n=32) did not have a feeding tube at transplant and were categorized as "No PEG;" and 34% (n=17) patients had feeding tubes at transplant. Sixty-six percent (n=33) patients in the study had a BMI less than 21 at the time of transplantation, and 60% (n=20) did not have a PEG at the time of transplantation. Those without PEG had a survival disadvantage compared to those with feeding tubes at the time of transplantation.

Conclusion: The presence of aggressive enteral support via feeding tube affects post-transplant mortality, particularly if the patient's BMI is less than 21 at the time of transplantation. Those patients with non-optimal BMIs should consider feeding tube placement prior to transplantation.

Survival estimate BMI <21 and presence of PEG at transplant



Kaplan-Meier survival estimates comparing BMI under 21 and the presence of a feeding tube in CF patients at the time of lung transplantation.

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PRE-TRANSPLANT COLONIZATION AND POST-TRANSPLANT SURVIVAL IN CYSTIC FIBROSIS LUNG TRANSPLANT RECIPIENTS

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Background: Cystic fibrosis is the third most common indication for lung transplant and the leading indication for bilateral lung transplantation. Many of these patients are colonized with *Pseudomonas*, MRSA and other resistant organisms at the time of transplant. Many recent studies have shown an association between colonization with these bacteria and poorer outcomes. Currently, none have definitely shown a negative impact on overall survival with the exception of infection with resistant strains of *Burkholderia*.

Objective: To determine if colonization with any of these resistant bacteria pre-transplant leads to poorer post-transplant survival in the cystic fibrosis population.

Methods: Retrospective cohort analysis of adult (18 and older) cystic fibrosis patients who underwent lung transplantation at Loyola University Medical Center between 2000 and 2011. Colonization was defined as two positive respiratory cultures at least one month apart. Primary outcome measure was death. Survival analysis was done using the Kaplan-Meier method assessed with the Breslow test for equality of survivors.

Results: Pre-transplant culture data included patients colonized with methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, and *Achromobacter xylosoxidans*. Colonization with *A. xylosoxidans* was associated with the worst overall survival when compared to colonization with any other single organism. Colonization with MRSA or *A. xylosoxidans* was associated with poorer survival than colonization with PA, MSSA, or *A. fumigatus*. Survival in patients colonized with PA, MSSA, or *A. fumigatus* was worse when those patients were also colonized with MRSA. Patients colonized with PA had worse survival when co-colonized with either MRSA or *Achromobacter* vs. with MSSA or *A. fumigatus*. Colonization with MRSA was associated with poorer survival than colonization with MSSA.

Conclusions: Pre-transplant colonization with either MRSA or *A. xylosoxidans* is associated with poorer post-transplant survival than colonization with *P. aeruginosa*, MSSA, or *A. fumigatus*.

EPIDEMIOLOGY & NEWBORN SCREENING

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IMPROVING THE POSITIVE PREDICTIVE VALUE OF THE VERY HIGH IRT NO MUTATION REFERRALS IN THE NEW YORK STATE CYSTIC FIBROSIS NEWBORN SCREENING PROGRAM: ONE YEAR POST-ALGORITHM CHANGE

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Background: Cystic fibrosis newborn screening (CF NBS) in New York state (NYS) started in October 2002. The initial algorithm used an immunoreactive trypsinogen (IRT) assay. Samples with IRT in the top 5% were tested for mutations. Screen-positive (2 mutations, 1 mutation, very high IRT no mutation (VHIRT): IRT>170 ng/ml and the top 0.2% infants, after removal of IRT values from other referral categories) were referred for sweat testing. In 2010, we reported an extremely low positive predictive value (PPV) of 0.4% in the VHIRT group from 2002-2009 (Kier et al, 2010), with a predominance of Hispanic infants (43%). Due to the low PPV of the VHIRT group, discussions ensued to determine whether to eliminate this category, consistent with CF NBS practices in other states. Given that NYS has an ethnically diverse population, there was concern that a disproportionate number of affected, non-Caucasian infants would remain undiagnosed, as they tend to have rare CFTR mutations. In March 2010, the algorithm was modified in an effort to reduce the number of false positive screens and capture true positive, pancreatic insufficient cases. VHIRT cutoff was raised from 0.2% to 0.1%, and day of birth specimens (21% of VHIRT referrals) are now only referred if ≥1 mutation is detected; otherwise, a repeat specimen is requested.

Objective: Compare pre- and post-algorithm CF referrals and diagnoses from the VHIRT group.

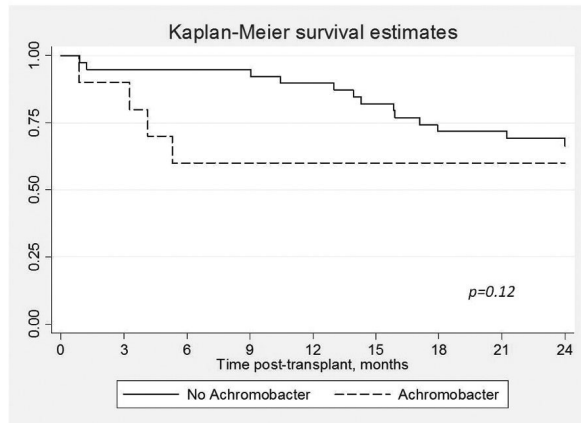
Methods: NBS records were reviewed for the VHIRT group between March 2003 and February 2010. A second cohort of records was reviewed between March 2010 and February 2011, following the new algorithm implementation. Fisher and chi-square tests were used as statistical analysis between the two cohorts.

Results: The number of screen positive infants (referrals) from the VHIRT group was significantly reduced and the PPV improved from 0.4% to 1.4% post-algorithm change. The total number of infants diagnosed with CF remained consistent. All three infants diagnosed with CF from the VHIRT group, post-algorithm change, were non-Caucasian race (identified as African-American, Asian and other), none were Hispanic.

Conclusions: After the algorithm change, VHIRT referrals decreased significantly (307%), and the PPV of the VHIRT group increased by more than three-fold, although it remains low (1.4%). The low yield for CF diagnoses in the VHIRT group necessitates careful and continued assessment of the algorithm, as all three infants diagnosed with CF were non-Caucasian.

Future Implications: Information from this study may be helpful in assessing the need for algorithm changes in other CF NBS programs. Analysis of mutations detected in these VHIRT infants may further inform future algorithm changes.

IRT Algorithm	Average Annual VHIRT Referrals (p<0.0001)	Average Annual Total Confirmed CF from all screen-positive groups (p=0.18)	VHIRT PPV (%), Confirmed CF (p=0.08)	VHIRT PPV (%), Possible CF and CRMS (p=0.03)
Initial (2003-2010)	776 (range 602-917)	45 (range 27-57)	0.4% (19/4378)	0.5% (22/4378)
Modified (2010-2011)	216	43	1.4% (3/216)	1.9% (4/216)



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COLONIZATION WITH *PSEUDOMONAS AERUGINOSA* FOLLOWING LUNG TRANSPLANTATION IN RECIPIENTS WITH CYSTIC FIBROSIS AND RISK OF DEVELOPING CHRONIC REJECTION

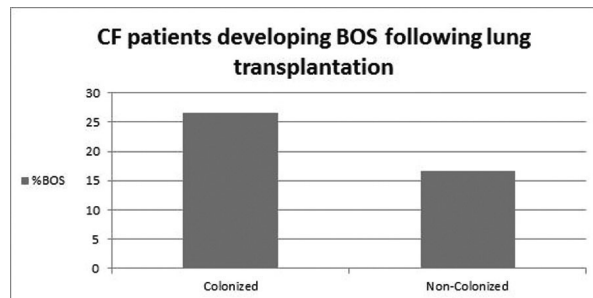
Bohle, R.J.; Mahoney, E.; Forsythe, S.; Lowery, E. *Loyola University Medical Center, Willowbrook, IL, USA*

Introduction/Rationale: Cystic fibrosis (CF) patients are commonly colonized with *Pseudomonas aeruginosa* before and after lung transplantation. *P. aeruginosa* is a chronic airway infection associated with reduced survival in CF patients. Approximately 73% of gram negative airway infections in CF patients after transplant are *P. aeruginosa*. Graft colonization with *P. aeruginosa* and its importance with respect to the development of chronic rejection, or bronchiolitis obliterans syndrome (BOS), is unclear, although we hypothesize that it may lead to an increased risk of developing BOS.

Methods: This is a retrospective cohort study evaluating all patients with cystic fibrosis who received lung transplants at Loyola University Medical Center from 2000-2010. All patients surviving a minimum of 4 months following lung transplant were included in the study. Colonization with *P. aeruginosa* was determined by evaluating bronchoalveolar lavage (BAL) cultures following transplantation, and patients were deemed colonized if 2 or more BAL cultures were positive for *P. aeruginosa*. Pearson's χ^2 was utilized to determine an Odds Ratio (OR).

Results: A total of 52 patients were included in the cohort. Re-colonization with *P. aeruginosa* following transplantation occurred in 65.4% (n=34) patients and 34.6% (n=18) did not become colonized with *P. aeruginosa*. A total of 26.5% (n=9) of the colonized patients developed BOS whereas 16.7% (n=3) of the non-colonized patients developed BOS (OR 1.8 [95% CI 0.42-7.7], p=0.507).

Conclusion: There was no difference in the development of BOS in the patients colonized with *P. aeruginosa*. This is despite concern that chronic airway infection may lead to an increased risk of developing BOS. Further investigation may show that continued prophylaxis for *P. aeruginosa* after lung transplant may be beneficial in assisting in clearance of *P. aeruginosa* from the airway following transplantation.



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CALIFORNIA CYSTIC FIBROSIS NEWBORN SCREENING: REVIEW OF THE FIRST 4 YEARS

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Objective: Evaluate the results from the first 4 years of cystic fibrosis (CF) newborn screening (NBS) in California (CA).

Methods: Starting 16Jul07 all infants born in CA were screened for CF using the CA model which has 4 steps: (1) measuring immunoreactive trypsinogen (IRT) levels in all newborn blood spot specimens, (2) specially designed CA (29-40) CFTR mutation panel testing of specimens with high IRT values (≥ 6 ng/mL, top 1.5%), (3) CFTR full gene sequence analysis of specimens found to have only 1 mutation in Step 2, and (4) referring infants with 2 or more mutations/variants to CF Care Centers (CFCs) for sweat chloride (SC) testing and follow up. Infants with only 1 mutation detected are not referred for sweat testing; their mothers are sent a letter describing the infant's carrier status and offered telephone genetic counseling. CFCs are asked to report all newly diagnosed CF cases not referred to them by the screening program (false negatives (FN)). Beginning 1Jul10, the program was expanded to include parent testing for families of Step 3 positive babies without a clinical diagnosis and without CFTR variants known to be in trans.

Results: Results will be updated to include at least one year of follow-up on all observations from the first full four years of screening. As of 5May12, 2,084,844 newborns were screened. In total, 830 newborns had positive CF NBS results: 169 had 2 mutations detected during Step 2 and 661 had 1 mutation detected during Step 2 and 1 or more additional CFTR mutations or variants detected during Step 3 (61 of these were novel). A total of 300 CF cases were successfully detected and 23 had FN screening results (n=11 Hispanic, n=9 White, n=1 Black, n=2 other race/ethnicity; case detection rate = 93%). Thirteen FNs had IRT levels below the cutoff, 7 FNs carried two CFTR mutations not on the CA panel, and 3 FNs had one panel mutation and one mutation not identified by DNA sequencing. The ratio of positive CF NBS infants to CF cases detected was 2.8:1. Fifty out of 300 CF cases were detected after an initial normal or borderline sweat test. CF prevalence was 1 in 6,455. CF cases identified at Step 2 were significantly younger at treatment initiation than CF cases identified by Step 3 (excluding meconium ileus (MI) cases, median ages: 25 vs. 53 days). From 1Jul10 to 1Jul11, 56 families had parent testing as part of the CA CF NBS program and six babies were found to have two mutations in cis.

Conclusions: In its first four years, the CA CF NBS model efficiently detected CF cases in a large, multi-ethnic population. The model uses comprehensive genotyping and continues to find novel CFTR mutations. With long term follow up and selective parent testing, the program has identified a range of CFTR-related disease in persons with two or more mutations and has acquired sufficient data to stop calling out some genotypes, like one panel mutation and (TG)11-5T, which are not disease causing.

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EARLY LIFE WEIGHT RECOVERY AND MAINTENANCE OF ADEQUATE GROWTH THROUGH AGE 6 ARE ASSOCIATED WITH BETTER PULMONARY STATUS AT AGE 12 IN CHILDREN WITH CF

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Background: We have previously shown that infants with CF who achieved catch-up weight gain and recovered their birth weight z-scores by age 2 years ("responders") have better lung function and chest x-ray scores (CXR) at age 6 years than infants who did not recover their birth weight z-scores by age 2. In addition, this early life weight recovery had a stronger effect on pulmonary status at age 6 than growth patterns during ages 2-6 (Pediatrics 2009;123:714). The present study examined whether responders experienced better pulmonary status at age 12, and whether growth patterns during ages 2-6 and 6-12 years had a significant effect on pulmonary status at age 12.

Methods: Children with CF who were pancreatic insufficient but did not have meconium ileus and were followed in the Wisconsin Randomized Clinical Trial of CF Newborn Screening through age 12 were studied. Responders were considered to maintain adequate growth through age 6 if their height and BMI z-scores at age 2 remained within one growth channel on the 2000 CDC growth charts through age 6. This criterion was also applied to responders during ages 6-12. Markers of lung disease severity included % predicted FEV₁ and CXR scores at age 12.

Results: Of 53 children followed through age 12, 68% were responders at age 2. Of these, 75% maintained adequate growth through age 6 and 47% maintained adequate growth through age 12. Responders had higher (96.5%), but not significant (p=0.12), mean FEV₁ at age 12 than non-responders (90.2%). Responders who maintained adequate growth through age 6 had better mean FEV₁ at age 12 (98.1%) than the remaining patients (93.2%), p=0.057. Additionally, responders who maintained adequate growth through age 12 years had better mean FEV₁ at age 12 (99.7%) than the remaining patients (92.0%), p=0.057. Subsequent analyses showed that the effects of the combined early life growth recovery and subsequent growth maintenance is mediated through a better FEV₁ at age 6 years, which was significantly associated with FEV₁ at age 12 years (p<0.001) and reduced the significance of the growth predictors (p-values became 0.1-0.2). Mean Wisconsin CXR scores at age 12 were better in responders than non-responders (17.1 vs. 22.7), in responders who maintained adequate growth through age 6 than the remaining patients (16.9 vs. 21.1) and in responders who maintained adequate growth through age 12 than the remaining patients (15.4 vs. 20.6). Similar trends were observed in Brasfield CXR scores. However, even though differences in CXR scores are large enough to be clinically meaningful, they did not reach statistical significance, p>0.13.

Conclusions: Our previous study demonstrated that weight recovery by age 2 is associated with significantly less severe lung disease at age 6. However, this association is lessened at age 12. In addition to recovering birth weight z-score by age 2, maintaining adequate growth through ages 6-12 and having a better FEV₁ at age 6 are needed to maximize lung function at age 12. (Supported by NIH-R01DK072126, R01DK034108 and CFF-SANDERS11A0).

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BRODY CHEST CT SCORES OF SEVERITY ARE ASSOCIATED WITH FEV₁ AND PULMONARY EXACERBATIONS 10 YEARS LATER

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Background: We have recently shown that Brody chest CT scores are significantly associated with PFTs and chest x-ray scores 5-7 years later, and more predictive of future lung disease severity than PFTs obtained at the time of the CT (AJRCCM 2011;184:816). Additionally, Loeve et al. (Chest 2011;140:178) have shown that bronchiectasis on chest CT is associated with the frequency of pulmonary exacerbations in the ensuing two years. In this study, we sought to (1) confirm our findings using data from participants of the Pulmozyme Early Intervention Trial (PEIT) in 1997-2000, and (2) determine if chest CT scores were associated with pulmonary exacerbations over the next 10 years.

Methods: Children ages 6-10 years with FVC $\geq 85\%$ predicted were enrolled in 1997-2000 into PEIT as described previously (J Pediatr 2001;139:813). For 64 subjects, chest CT scans were obtained during a period of clinical stability and scored using the Brody scoring system. Data from 1999-2009 for these patients was obtained from the CF Foundation Patient Registry. Multivariable linear regression was used to determine the association between Brody chest CT scores and FEV₁ (% predicted) in 1999 and the most recent FEV₁ and the number of pulmonary exacerbations between 1999 and 2009.

Results: The mean (SD) chest CT score was 3.8 (1.9), out of a possible 40.5. The mean (SD) bronchiectasis subscore was 0.6 (0.8) out of a possible 12. At the time of the chest CT, the mean (SD) age was 10.5 (1.7) years, the mean (SD) FEV₁ was 99.4 (13.9)% predicted, 39% were female, 50% were homozygous F508del, and 48% had cultures positive for *P. aeruginosa* (PA). Mean (SD) age at the most recent observation was 20.3 (1.8) years; mean (SD) FEV₁ was 78.3 (24.2)% predicted. The most recent FEV₁ and the number of pulmonary exacerbations between 1999 and 2009 were significantly

associated with overall Brody scores and bronchiectasis subscores (See Table). Comparing the strengths of association, Brody scores were more strongly associated with FEV₁ in 2009 and pulmonary exacerbations in 1999-2009 than FEV₁ in 1999 ($p < 0.01$ by F-test).

Conclusion: There is a significant association between overall chest CT scores and bronchiectasis subscores with FEV₁ and pulmonary exacerbation number up to 10 years later. This association is stronger than for FEV₁ obtained at the same time as the CT. This confirms and extends previous findings to indicate the predictive potential of chest CT scores in children with mild PFT abnormalities. (Supported by CFF-SANDERS11A0.)

Table. Regression estimates of group mean differences in most recent FEV₁ and the number of pulmonary exacerbations between 1999 and 2009 associated with a 1 point change in FEV₁ % predicted, overall Brody chest CT score, and bronchiectasis subscore in 1999

Lung disease measure	FEV ₁ % predicted in 1999		Overall chest CT score		Bronchiectasis subscore	
	Parameter estimate (SE)	P-value	Parameter estimate (SE)	P-value	Parameter estimate (SE)	P-value
FEV ₁ (% predicted) in 2009	0.55 (0.21)	0.01	-5.1 (1.5)	0.001	-8.8 (3.7)	0.02
Pulmonary exacerbation number, 1999-2009	-0.07 (0.12)	0.54	3.92 (0.83)	<0.001	5.85 (2.22)	0.01

Models adjust for PA, mucoid PA, gender, genotype, and FEV₁ in 1999

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CHILDREN AND YOUNG ADULTS IN THE US HAVE IMPROVED LUNG FUNCTION COMPARED TO THE UK
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Rationale: People with CF are managed in entirely different health care systems in the US and UK. National comparisons are now possible with the use of a unified data set created by CF Foundation and CF Trust researchers.

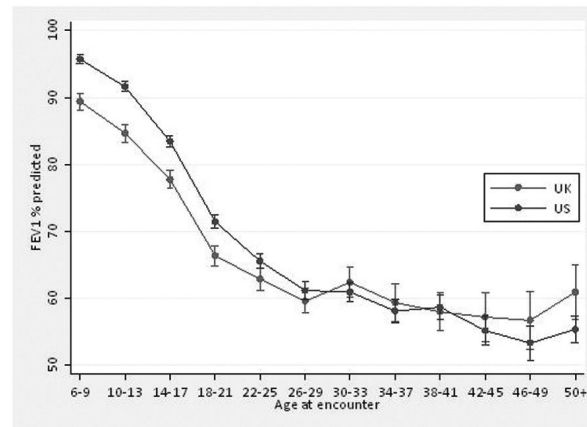
Objectives: To compare cross-sectional demographics and clinical parameters between US and UK CF patients.

Methods: This was a cross sectional study using 2010 data from patients in the US CF Foundation Patient Registry and the UK CF Trust registry. Using a random selection approach, US CF patient non-exacerbation encounters were matched with UK Trust registry patients. Data elements were unified with BMI and FEV₁ % of predicted^{1,2}. Descriptive statistics and two sample comparisons were performed. FEV₁ comparisons were limited to self identified Caucasian patients.

Main Results: The study cohort included 13777 children and 11058 adults from the US and 3984 children and 3949 adults from the UK. Median age of diagnosis was earlier in the UK (0.3 vs 0.4 years, $p < 0.001$). In children, mean BMI percentiles were similar except for male children ages 6-9 yrs, 10-13 yrs, and 14-17 yrs [US children 3.03 (95% CI's: 0.05, 6.01); 3.06 (0.23, 5.89) and 3.44 (0.52, 6.36) points higher, respectively]. The magnitude of this difference was small. In adults, median BMI was similar between the two populations except for women ages 34-37 yrs and 42-45 yrs [median difference 0.7 (95% CI: 0.1, 1.4), 1.1 (0.2, 2) higher in UK, respectively]. The magnitude of the lung function difference was significant (Figure 1) with US patients having higher FEV₁ % pred. through age 25 for both males and females. Hypertonic saline [US +32.5% (95% CI: 31.3%, 33.7%)] and dornase alfa [US +41.7% (41.1%, 43.4%)] use was much more common in the US children.

Conclusions: Children and young adults with CF in the US have better lung function than children and young adults in the UK despite similar nutritional status. The causes of these differences need further exploration including use of longitudinal data.

1. Wang X, et al. *Pediatr Pulmonol* 1993 Feb;15(2):75-88. 2. Hankinson JL, et al. *Am J Respir Crit Care Med*. 1999 Jan;159(1):179-87



FEV₁ % predicted by Age Category in 2010

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HIGH VARIABILITY IN FEV1 HELPS IDENTIFY CYSTIC FIBROSIS PATIENTS AT RISK OF GREATER LOSS OF LUNG FUNCTION

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Background: Unsurprisingly, a high rate of decline in lung function as measured by forced expiratory volume in 1 second (FEV1) is a predictor of subsequent loss of lung function in patients with cystic fibrosis (CF). VandenBranden et al. (*Pediatr Pulmonol* 2012; 47:135-143) showed that variability around the FEV1 trend line in adolescence independently predicted subsequent drop in FEV1. Our objective was to assess several measures of FEV1 variability as predictors of lung function decline.

Methods: Using data from the Epidemiologic Study of CF, we identified patients aged ≥6y from 1994-2002 with ≥4 FEV1 % predicted values spanning ≥366 days in a two calendar year baseline period. Individual regression lines were fit to the FEV1 % predicted values. The measures of variability were the median and maximum deviation from the best FEV1 in the period, and three measures of deviation around the fitted line: standard deviation and the median and maximum absolute deviations. Patients were divided into four lung disease stage groups (<40, 40-70, 70-100, ≥100 % predicted) by best FEV1 during baseline. Measures of FEV1 variability, FEV1 level, and FEV1 slope during baseline were used to predict the change from the best baseline FEV1 to the best in the subsequent two-year period using regression controlling for demographic and clinical factors. Patients contributed data for up to 5 periods.

Results: We identified 31,962 episodes from 13,907 patients. Across the four disease stages, median deviation from the best FEV1 added the most predictive power to the level and rate of FEV1 decline. Other measures of variability provided additional predictive power (except for patients with FEV1 < 40% pred), with median absolute deviation around the fitted regression line providing the largest increments (see Table).

Conclusion: Even after controlling for level and rate of decline in FEV1 and multiple patient characteristics, several aspects of variability in the baseline period add substantially to the ability to predict the change in the best FEV1 from one two-year period to the next. Median deviation from the best FEV1 during the baseline period is the best single predictor, but measures of the variability around the fitted trend line add additional explanatory power. When identifying patients at risk for a substantial decline in lung function, clinicians should consider not only the level and rate of change of FEV1 but also the amount of variability.

This study was supported by Genentech, Inc.

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Table

Variability Measure	FEV1 < 40% N=1765	FEV1 40-70% N=7048	FEV1 70-100% N=12,998	FEV1 100-150% N=10,151
	F stat (P value)	F stat (P value)	F stat (P value)	F stat (P value)
Median absolute deviation from line	1.5 (.23)	13.9 (<.001)	76.1 (<.001)	63.1 (<.001)
Maximum absolute deviation from line	7.1 (.008)	0.4 (.53)	0.9 (.35)	10.9 (.001)
Maximum deviation from best	2.1 (.15)	1.6 (.21)	1.0 (.33)	11.0 (<.001)
Standard deviation from line	2.3 (.13)	14.4 (<.001)	40.6 (<.001)	26.1 (<.001)

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RELATIONSHIP OF SOCIOECONOMIC STATUS AND ENVIRONMENTAL TOBACCO SMOKE EXPOSURE WITH DISEASE OUTCOMES IN THE EPIC OBSERVATIONAL COHORT

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Background: Socioeconomic status (SES) and environmental tobacco (ET) exposure are both strongly associated with disease outcomes in CF, and CF children from low SES families are disproportionately exposed to ET smoke (ETS). Our objective was to evaluate the degree to which the negative effect of SES on outcomes is mediated by ETS exposure in the well-characterized EPIC Observational cohort.

Methods: The ongoing EPIC observational study (EPIC OBS) enrolled *Pseudomonas*-negative CF patients <13 years of age between 2004- 2006. A survey completed by families at enrollment assessed pre- and postnatal tobacco exposure and SES. Outcomes 4 years after enrollment were extracted from study-specific encounter forms and the CFF National Patient Registry. Univariable and multivariable linear or logistic regression models with robust variance estimates were used to evaluate the association of SES, ET exposures at enrollment and outcomes 4 years later, adjusting for potential confounders (age, gender, race, ethnicity, CFTR genotype risk group, and diagnosis by screening).

Results: Of 1797 children enrolled in EPIC OBS, 1375 had follow-up data available at year 4. Mean age at enrollment was 5.7 years (SD 3.5). Maternal education was high school education or less in 28.1%; 26.8% had household income <\$40,000; 43.3% had Medicaid insurance. Prenatal tobacco exposure was reported in 13.7%; 24.3% had a mother who smoked at any time after birth (MSMK); 29.1% had a household member who smoked; 21.7% were exposed to at least 1 cigarette per day in the home during the week prior to enrollment; 17.5% were around people smoking in the 3 months prior to enrollment. ET exposure was more prevalent in low SES households: for example, 43% of children with household income <\$40,000 had a MSMK compared to 16% of children with higher household income. In independent models, both SES and ET exposure status were significantly associated with FEV1, weight percentile, body mass index, presence of crackles/wheeze, and occurrence of pulmonary exacerbations 4 years later. When different measures of ET exposure were added to a multivariable model of the association between SES (maternal education, household income, insurance status) and outcomes, the estimate of the effect of the SES variables changed only minimally but the effect of ET exposure was significantly reduced in most of the combined models.

Conclusion: ET exposure disproportionately affected low SES families in this large cohort of children with CF, and both lower SES and ET exposure had significant adverse effects on disease outcomes 4 years later, including FEV1, nutritional status, crackles/wheeze on exam and pulmonary exacerbations. The effect of SES on disease outcomes appears to be independent of ET exposure in the EPIC observational cohort. CFF EPIC09K0 (Rosenfeld).

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REDUCING QNS SWEAT TEST RATES IN OUR CF CENTER'S NEWBORN SCREEN POSITIVE BABIES

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Background: Illinois began CF newborn screening (CF NBS) in 2008, using the IRT (immunoreactive trypsinogen)/ DNA system. A CF NBS is reported as positive if the IRT value is in the top 4% IRT (high) with at least 1 DNA mutation (panel of 40), or if the IRT is > 170ng/mL (ultrahigh) in the absence of CFTR mutations. A baby with a + NBS is referred for sweat testing (ST). The CF Foundation (CFF) recommends < 10% QNS (quantity non sufficient) rate for sweat testing (ST) in infants <3 months old. Our center's QNS rates in NBS+ infants were 8% in 2008, 5% in 2009, 24% in 2010, and 23% in 2011. Our center's overall ST QNS rate in all ages remained at 3%. The purpose of our project was to reduce ST QNS rate to <10% in infants testing + for CF NBS. Our lab uses Wescor Macroduct method for sweat testing.

Methods: Our CF Center joined the Illinois NBS Quality Improvement (QI) Collaborative when it was formed in August 2010. We have focused on decreasing ST QNS rates. Our local CF NBS Team met regularly to address our QNS rate and we systematically addressed multiple hypotheses as to why our rate was high. Hypotheses grouped into four main areas: equipment factors, patient selection factors, staff training, and sweat collection technique. We confirmed with on-site consultation with Wescor that our equipment and techniques were good. We determined that babies with QNS ST did not differ significantly in age, weight, acuity of illness or reported skin color from babies who had sufficient sweat. We also determined that QNS ST did not localize to particular technicians. In November, 2011 and January 2012 we had site visits from Drs. S. McColley and V. LeGrys, who reviewed each step of our ST process. From November 2011, we began implementing small cycles of change that included use of warming blankets to swaddle during the collection; use of disposable stretch tape to secure both the stimulation gels and collection device to the infant's arm; discontinued the use of the inner thigh as a collection site; changed the position of the stimulating electrodes to the more distal volar forearm (near the wrist); and began collecting from each forearm on each patient.

Results: Our QNS rate dropped to 0% in December 2011 – February 2012.

Discussion: Dogged persistence by the lab team to investigate, trial suggestions, remain focused on the patient, and keep an open mind to possible critiques all led to what appears to be improvement in our QNS rate. The lab team felt that those processes that had the most impact on collection was the use of disposable stretch tape to secure the stimulation gel and the collection device, the use of the forearm rather than the thigh, and the positioning of the electrode toward the wrist instead of the elbow. We are monitoring QNS rates to verify these initial improved results.

This project was supported by a grant from the CF Foundation supporting the Illinois NBS QI Collaborative (MCCOLL11Q10).

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DETERMINISTIC LINKAGE BETWEEN CYSTIC FIBROSIS PATIENT REGISTRY DATA AND MEDICAID ANALYTICAL EXTRACT DATA

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Medicaid (MAX) data contain comprehensive reimbursed health services claims but lack clinical parameters. CF Patient Registry (CFPR) collects clinical information but provides quarterly updates on selected health services such as drug exposure. Because CFPR only collects limited non-unique direct patient identifiers, linking to other data sources presents a methodological challenge. We present a method illustrating the use of deterministic patient-matching algorithm to link CFPR and MAX.

MAX (at least 2-CF claims) and CFPR patients born between 01/01/1981 and 12/31/2006 were included. The following identifiers were examined for linking plausibility: date of birth, last four digits of social security number, zipcode, gender, date of sweat test, date of gene testing and date of hospital service by assessing the percentage of unique records in each dataset for each variable and percentage of uniqueness of various combination. Only variable combinations with a 99% level of uniqueness were considered for defining the deterministic rules; a total of nine linkage rules were established. We examined the linking performance of each rule as the proportion linked one-to-one to the registry and ascertained the validation parameters of these rules in the context of a selected gold standard (rule with the highest linkage performance) (see table).

We assessed 14,515 and 15,446 patient records in MAX and CFPR respectively. Linkage rule performance ranged from 1.4% (95% CI: 1.2–1.6) to 32.0% (31.3–32.8). As expected rules with lower linkage performance had fewer or no duplicate records. Using the selected gold standard, sensitivity of the other rules ranged from 4.3% (3.8–4.9) to 73.3% (72.0–74.6) with specificity of 88.2% (87.6–88.9) to 99.9% (99.8–99.9); the positive predictive value (PPV) ranged from 68.2% (62.6–73.4) to 99.0% (96.5–99.8).

The defined linkage rules exhibited varying operational characteristics suggesting that relying on multiple linkage rules may be necessary to optimize linkage performance.

We acknowledge the CF Foundation for the provision of CFPR data.

Linking rule performance, sensitivity, specificity and positive predictive value of linking rules

Rules	Linking rule performance (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)
R1*: Gender, DOB, SSN	32.04	-	-	-
R2: Gender, ZIP, SSN	24.14	73.34	88.27	97.35
R3: Gender, Sweat test date, SSN	1.42	4.39	99.98	99.03
R4: Gender, Genotype test date, SSN	1.46	4.41	99.93	96.70
R5: Gender, Hospitalization event date, SSN	14.14	43.02	99.48	97.51
R6: Gender, ZIP, DOB	31.47	73.34	88.27	74.67
R7: Gender, Sweat test date, DOB	2.43	4.32	98.47	57.10
R8: Gender, Genotype test date, DOB	2.06	4.39	99.04	68.23
R9: Gender, Hospitalization event date, DOB	18.56	42.89	92.91	74.05

*linkage rule with the highest linkage performance

Sensitivity: For a sensitivity of 73.3%; there are about 27% more records linked by R2 rule that are not linked by the gold standard rule.

Specificity: For a specificity of 88.3%; there is a 12% difference in the number of records not linked by the R2 and the gold standard rule.

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PREVENTION OF ACUTE KIDNEY INJURY IN HOSPITALIZED CHILDREN WITH CYSTIC FIBROSIS
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Introduction: Aminoglycosides (AGs) are commonly used to treat cystic fibrosis (CF) related lung infections. AGs are nephrotoxic and are an important risk factor for acute kidney injury (AKI) in hospitalized CF patients. In June 2009, a new clinical protocol was implemented to reduce the incidence of AKI by standardizing monitoring and AG treatment in all CF patients admitted to Children's of Alabama for pulmonary exacerbations. This protocol includes twice weekly monitoring of serum creatinines (SCr) and AGs pharmacokinetics.

Methods: A retrospective chart review was performed using data from the Children's of Alabama's Cystic Fibrosis Center database and hospital records for all admissions of CF patients with pulmonary exacerbations from July 2007 to April 2011. These data include demographics, co-morbidities, and serum creatinine. Hospitalized costs were obtained from Chil-

dren's of Alabama for cost analysis. AKI was defined according to the 2011 Kidney Disease Improving Global Outcomes (KDIGO) as a rise in SCr of 0.3 mg/dL or 50% rise from a baseline value. Data analysis was performed using SPSS® software. IRB approval for the study was obtained.

Results: The incidence of AKI was lower in the pre-protocol group 96/631 (15.2%) compared to the post-protocol group 113/475 (23.8%) (p<0.001). Children in the pre-protocol group had less SCr values performed than those in the post-protocol group (2.1 vs. 5.4; p<0.001). Length of stay for the two groups was similar. The median hospital cost was higher for AKI vs. no AKI in the pre-protocol (\$68,770 vs. \$61,551 p<0.05) as compared to the post-protocol AKI vs. no AKI (\$70,378 vs. \$80,816; p<0.05).

Conclusions: Despite protocols to decrease AG toxicity, the incidence of AKI in CF patients admitted for pulmonary exacerbations is high. Our protocol, though designed to reduce the rates of AKI in this population, has illuminated the problem and suggests that the incidence of AKI is higher than what is reported in the literature. Through increased screening with SCr, CF patients with AKI can be more readily identified, prevention of severe AKI can be avoided, and complications of AKI can be better managed. Studies are needed to find preventive strategies to reduce AKI, thereby decreasing morbidity, and to assess the cost-benefit ratio of such screening.

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NORMAL IRT IS A POSITIVE PROGNOSTIC FACTOR IN COMPOUND HETEROZYGOUS NEWBORNS CARRYING NON CF-CAUSING MUTATIONS
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CF carrier testing is nowadays offered to many couples, regardless of a negative CF family history. Such testing is not infrequently performed by sequencing all or most exons or by employing panels which include mutations of unclear clinical liability. Hence, carrier couples are occasionally detected where one or two partners carry a mutation whose potential for causing CF is unknown or unclear. It is virtually impossible to predict if their children carrying both the maternal and the paternal mutation will be affected by CF. Most of these children have intermediate or even negative borderline sweat chloride values at birth, and years of follow-up may be necessary to find out if they will develop clinical characteristics compatible with CF or a CFTR-related disorder.

Immunoreactive trypsinogen (IRT) levels are measured in all neonates undergoing CF newborn screening (NBS), which has now been almost universally adopted in Western countries. Elevated IRT suggests with good sensitivity and modest specificity potential CF or a CFTR-related metabolic syndrome.

In Northeastern Italy both NBS and carrier testing for the general population have been performed for several years, and some newborns carrying two mutations of which at least one is not clearly labelled as CF-causing have been detected and followed-up.

The aim of this study was comparing longitudinal data on sweat chloride and cultures from pharyngeal suction in 47 compound heterozygous children with at least one mutation not acknowledged as CF-causing and either raised IRT at birth (IRTpos group, 34 individuals) or IRT below the 99.5th centile cutoff (IRTneg group, 13 individuals). Children with more than one sweat chloride measurement were considered, and the mean sweat chloride was calculated for each individual. The median age at the first observation was lower in IRTpositives (0.1 yrs, range 0.1-0.3) than in IRTnegatives (0.2 yrs, range 0.1-0.4), p=0.01. The children were usually seen every 6 months, and the median follow-up was 2.3 yrs, range 0.2-24.2 in the IRTneg group and 2.7 yrs, range 0.6-6.3 in the IRTpos group.

The mean sweat chloride value was 31.0 mmol/L (SD 13) in IRTpos and 15.6 mmol/L (SD 6) in IRTneg children, p<0.01. In the first 6 months of age, the mean chloride value was 27.7 mmol/L (SD 12) in IRTpositives and 14.1 mmol/L (SD 5) in IRTnegatives, p<0.01. Three patients in the IRTpos and none in the IRTneg group had at least one chloride value above 60 mmol/L. In the IRTpos group 21% had at least one culture positive for *Pseudomonas aeruginosa*, none in the IRTneg group (p n.s.). *Staphylococcus aureus* was

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detected in at least one culture in 68% of the IRTpositives and 77% of the IRTnegatives (p n.s.).

These preliminary results suggest that the clinical evolution of heterozygous newborns carrying at least one non CF-causing mutation is mild or even normal if IRT at birth is not raised. These newborns may need a looser follow-up than those with elevated IRT.

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SOCIOECONOMIC STATUS AND USE OF CF CARRIER SCREENING

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In northeastern Italy, CF neonatal screening has been performed for many years, and is regularly used to monitor CF birth prevalence. A significant progressive decrease of CF birth rates has been recorded in a sub-area where a CF Carrier Screening (CS) programme has been implemented (CS area), but not where CS is not in place (NO CS area). In the CS area a negative correlation has been found between CF incidence and the number of carrier tests performed, suggesting that CF CS may be connected with a downward birth prevalence trend.

The CS strategy used private specialists, mainly gynaecologists, to offer the test to individuals of reproductive age. It could be speculated that economically disadvantaged people, living in more deprived areas, are less likely to access expensive private practice, and therefore to be offered the CF carrier test.

This study aims at evaluating if CS based on genetic test offered through private practice may over time select a poorer population of CF patients.

CF neonates born in the 2002-2010 period were attributed a socioeconomic status (SES) score according to their parents' residence at the time of the child's birth. The score distribution was divided into four SES groups, identified at the 20th, 50th and the 80th percentiles. The four SES groups ranged from SES 1-affluent to SES 4-deprived.

CF incidence was significantly lower in the CS area than in the NO CS area for the overall sample (1.5 vs. 2.1 per 10,000 neonates, respectively) and for births in the more affluent municipalities (0.9 vs. 2.4 per 10,000 neonates, respectively). No significant differences were recorded between the CS and the NO CS area for SES 2, SES 3, SES 4 (table).

When the SES Index was used as a continuous variable, a quadratic effect was shown only for the CS area, where more affluent and more deprived municipalities had lower incidence rates.

These results suggest that prospective parents from affluent social backgrounds, with higher socioeconomic status, are more likely to be reached by a CS offer based on private practice. If confirmed, these conclusions may have implications for the planning of CF CS strategies.

	NO CS area	CS area	NO CS / CS areas difference
SES 1	2.448 (1.399-3.975)	0.924 (0.339-2.011)	1.524 (0.115;2.933) p<0.05
SES 2	1.818 (0.968-3.108)	1.733 (0.970-2.858)	0.085 (-1.236;1.406)
SES 3	2.003 (0.916-3.803)	1.927 (1.177-2.976)	0.076 (-1.481;1.633)
SES 4	1.764 (0.214-6.371)	0.910 (0.334-1.981)	0.854 (-1.697;3.405)
All	2.071	1.463	0.608 (0.046;1.262) p<0.05

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PATTERNS OF HEALTH CARE UTILIZATION IN CHILDREN WITH CYSTIC FIBROSIS ENROLLED IN A STATE PROGRAM FOR CHILDREN WITH SPECIAL HEALTH CARE NEEDS

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Background: Guidelines for the care of patients with cystic fibrosis (CF) are available. These start with a strong recommendation for quarterly multidisciplinary visits at specialized centers. Despite this, children with CF are not being seen at CF care centers as frequently. California has a Title V program, California Children's Services (CCS), to provide coverage for essential health care services to children with special health care needs

whose family income is below a financial threshold. We aim to describe and investigate the patterns of outpatient care and the impact on morbidity, using hospitalization as a surrogate, in patients with CF enrolled in CCS.

Hypothesis: Patients with CF who do not utilize outpatient care at the recommended frequency are more likely to be hospitalized for pulmonary exacerbation than patients who are seen regularly.

Methods: This is a retrospective cohort study of the CCS claims data including outpatient clinic visits, hospitalizations, and outpatient medications for fiscal year 2007 through 2009. Cases were identified by CCS eligibility diagnosis for cystic fibrosis by ICD-9 code, 277.0x. Clinic visits were defined as an outpatient encounter with a pediatric or adult pulmonary provider. Hospitalizations were defined by ICD-9 codes for respiratory disease or symptoms.

Results: During the 3-year study period, 774 children with CF were enrolled in the CCS program. The patients were mainly Hispanic (41%) and white non-Hispanic (33.5%). Eighteen percent of patients had ≤ 1 clinic visits/year and 60% had ≥ 4 clinic visits/year; 48% of the patients were hospitalized at least once during the study period, and 15.8% of patients were hospitalized more than 3 times. The children who were not hospitalized had 3.3 (± 2.9) clinic visits per year and children who were hospitalized more than 3 times had 7.3 (± 5.7) clinic visits per year ($p < 0.0001$). Among those who were hospitalized > 3 times, 90% were seen in pulmonary clinic ≥ 4 times a year. For children who had more than 1 hospitalization during the study period, 41% were re-hospitalized within 90 days of the index hospitalization.

Conclusion: Despite enrollment in CCS, 40% of children were not seen at the frequency recommended by the CF care guidelines. Refuting our hypothesis, children who were hospitalized frequently also had high utilization of outpatient clinic visits. A significant proportion of re-hospitalizations occurred within 3 months of the prior hospitalization. These findings may reflect underlying disease severity as opposed to lack of access to care. Future studies will account for disease severity as a confounder for health care utilization.

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CHANGES IN DEMOGRAPHICS AND OUTCOME VARIABLES - NORTHERN IRELAND ADULT CF PATIENTS 2000 - 2011

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Life expectancy in CF has increased over the last four decades, and based on data from the UK CF registry, median predicted survival is continuing to improve. It is likely that improved survival in CF is associated with an improvement in other outcome variables in CF, such as lung function and nutritional status, and has also resulted in a change in demographics of the CF population.

We used data from the UK CF registry to analyse how demographics and outcome variables including BMI and FEV1 % predicted have changed for adult CF patients in NI between 2000 and 2011. Age and gender were obtained using end of year survey data. A search strategy was applied to obtain annual review BMI and FEV1 % predicted values for patients attending the adult CF clinic between 2000 and 2011. Median values were calculated and the Mann Whitney test was used to compare 2000 and 2011 values.

There are approximately 240 patients currently attending the adult CF clinic in NI. In 2011, 198 (83%) had data uploaded to the registry. In total, 265 patients had data submitted at least once between 2000 and 2011; 237 (89.4%) of these were represented in 2 or more years during this time period. Median age has increased from 26.13 in 2000 to 29.12 in 2011 ($p = 0.004$); this may be a result of improved survival and increased diagnosis of older patients. There was a greater proportion of male patients in all years except 2001; this is consistent with previous studies and most likely reflects higher median survival in males with CF.

Median FEV1 % predicted was not different between 2000 and 2011 values ($p = 0.9$). In this context, values may have been influenced by a decline in the FEV1% of patients who are represented in multiple years. It is also possible that greater improvements in FEV1 % predicted occurred

prior to 2000 and the values have now plateaued. BMI increased, with median values rising from 21.3 in 2000 to 22.8 in 2011 ($p = 0.001$). This may be due to more aggressive nutritional input e.g. pancreatic enzyme use and supplementary feeding, or perhaps a general increase in BMI in the general population.

In conclusion, over the last decade it appears that the adult population with CF in NI has increased in age; this has implications for service provision. BMI has increased, while FEV1 % predicted has not shown any significant change.

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FALSE POSITIVE DIAGNOSIS OF CYSTIC FIBROSIS – AN UNDERREPORTED PROBLEM

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Objectives: False-positive cystic fibrosis (CF) diagnoses are rare and has been reported by single centre reports from UK and US, mainly before 1990. We investigated patients with false-positive diagnoses of CF in Germany based on the German registry, and compared patients diagnosed before and after 1990.

Methods: We analysed data from the German CF quality assurance project (Data set 01.06.2003) and rechecked the data with CF-centre directors and compared the diagnostic criteria with the consensus statement (Rosenstein 1998).

Data: Between 1989 and 2004, CF diagnoses were withdrawn in 51 patients after 5.79 years (median) (range 0.41 -24.96 years), whose diagnosis was based on nonspecific symptoms between 1979 and 2001 at the median age of 4.33 years (range 0.07-52.94 years). CF transmembrane conductance regulator (CFTR) dysfunction was indicated by unreliable sweat tests (45.1%), pathologic sweat chloride (37.3%), genetic tests (3.9%), and nasal potential difference measurements (13.1%). Patients diagnosed after 1990 were older (6.13 vs 1.21 years), more frequently fulfilled the diagnostic criteria (77.4% vs 20%) and experienced respiratory symptoms (83.8% vs 50%).

Conclusions: To detect CF patients with false-positive diagnoses, CF-centres should re-evaluate patients with atypical courses and new or transferred patients. The commitment to standardization and continuous quality control for all diagnostic tests according to the guidelines could help to minimize these tragic cases.

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PREDICTORS OF PARENTAL KNOWLEDGE AND PATIENT OUTCOMES IN PATIENTS DIAGNOSED BY NEWBORN SCREENING

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Introduction: Despite adoption of newborn screening (NBS) for cystic fibrosis, clinical outcomes remain diverse. Parental education is an important component of the CF NBS program. Our center has developed a 10-point knowledge test to identify areas of follow-up educational emphasis. We have also developed a 10 point clinical score to quantify the CF health of the NBS population. This study seeks to identify predictors of parental knowledge and the effect of parental understanding on CF clinical outcomes.

Hypothesis: Increased CF knowledge is associated with improved CF clinical score at six months of age.

Methods: The Children's of Alabama/University of Alabama (COA/UAB) pediatric CF center has an open IRB protocol to collect socioeconomic status, parental knowledge and clinical data on infants diagnosed by NBS. From the clinical data collected on these infants, we developed a 10 point clinical score to combine assessment of nutrition (weight for length %), microbiology (acquisition and eradication of PA) and respiratory symptoms (cough, pulmonary exacerbations, and hospitalization) into one clinical score (higher score indicative of milder disease). We utilized a 10 item true/false and multiple choice questionnaire to assess parental knowledge of CF (higher score indicative of increased knowledge). Analysis of the corre-

lation between knowledge score and clinical score utilized Pearson's r calculation. Dichotomous comparison of clinical outcomes in high vs. low knowledge patients utilized t-test analysis. Statistical significance was assumed at $p \leq .05$.

Results: Twenty parents of the 32 CF NBS infants partook in the knowledge assessment. Patient age at testing was at $6.2 \pm .7$ months. On average, parents answered 8/10 questions correctly ($81.3\% \pm 2.4\%$) with a range of 58-100%. Maternal education was modestly correlated with test performance ($r = .51$, $p < .05$). Other measures of socioeconomic status such as paternal education, income level or insurance status were not associated with the test score. Measures of clinical interaction such as the number of clinic visits, previous hospitalizations, parent age at testing or an older sibling with CF did not alter the knowledge score. Higher caregiver knowledge was not associated with improved clinical score, either by correlation analysis (slight inverse correlation, $r = -0.44$, $p < .05$), or dichotomous comparison (Above average knowledge: 7.3/10; Below average knowledge: 8.4/10, $p = 0.14$).

Discussion: Assessments of parental knowledge can provide opportunity to continue CF education. Parental understanding of CF disease can be difficult to predict, with only maternal level of education having a modest correlation with knowledge score. Similarly, clinical outcome can be difficult to predict, with above average parental understanding of CF disease not associated with improved clinical score at age six months. To improve upon these initial measures, we will utilize the knowledge assessment to address areas of improvement in CF education and the 10-point clinical score to track clinical outcomes. Future longitudinal studies will assess whether improving CF knowledge alters clinical outcomes.

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IMPROVING SWEAT TEST QNS RATES FOR INFANTS WITH POSITIVE CF NBS IN ILLINOIS

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Submitted on behalf of the Illinois Newborn Screening Consortium.

Illinois initiated newborn screening in 2008 using the IRT/DNA method. The Illinois Newborn Screening Program tracks a variety of metrics, including sweat test quantity not sufficient (QNS) rates. Due to consistently high QNS rates, a consortium of 15 programs in IL and MO that are responsible for provision of sweat testing and related care for babies born in IL was formed in August 2010. Our specific aim for this project was to reduce the proportion of QNS sweat tests to $< 10\%$ at all CF NBS follow-up programs.

Methods: We used a number of strategies to improve sweat testing:

- 1) Monthly conference calls, held at two different times most months, were used to review published sweat testing best practices, review quarterly QNS results, and to discuss progress and offer advice about problems at individual sites.
- 2) An email Listserv was created and is maintained by the Illinois Department of Public Health to allow easy communication and dissemination of information to all collaborative members.
- 3) One consortium site (Peoria, IL, Center Director) had a site visit from Wescor and shared a detailed list of ways to reduce QNS when using the Wescor Macroduct™ collection system.
- 4) Two consortium sites were visited by sweat testing expert Dr. Vicky LeGrys, whose recommendations were disseminated to all sites via the Listserv.

Results: Overall, there has been improvement in sweat test QNS rates. As a state, we improved, and have achieved acceptable QNS rates of $< 10\%$ for one year. The last six quarter QNS rates are: 10/10-12/10 14%; 01/11-03/11 23%; 04/11-06/11 9%; 07/11-09/11 9%; 10/11-12/11 7%; 01/12-03/12 9%.

Several sites have had marked reductions in their QNS rates. Individual site improvement is noted in QNS rate ranges in the Table.

Conclusion: Collaborative learning utilizing a variety of methodologies, including sharing results of expert program review, can improve sweat test rates at the state level. Ongoing work, and monitoring, is required to assure that all sites meet the national benchmark for QNS rates.

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Table: QNS rates at individual NBS sites

QNS range	Number of sites 1st quarter 2011	Number of sites 2nd quarter 2012
≤10%	N=5	N=8
11-15%	N=0	N=5
>15%	N=10	N=2

Among the sites that had <10% QNS range at the start of the project, no site has increased its QNS rate to >10%.

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A STUDY OF SWEAT TESTING USING THE CF QUANTUM® TEST SYSTEM

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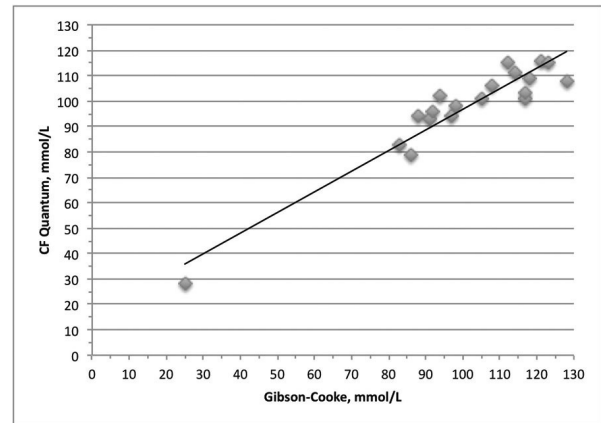
Background: The approved methods for sweat testing are Gibson-Cooke pilocarpine iontophoresis (GCQPIT) or the Wescor Macroduct® Sweat Test system. There are many steps in these methods that can and do lead to errors. With the universal adoption of newborn screening, there are approximately 10,000 infants per year in the United States who require sweat testing. The GCQPIT and Macroduct® methods can result in quantity not sufficient sweat test samples exceeding 20% and delays in diagnosis. Thus, there is a critical need to improve sweat testing technology.

Methods: The CF Quantum® Sweat Test System consists of: a) a controller and electrode set that is worn on the arm for pilocarpine iontophoresis; b) a chloride test patch that collects the sample. Chloride ions in the sweat sample come into contact with silver chromate in the test patch, and an ion exchange reaction occurs which creates silver chloride, an insoluble white precipitate formed in the center of the patch; c) a CF Analyzer utilizes a camera and proprietary software that scans the reacted chloride test patch and calculates the concentration of chloride and volume of sweat collected. Herein are preliminary results of a multicenter study (final subject number will be 170) in which patients have concurrent sweat tests performed.

Results: Thus far after successful instrument and method development, ten subjects (nine with CF and one infant with a positive newborn screen) had bilateral GCQPIT and CF Quantum® Sweat tests. There was excellent agreement of sweat chloride values between the GCQPIT and CF Quantum® Sweat tests with a Pearson correlation coefficient of $r=0.949$. The sweat collection time for the CF Quantum® sweat test was a mean of 8.5 minutes (range: 3 to 19 minutes) and the sweat rates ranged from 1.1 to 8.8 gm/m²/min. In a post-test questionnaire administered to the subjects (or parents), all of the subjects preferred the CF Quantum® sweat test over the GCQPIT.

Conclusions: The impressive results of this study in progress are that the CF Quantum® Sweat test yields sweat chloride values that are equivalent to that of GCQPIT sweat testing. The CF Quantum® Sweat test has a much faster collection and analysis of sweat compared to GCQPIT or Macroduct®, has an easier procedure to analyze sweat chloride, and is preferred by patients/parents. Our goal is for one-half of the subjects (85) to complete concurrent sweat tests during the next 6 months.

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COLORADO NEWBORN SCREENING QUALITY IMPROVEMENT PROGRAM: IMPROVING SWEAT TEST PERFORMANCE

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Background: The need for quality improvement (QI) in sweat testing for newborn screening programs has been recognized. Quantity not sufficient (QNS) rates for sweat collection are a particularly vexing problem for newborn screening programs. A ten percent QNS rate has been established as a target for sweat testing in infants < 3 months of age. In the year preceding this QI project, our QNS rate for sweat tests in infants < 3 months of age was approximately 20%. This provides rationale for our proposed QI program to improve sweat test performance in infants.

Objective: To improve sweat test performance through application of QI approaches.

Methods: We are applying known QI techniques to our local sweat test process in order to decrease QNS rates. These techniques consist of understanding our CF Center and sweat testing microsystem (with the help of an external consultant, Dr. Vicky LeGrys), establishment of an oversight committee with regular meetings and goals, review of existing standard operating procedures and techniques, utilization of Plan-Do-Study-Act improvement models, and ongoing tracking of data with mechanisms in place to amend protocols as needed in order to sustain improvement gains. Our primary outcome measure is QNS rate over time in infants < 3 months of age.

Results: The *Plan* phase began with the establishment of a sweat test oversight committee comprised of key CF and laboratory personnel. This committee reviewed national guidelines, and identified inconsistencies in our local sweat testing protocols and processes. Dr. LeGrys, a world renowned expert in sweat testing, completed a site visit to our institution and comprehensively evaluated our sweat testing microsystem and processes. This feedback was used to guide the *Do* phase in which we have incorporated changes in our sweat testing procedures to exactly match national standards. During this phase, we implemented changes in sweat result reporting in our electronic medical record, decreased the number of staff performing sweat collection, implemented infection control procedures for sweat collection, developed a sweat testing data collection form to examine possible risk factors associated with QNS rates, and developed a burn protocol for sweat collection. The *Study* phase has begun with monthly monitoring by the sweat test oversight committee of the QNS rates and other key metrics. Through the first four months of 2012, our QNS rate in infants < 3 month of age is 8% (1/13 QNS samples).

Conclusions: We are successfully applying known QI techniques to improve sweat test performance at a large tertiary pediatric hospital. Preliminary results indicate a reduction in QNS rates in infants < 3 month of age.

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A COMPARATIVE ANALYSIS OF NEW YORK STATE CF NEWBORN SCREENING MUTATION PANEL AND CFF REGISTRY DATA

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Background: Cystic fibrosis newborn screening (NBS) began in New York state (NYS) in 2002. The current NYS algorithm includes molecular testing for a panel of 40 mutations on specimens with elevated immunoreactive trypsinogen (IRT) levels (top 5% daily). Infants are referred for sweat testing if they carry 1 or 2 panel mutations or have very high IRT (VHIRT; top 0.1%) and no panel mutations.

Purpose: To evaluate the utility of the current mutation panel.

Methods: Mutations detected and their associated allele frequencies from 3 data sources were compared: 1) mutations reported to the US CF Foundation (CFF) Patient Registry for patients born in NYS (2002-2010); 2) mutation and race/ethnicity data from the NYS NBS database; 3) the 2010 CFF Patient Registry Annual Report.

Results: Genotype data was reported to the CFF registry for 402/422 (95%) infants with confirmed CF born in NYS (2002-2010). *Panel Mutations:* delF508 and 31 other mutations currently on the NYS newborn screening panel accounted for 606/804 (75.4%) of reported alleles. Remaining panel mutations may have been categorized under other or unknown, or may not have been detected. DelF508 was the most common mutation, noted in 425/804 alleles (52.9%). This is substantially lower than the reported national delF508 allele frequency of 70%. While the majority of CF patients were White (79.6%), 11.8% were Hispanic, 3.8% were Other/Unknown, 3.2% were Black, and 1.6% were Asian. *Non-Panel Mutations:* 67 alleles not on the current NYS mutation panel were reported to the registry. The most common were the IVS8-5T variant (N=8, 1%) and the benign polymorphism M470V (N=7, 0.87%). The NBS program only tests IVS8-5T as a reflex in R117H carriers; it is not reported in isolation. Excluding IVS8-5T and M470V, the most common non-panel mutation reported to the registry was L206W (N=5, 0.62%), described in the literature as a variable expression mutation. Among the remaining reported non-panel mutations, only one (1154insTC, 3 alleles, 0.37%) was found in more than 2 alleles, 12 were found in 2 alleles (0.25%, collectively, 3.0%), and 20 were reported in one allele (0.12%, collectively, 2.5%). A total of 131/804 (16.3%) of CF alleles were reported as "other" or "unknown."

Mutations in CFF<u> Registry	Proportion of CF Alleles<u>
NYS NBS Panel Mutations	Total: 75.4%
delF508	52.9% (425/804)
additional 31 of 40 mutations	22.5% (181/804)
Non-Panel Mutations (36)	8.3% (67/804)
"Other"	7.5% (60/804)
"Unknown"	8.8% (71/804)

Conclusions: The NYS CF population differs from the national dataset in terms of delF508 frequency. This may be due in part to the NYS ethnic composition. The NYS screening panel's overall detection rate of 75.4% is consistent with other reported CF screening panel detection rates. Most of the non-panel mutations reported to the CFF registry were rare. Further investigation of the reported non-panel mutations is warranted.

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INCIDENCE OF INITIAL PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS PATIENTS IN THE UNITED STATES: A SEASONAL AND CLIMATIC PERSPECTIVE

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Background: *Pseudomonas aeruginosa* (Pa) isolates from initial infection in cystic fibrosis (CF) patients often exhibit phenotypic characteristics resembling those of environmental isolates, suggesting that the majority of

initial acquisition is from the environment. The purpose of this study was to evaluate whether season and climate zone were associated with initial Pa acquisition in young U.S. CF patients.

Methods: We conducted a retrospective cohort study of initial Pa acquisition in children with CF born between 2002 and 2009 and diagnosed prior to age two, using respiratory culture data (generally oropharyngeal) from the Cystic Fibrosis Foundation National Patient Registry. Incidence rates (initial acquisitions / number at risk for initial acquisition) were calculated for season, defined as: spring (March-May), summer (June-August), fall (September-November), and winter (December-February); as well as for climate zones, as defined by the Koppen Climate Classification (dry, temperate or continental). Incidence rate ratios (IRR) were used to compare initial Pa acquisition between seasons, considering winter as the reference season; and between climate zones, considering temperate climate as the reference. Additionally, we evaluated the potential interaction between season and climate.

Results: A total of 5,050 subjects were included in the cohort, of which 2,689 (53%) acquired Pa over the study period. The overall Pa incidence rate was 22.2/100 person-years and the median age of acquisition was 1.3 years. The incidence rate in winter was 1.5/100 person-months and 1.5, 2.1, 2.2/100 person-months for spring, summer, and fall respectively. Compared to winter, a significantly higher Pa incidence was observed in summer (IRR: 1.44; 95% CI: 1.28-1.62) and fall (IRR: 1.50; 95% CI: 1.33-1.68), while no difference was observed for spring (IRR: 1.07; 95% CI: 0.97-1.20). A significant difference in Pa incidence rates between climate zones was not detected.

A different pattern of seasonal acquisition was observed within each climate zone. In the temperate and continental climate zone, initial Pa acquisition rates were significantly higher in the summer and fall compared to winter. No difference in seasonal acquisition was observed within the dry climate zone.

Conclusion: In this U.S. national study, differential rates of initial Pa acquisition in young CF patients were observed by season, in addition, these seasonal patterns differed by climate zone. The results of this study, are concordant with the recent study of Collaco, et al¹, and suggest that environmental factors, including geography and climate, may be associated with Pa acquisition. Future studies should seek to evaluate the role of these factors, while considering spatial and temporal relationships, in initial Pa acquisition.

Reference: ¹Collaco JM, McGready J, Green DM, et al. (2011) Effect of temperature on cystic fibrosis lung disease and infections: a replicated cohort study. PLoS ONE 6(11).

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CYSTIC FIBROSIS NEWBORN SCREENING IN ITALY: EDUCATIONAL ASPECT

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Background: Since the inception of cystic fibrosis (CF) newborn screening (NBS) programs there has been concern about adverse psychosocial events. Negative consequences have been attributed generally to insufficient information about the screening process and confusion about test results. The intensity of parents' initial reactions seemed to be compounded by their level of knowledge regarding NBS and CF. Every newborn screening program has in the model of communication/information the most critical moment which can profoundly affect its results.

The Cystic Fibrosis Foundation and European CF Society have issued directives for the implementation of CF newborn screening programs. In Italy, although mandatory since 1992 CFNBS was implemented in different years (1992-2009) and there are different regional approaches and programs.

Aim: Describe the state of information about CFNBS in Italy.

Materials and Methods: Within an audit study on CFNBS in Italy we collected data on educational programs in 15/15 labs and 16/16 CF care centres involved in CFNBS.

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Results: Information before screening: only 9/15 regions inform parents by written educational materials (brochure) that are distributed in nurseries immediately before (3/15 regions) and immediately after delivery (6/15 regions). Eight of 15 regions organize regular meetings for health staff with different intervals (ranging from 1 meeting a year to 1 meeting every 2-3 years). Four of 15 regions organize training session on CFNBS for expectant parents. No other educational materials are distributed.

Information after screening: Primary care professional (PCP) is not informed about screening results by labs and no labs provide educational material about CFNBS to PCP.

Information post-diagnosis: Twelve of 15 CF care centres provide a brochure about CF to parents; five of 16 in case of CFTR metabolic syndrome (CRMS). Four of 16 CF centres provide written information about CF and three of 16 about CRMS to PCP.

Conclusion: In regions where newborn screening is active there is still a lack of information in the community, but also among health professionals who interact directly or indirectly in the newborn screening process.

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LOW PREVALENCE OF CHRONIC *PSEUDOMONAS AERUGINOSA* INFECTION IN CYSTIC FIBROSIS PATIENTS DIAGNOSED BY NEWBORN SCREENING (NBS) AND TREATED WITH EARLY ERADICATION TREATMENT: 19 YEARS OF EXPERIENCE

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Background: NBS for cystic fibrosis (CF) has been carried out on all newborns in the region of Tuscany since 1986 and up to now. Since chronic lung infection by *P. aeruginosa* (PA) is an unfavorable prognosis for CF patients, the Regional CF Center of Tuscany introduced in 1993 the practice of early eradication treatment in all patients with initial PA infection. The aim of the present study is to evaluate the current prevalence of chronic PA infection in patients diagnosed by NBS who are regularly followed up at the Tuscan CF Center since 1993.

Methods: All CF patients diagnosed by screening (IRT/meconium lactase/IRT or IRT/DNA/IRT) were confirmed with the sweat test. All patients underwent quarterly microbiological follow-up and were segregated according to their microbiological status. Samples were taken using pharyngeal swab in patients who could not produce expectorant and with sputum culture in the others. Patients attended the CF Center at least every three months, according to the European CF Society standards. Attendance at the CF Center and age of first PA infection were analyzed by reviewing patients' clinical charts.

PA eradication was carried out using inhalatory antibiotics (colimycin or tobramycin) associated with oral ciprofloxacin. The current microbiological status of the patients was evaluated by using the Leeds criteria on cultures made in the last year (from January 2011-January 2012).

Results: Since 1993 up to now, 130 patients have been diagnosed with CF according to NBS (the incidence of the disease in Tuscany is 1:3704). Of these 130 patients, 112 (86%) (44 females, 68 males) diagnosed by NBS have had regular follow-up (3 have died and 15 were lost to regular follow-up). The mean age of these patients is 8.09 ± 1.4 yrs (median 7.2 yrs). Twenty-seven (24%) of these patients have never been colonized with PA, 85 (76%) have been colonized and treated with early eradication treatment. The mean age $\pm$ SD of these patients at first PA infection was 8.8 ± 12.3 years.

During the last year a mean of 5 clinical check-ups per patient and a mean of 4.4 ± 1.47 cultures per patient have been carried out. Ten (8.9%) of 112 patients are chronically infected by PA, 27 (24.1%) have intermittent colonization, while 75 (67%) are currently free of PA infection.

Conclusions: NBS is an opportunity to prevent progressive lung disease in CF patients through microbiological monitoring of PA acquisition. Microbiological monitoring from time of diagnosis and the administration of early eradication treatment for initial PA infection provides the most opportune management of the disease. In our long experience we have been able to rapidly identify patients with initial PA infection and early eradica-

tion treatment has maintained a low (8.9%) prevalence of chronic infection in our patients who have been diagnosed by NBS.

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GENDER DIFFERENCES IN THE UK CF POPULATION

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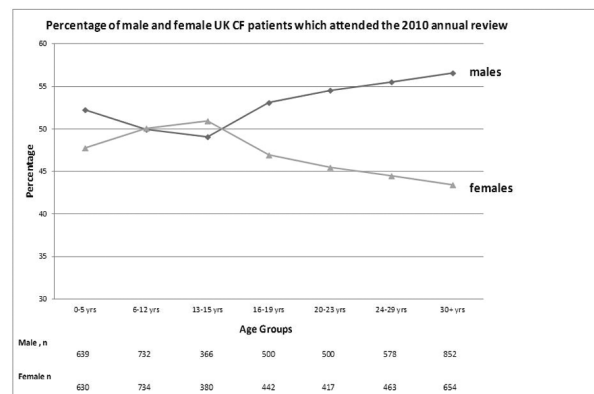
The literature is conflicting with regards to gender differences in the CF population with some studies reporting poorer prognosis in CF females. Variations in study design (cross-sectional vs longitudinal and single vs multi-centre), study populations (paediatric vs adults) and small sample sizes may in part explain these differences. In an attempt to overcome some of these limitations we analysed the UK CF Trust Registry database, which collates data on the paediatric and adult UK CF population for evidence of gender differences.

Methods: We used the 2010 annual review data and compared males and females in age-groups 0-5, 6-12, 13-15, 16-19, 20-23, 24-29 and 30+ years for lung function (FEV1 and FVC (% predicted)), infection status, treatments (rhDNase, O₂ therapy, Non-Invasive Ventilation (NIV), IV antibiotics, supplementary feeding and pancreatic supplements), body mass index and associated complications (CF related diabetes, osteoporosis, liver disease).

Results: Of the 7937 patients attending annual review in 2010 47% were female. The sex distribution differed by age whereby the proportion of females dropped from 16 years onwards (Figure 1). The mean FEV1 was significantly ($p < 0.001$) lower in female teens aged 16-19 years compared to males (females: 71.0 (confidence interval 68.8 - 73.2), males 76.7 (74.6 - 78.8), but not in any other age group. Similar results were observed for mean FVC for which females aged 20-23yrs also had significantly lower values than males. Teenage females also had a significantly ($p < 0.05$) higher use of rhDNase, gastrostomies, O₂, had more days/yr on IV antibiotics in hospital and had a significantly ($p \leq 0.001$) higher rate of CFRD. In addition, a significantly ($p < 0.05$) higher percentage of females older than 13 had more home IV antibiotics. We did not detect any gender differences with respect to chronic bacterial colonisation (PA, SA, *B. cepacia* and MRSA).

Conclusions: There is evidence of a more severe lung function deficit in teenage years. The reduced proportion of females in the adult cohorts is consistent with a survival difference. Further longitudinal analyses will help characterise the identified gender gap and effects on disease progression.

We thank the UK CF Trust for the registry data.



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MECONIUM ILEUS PREVALENCE SCORE AS NEW PHENOTYPIC MARKER FOR THE SEVERITY OF CFTR MUTATIONS

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Introduction: We calculated meconium ileus prevalence scores (MIP) for the most frequent CFTR mutations as the ratio between CF patients with MI and all CF patients (MI and no MI). Initial analysis focused on patients carrying F508del on one allele allowing each mutation on the second allele to be assigned a MIP score.

Methods: MIP scores were established by chart review using a Canadian cohort (representing 70% of the Canadian CF population; n=2492) sampled for ongoing modifier studies in CF. MIP scores were compared to those derived from other registries including the US CF Foundation (n=43,432), Italy (Veneto/Trentino/Alto Adige) (n=1,786) and Germany (n=3,596). The pancreas insufficient prevalence (PIP) score was calculated based on chart review of the same Canadian cohort (Ooi et al. Gastroenterol 2011).

Results: The overall prevalence of MI (16%) in the Canadian population was similar to that observed in other registries 17% (US), 10.4% (Italy), 20% (Germany). The different mutations showed variable MIP scores. Those with high PIP scores (high prevalence of “pancreas insufficiency”) showed a wide range of MIP scores (0.06 to 0.28), whereas mutations with low PIP scores (low prevalence of “pancreas insufficiency”) showed a MIP score of zero. Notably the MIP score for G85E with intermediate PIP score was higher than that observed for mutations with lower PIP scores. MIP scores for each mutation in the other registries were remarkably similar to those observed in the Canadian database.

Conclusion: Preliminary results demonstrate considerable variation of MIP score among different CFTR mutations following the same trend as the corresponding PIP scores.

MIP scores may help to more accurately predict the genotype/phenotype correlation of CFTR mutations, and thus the gene-dose effect of a specific CFTR mutation. This knowledge is essential in studies investigating the role of modifier genes in CF.

CFTR mutation # with F508del / x	MIP score (# of patients with specific mutation)				PIP score
	Canada (2,117)	US (27,494)	Italy (860)	Germany (3,438)	
G542X	0.28 (72)	0.20 (928)	0.14 (43)	0.24 (80)	0.99
W1282X	0.23 (13)	0.24 (417)	na	0.10 (20)	1.0
F508del	0.18 (1,265)	0.19 (14,644)	0.14 (263)	0.16 (1,888)	0.99
621+IG>T	0.17 (88)	0.17 (354)	na	na	1.0
G551D	0.06 (53)	0.06 (939)	na	0.04 (85)	0.96
G85E	0.04 (24)	0.09 (133)	0.00 (15)	na	0.50
2789+5G>A	0.00 (18)	0.01 (265)	0.00 (22)	na	0.28
3849+10kbC>T	0.00 (14)	0.01 (296)	0.00 (24)	0.00 (42)	0.07

na: too few patients (<10) to calculate MIP score

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IMPROVED QUANTITY NOT SUFFICIENT (QNS) SWEAT CHLORIDE TEST RATES THROUGH QUALITY IMPROVEMENT INITIATIVES

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Objective: Reliable sweat chloride testing is essential for an efficient diagnosis of CF. High QNS rates delay diagnosis and initiation of therapies for patients with CF and result in increased anxiety for families. Quality improvement programs at this CF Center were begun as a joint effort between respiratory therapy and the CF pediatric program providers to decrease the QNS rate for the Center.

Methods: Data were collected for QNS rates for both the greater than 3 month-old as well as less than or equal to 3 month-old age groups starting July 2008. Information was solicited from other CF Centers regarding their sweat chloride programs—procedures, systems used, QNS rates—and compared to our Center's practices. A mock sweat chloride site visit was performed in 2009 by the CF Center Director, and problems within our system were identified and addressed. Best practice goals and guidelines were set for our Center and adherence to these guidelines and competence were tracked regularly in 2010. Outside review of sweat chloride testing process in 2010 was performed to increase our progress towards the goal of < 5% QNS, and recommendations were implemented.

Results: Sweat chloride rate for fiscal year (FY) 08-09 was 6.17% (5.12% > 3 months). In FY09-10 when the initial changes were implemented, rate was 6.73% (4.22% > 3 months). In FY10-11 with stricter adherence to the Center's guidelines, the rate was 4.34% (3.05% > 3 months). These rates are absolute rates and have not been modified based on patient status at the time of testing. FY11-12 data are being collected currently.

Conclusions: By setting internal standards for our Center's sweat chloride testing and tracking outcomes as well as adherence to these standards, the Center's sweat chloride overall QNS rates have decreased by approximately 30% from FY08-09 to FY10-11. Continuing to focus on improvement in QNS rates, now with a focus on the < 3 month-old age group will be an essential part of our Center's mission. Ongoing review of patients to determine the best timing for testing in an individual patient is in progress. As we continue to track these data, identifying characteristics which may predict an individual patient's ability to provide an adequate sweat sample will allow for better utilization of the sweat chloride lab resources and less frustration for the families and patients.

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PANCREATIC ENZYME REPLACEMENT THERAPY IN CHILDREN WITH CYSTIC FIBROSIS: A TALE OF TWO MODELS

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Background: The impact of nutrition on outcomes in children with cystic fibrosis (CF) is well known. Little published data demonstrates the role of pancreatic enzyme dosing. The CF Foundation Patient Registry allows for such an analysis. Criticisms of large database studies, particularly at the patient level, include selection and information bias and confounding by indication. Novel approaches, such as center level analysis and risk adjustment, are necessary to overcome such biases. The impact of risk adjustment on such models, however, is not known.

Purpose: To compare the outcome of a large epidemiologic study on the role of enzyme dosing on nutritional status in children with CF using a patient level risk adjusted and non-risk adjusted model.

Methods: A retrospective analysis of the CF Foundation Patient Registry from 2005 to 2008 of patients 2 to 20 years old taking enzyme supplementation was performed. Centers with fewer than 10 patients were excluded. Center level BMI-percentile performance quartiles were established according to the center level mean BMI percentile from 2008, calculated

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from each patient's best BMI percentile that year. Descriptive analysis was performed at the individual level for all patients within their respective center's BMI performance quartile. Continuous variables were compared between the top and bottom performing quartiles with a nested covariance model. Multivariable analysis with repeated fixed effect of years nested within patients within centers was used to predict the effect of enzyme dose on the presence in the top or bottom quartile. The model was recreated using CFF risk adjusted patient level BMI data, resulting in new BMI performance quartile definitions.

Results: Higher enzyme dose was associated with higher BMI performance quartile in the unadjusted model. Despite little intraquartile movement of centers using risk adjusted data, higher enzyme dose was no longer associated with higher BMI percentile quartile after risk adjustment.

Conclusion: Using risk adjusted and non-adjusted patient level data may result in significantly different conclusions in large scale epidemiologic outcome studies. In this study, enzyme dose was no longer associated with better BMI after risk adjustment. Further investigation is needed to understand the role of such adjustments and how they apply to patient level management decisions.

Year	Lowest Quartile Center Enzyme Dose (lipase units/kg/meal)	Highest Quartile Center Enzyme Dose (lipase units/kg/meal)	p-Value (Wilcoxon Rank Sum)
2005	1571	1686	0.287
2006	1546	1682	0.042
2007	1539	1668	0.036
2008	1603	1699	0.059
Year	Risk Adjusted Lowest Quartile Center Enzyme Dose (lipase units/kg/meal)	Risk Adjusted Highest Quartile Center Enzyme Dose (lipase units/kg/meal)	p-Value (Wilcoxon Rank Sum)
2005	1653	1697	0.639
2006	1627	1678	0.537
2007	1636	1660	0.569
2008	1700	1727	0.713

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FEASIBLE STATISTICAL MONITORING TO IDENTIFY ONSET OF RAPID FEV1 DECLINE AND FACILITATE CYSTIC FIBROSIS CARE DELIVERY

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Objective: To identify rapid disease progression in cystic fibrosis (CF) through statistical implementation and determine the clinical utility of this approach.

Methods: We conducted a retrospective longitudinal cohort study using over 150 CF patients receiving care at Cincinnati Children's Hospital Medical Center (CCHMC). Patients less than 6 years of age or with no pulmonary function testing data were excluded; longitudinal data obtained after organ transplantation were excluded. Patient-specific FEV1% slopes were calculated over two-year increments and measured as % predicted/year. Data management and statistical analyses were conducted using SAS 9.2 and R 12.2.

Results: The monitoring results were further refined based on chart conference meetings at CCHMC. The example output in the accompanying figure has been used during CCHMC CF Chart Conferences to discuss each patient's health outcomes and make individualized treatment decisions. This is a plot of FEV1% over time (age and calendar year) for an individual CCHMC CF patient with 2-year, overlapping linear slopes used to depict intermittent rate of decline. Black dots represent actual FEV1% values from clinical visits. Red dots represent FEV1% values from hospital visits due to exacerbations. Solid lines represent overlapping, two-year, linear regression slopes based on the clinical data only. The blue open squares represent extrapolated values based on the patient's last two-year slope. The green open triangles represent extrapolated values based on an improvement of 5% predicted in the patient's last two-year slope.

Conclusions: Biostatistics approaches may be tailored to advance care in CF. This pilot study feasibly incorporated existing sources of biomedical data into everyday clinical practice and provided a standardized approach for monitoring the clinical course in this chronic disease.

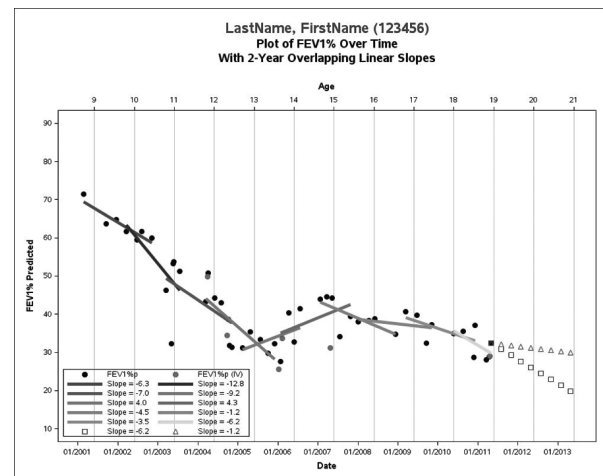


Figure: Lung function decline for CCHMC CF Patient

QUALITY IMPROVEMENT

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ELECTRONIC REMINDERS TO ADDRESS ADHERENCE: AN EVIDENCE BASED PROJECT

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Objective: Describe an evidence based project that utilized recent literature regarding the impact of electronic reminders, such as text messages, on adherence to outpatient appointment completion and/or behavior change such as medication adherence to formulate a best practice recommendation.

Method: Utilizing the Iowa Model of evidence based practice, which provides a framework for the development of Evidence-Based Practice in a clinical agency, the following clinical question was developed: Among pediatric patients and families, does the use of electronic reminders such as text messaging versus standard care (no electronic reminders) improve adherence to taking medication and attending outpatient appointments? All articles were critically appraised utilizing the evaluation system developed at Cincinnati Children's Hospital Medical Center known as LEGEND ("Let Evidence Guide Every New Decision").

Conclusions: A total of twelve relevant articles contributed to a high grade of evidence creating a strong recommendation that electronic reminders be offered to reduce no-show rates in the outpatient clinic setting as well as to positively impact behavior change and improve treatment adherence. Social workers and other members of the CF team can advocate that electronic reminders for appointments be offered to their CF patients and families, and can connect those interested in daily medication reminders with applicable resources such as www.CFFone.com and www.mymedschedule.com

Implementation: As a part of a quality improvement project, an anonymous survey was given to all pulmonary patients in February 2012. Of the 53 CF patients and their families who participated, 44.3% preferred text message appointment reminders over phone calls, email, or postal mail. However, only 7.5% of CF patients and families were interested in daily medication reminders versus 14.2% of the total population, suggesting that daily medication reminders may be more desirable in the non-CF pulmonary population.

450★

PRELIMINARY RESULTS ON THE EFFICACY OF AN ONLINE SOCIAL NETWORK FOR ADOLESCENTS WITH CF: AGE AND DISEASE SEVERITY GROUP COMPARISONS

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Objective: Adolescents with CF have significant challenges, including disease progression, decreased adherence to medical regimens, and transitions toward greater independence. Given recent infection control guidelines, they are not permitted to meet face-to-face to obtain support from same-illness peers. This study evaluated the effects of an online intervention, CFfone, on perceived social support, knowledge of disease management, anxiety and depression, and health-related quality of life (HRQOL) over a 6-month period.

Methods: Participants included 88 adolescents and young adults with CF (63% females), randomly assigned based on age (age mean = 15.95 years) and pulmonary function (FEV1% predicted mean = 80.28%) to either: 1) CFfone (Intervention) or 2) an educational website for patients with CF (Control). Participants completed the following measures at baseline and 2 additional assessments occurring every 3 months post-intervention: 1) Perceived Adolescent Social Support Cystic Fibrosis (PASS-CF); 2) Knowledge of Disease Management (General Health, Lung Health, Nutrition, Treatments); 3) Hospital Anxiety and Depression Scale (HADS; Anxiety and Depression); and 4) Cystic Fibrosis Questionnaire-Revised (CFQ-R; Treatment Burden, Social Functioning, Respiratory Symptoms).

Results: Preliminary results compared outcomes between the two groups (CFfone Intervention vs Control) across 3 assessment points. Compared to the Controls, Intervention participants reported significantly more friends with CF ($F(85) = 4.18, p < .05$) and fewer depressive symptoms ($F(86) = 4.19, p < .05$). Number of friends without CF decreased similarly in both groups ($F(22) = 4.31, p < .05$) from baseline to the second assessment; however by the third assessment, number of friends without CF increased for the Intervention but not the Control group. A significant interaction between assessment visits and treatment condition was found for the Nutrition Knowledge scale ($F(22) = 4.06, p < .05$), with the Intervention group scoring higher over time on this scale than the Control group. However, by the third assessment, the Control group also improved. The Intervention group also demonstrated significant improvements from second to third assessment in Social Functioning on the CFQ-R; this improvement was not found in the Control group.

Conclusions: Preliminary findings suggested that CFfone may be a promising intervention, with benefits in perceived support, knowledge, depressive symptoms and HRQOL. Future directions include examining longer term effects of the intervention with the full sample. Subsequent analyses will also examine the impact of CFfone on adherence.

451★

TWO YEARS' EXPERIENCE WITH A PATIENT PORTAL FOR CF PATIENTS AND FAMILIES

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Purpose: Patient portals are being suggested as a tool to enhance communication between patients and the medical team and to allow patients additional ways to access their medical record. In patients with chronic diseases, it is hoped that patient portals will facilitate adherence and enhance outcomes. *My Nemours* is a patient portal that is available to parents/patients enrolled in the Nemours health system. This free, online application allows participants to schedule/change appointments, request medication refills, ask non-urgent medical questions and view selected portions of the medical record (i.e. staff reviewed lab results). Patient portals have not been evaluated in the management of CF. Although many families in our CF care center

use *My Nemours* regularly to actively participate in their child's care, it appears that many do not. Our primary aim is to assess parents' satisfaction with the *My Nemours* website to determine ways to improve access to and meaningful use of this tool. The second aim of this quality initiative is to improve the *My Nemours* site by sharing information obtained from this focused population, with the central *My Nemours* team to potentially benefit all patients enrolled in similar programs.

Methods: The *My Nemours* program has been available to our clinic population for approximately 2 years. An IRB approved 15 item-questionnaire, including items to assess enrollment in *My Nemours*, mode of interaction, portions of program accessed, ease of utilization, and satisfaction with the site was distributed to families over a 3 month period during quarterly CF clinic visits.

Results: Fifty-four (53 parents, 1 adult patient) out of 60 individuals surveyed returned a completed questionnaire. Patients who returned questionnaires have been cared for at our CFF-accredited care center for a range of 6 months to 17 years. Seventy-two percent of participants are enrolled in the *My Nemours* program obtaining access via computer, smartphone or notebook. Of those who do not access *My Nemours*, 86% are aware of the program but choose not to participate. Of those who use *My Nemours*, 51% have a college degree or higher versus 33% for those who do not use it. Families who access *My Nemours* agree or strongly agree that information available from the site was comforting (64%), easy to use (61%), easy to understand and was useful (both 54%). Those who use the program disagree or strongly disagree that the information contained on the site is confusing (64%) or frightening (61%). All respondents that use *My Nemours* indicated that they feel the site is helpful. About 50% of the respondents that use the website indicate that they have used the site for 1 year or longer.

Conclusions: Families appreciate another means of access to their child's medical record and care team. Information accessed by families enrolled in the *My Nemours* program is felt to be helpful rather than confusing or frightening. This program provides an alternative and complementary mode of communication with families to facilitate care of their children. Additionally it provides opportunities for education regarding overall care and also ongoing outreach to those who are not using the tool.

452★

CYBER CF A TELEHEALTH ADOLESCENT SUPPORT GROUP

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Background: Cystic fibrosis (CF) is a life limiting, genetic disorder that affects approximately 30,000 people in the U.S. Adolescence can be an especially difficult time for individuals with CF. Teens with cystic fibrosis are medically counseled not to be in the same room with each other in order to prevent cross contamination with life threatening, opportunistic infections. Yet, many youngsters wish to interact with others who also experience this disease.

Objective: The objective of this group intervention is to remedy the isolation of teens with CF; to provide for them a vehicle to communicate with each other in real time; and to provide emotional support and health education.

Methods: A support group for adolescents with CF using Telehealth equipment was started in 2009 by the University of New Mexico (UNM) cystic fibrosis team in conjunction with a UNM child psychiatrist. The "Cyber CF Support Group" is composed of teenagers from a variety of sites in New Mexico – one teen per site. The teens meet via tele-video communications for 45 minutes every two weeks with the assistance of a group facilitator, the UNM CF team social worker. To achieve a successful outcome, support staff (nurse or social worker) are provided at each site; parents of the teens are encouraged to become informed about the nature of the group; and technical challenges are overcome. A satisfaction survey was developed to assess contentment with the program as well as to draw out attitudes and concerns.

Results: The Cyber CF Support Group has been in practice for over two years. It reaches into multiple communities, urban and rural, giving teens the opportunity to express themselves and develop relationships with peers. The themes that emerge from group discussions have provided insight regarding emotional needs and physical concerns of young people with CF.

Conclusion: For teenagers with cystic fibrosis a "cyber group" can provide a venue to meet, exchange ideas, and share concerns while seeing and

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talking to each other in real time. It has the capacity to dispel isolation and cement friendships. This unique group has the potential to be replicated in other regions for teens and young adults with CF as well as for those with other medical conditions.

453★

UTILIZATION OF QUALITY IMPROVEMENT INITIATIVES TO INCREASE ADHERENCE WITH ROUTINE CLINIC APPOINTMENTS FOR PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Routine care is paramount for maintenance of health for patients with cystic fibrosis. As part of the CF Foundation's recommendations for maintenance of care, quarterly visits at an accredited CF center are recommended for all patients. Despite this recommendation, compliance with quarterly visits remains one of the lowest achieved recommended metrics tracked by the CF Foundation. In 2010, the national average for completion of quarterly visits (with 4 cultures and 2 PFTs) was 28.1% for patients ≥ 18 years of age. The Drexel Adult CF Center has traditionally achieved compliance with this metric significantly below the national average. In the year preceding this Quality Improvement Initiative, our center had 16.1% of eligible adults attain this goal. The purpose of this project was to utilize strategies developed as part of the AQI2 Program to increase patient adherence with this valuable metric.

Methods: Training in quality improvement techniques was provided to a core team of care providers at the Drexel Adult CF Center along with members from 12 other accredited adult CF centers from around the country. As part of the training, education developed from the Dartmouth Institute for Health Policy and Clinical Practice was provided in the form of webinars, discussion groups, mentored team meetings, and face-to-face collaboratives with involved centers. The Drexel Lead Team used brainstorming techniques and fishbone diagrams to identify perceived obstacles to adherence with routine clinic appointments. From this list, team voting was used to identify the most significant issues facing our patients. In conjunction with patient and family representatives, educational programs were developed to ascertain CF-specific knowledge bases of our patients in order to determine areas of deficiency to target educational programs. It was the consensus belief of the Lead Team that by engaging patients through education, we would be able to positively affect patient adherence to regular follow-up.

Results: Our center's compliance with routine quarterly visits for the calendar year 2009 was 16.5% ($n=16/97$). In the calendar year 2010, compliance increased to 26.1% ($n=24/92$). In the calendar year 2011, 72% of our patients ($n=48/67$) achieved quarterly visits as recommended by the CF Foundation. During the Quality Improvement Initiative, our center was able to increase the compliance with recommendations by 275%.

Discussion: Through utilization of quality improvement techniques, our adult CF center was able to dramatically increase adherence to quarterly clinic visit to a percentage in line with the top 10 performing centers. The global aim of the project was to improve both pulmonary and nutritional outcomes for our patients without cystic fibrosis. By increasing patient visits, we believe that subsequent improvement in our global aim is possible.

Funding: This project was supported by the Cystic Fibrosis Foundation's Adult Quality Initiative 2 Program.

454★

IMPROVING RAPID FEV1 DECLINE THROUGH QUALITY IMPROVEMENT

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Background: Almost a quarter of children with CF have a year-to-year FEV1 decline of 7% or greater. A unique clinical approach is needed to treat patients with rapid FEV1 decline.

Methods: An algorithm for how to assess and treat patients with rapid FEV1 decline was designed by a local quality improvement team. The algorithm included empiric antibiotic therapy, initiation of evidence-based pulmonary therapies, respiratory cultures obtained by sputum or BAL, increased clinic visit frequency, and assessment of CF regimen adherence and airway clearance techniques. All FEV1 values not associated with pulmonary exacerbations from our CF database from 2010-2011 were used to calculate 2-year regression slopes for FEV1 for each patient in our CF Center.

Results: In the first quarter of 2012, eight patients with a calculated FEV1 decline of 9% per year or greater were treated with the FEV1 decline algorithm. All patients received antibiotics (six IV and two oral). Two patients had new infections identified with *Pseudomonas*. After the first quarter of the project, three patients had an improvement in their FEV1 of 10% or greater, four patients had stable lung function without further decline, and one patient had continued FEV1 decline. The average FEV1 improved by 5.8%. The average 2-year FEV1 slope on regression improved from -10.6% to -8.0%. The average 2-year peak-to-peak FEV1 slope improved from -11.4% to -7.2%. Seven out of eight patients recovered to within 10% of their best FEV1 in the prior 12 months, and five out of eight patients recovered to within 5% of their best FEV1 in the prior 12 months.

Conclusions: A systematic center-level approach to identify and treat patients with rapid FEV1 decline in CF results in improved pulmonary outcomes.

455★

EVALUATION OF RISK OF SLEEP DISORDERED BREATHING IN ADULT PATIENTS WITH CYSTIC FIBROSIS

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Background: Patients with end stage cystic fibrosis are known to have decreased oxygen saturations at night and increased risk of hypoventilation. The use of bilevel positive pressure support has been shown to improve oxygen saturation, decrease pCO₂, and improve alveolar ventilation. When transitioning to an electronic medical record, our practice became less diligent about screening adult patients with cystic fibrosis for sleep related breathing disorders. In the 2 year period following our transition to an electronic medical record, we identified that only 21% of adults with baseline FEV1<40% had been evaluated for sleep disordered breathing. However, of those patients 79% had an indication for initiating treatment with bilevel positive pressure ventilation. We set in place a quality improvement initiative to ensure that patients at increased risk of nocturnal hypoventilation would be appropriately screened.

Methods: Patients were identified as high risk if they had an FEV1<50% in the CF Registry Database on two separate occasions within the last year. Patients were screened at a routine cystic fibrosis clinic visit for increased risk of sleep disordered breathing using the Epworth Sleepiness Scale (ESS) to assess level of sleepiness. They were also asked to complete a brief questionnaire to target symptoms associated with sleep disordered breathing. This included questions about snoring/gasping at night, AM headaches and sleep disruptions. Patients admitted to the hospital during this time frame were screened while in house. All patients with daytime oxygen saturation of 94% or less and all patients with an ESS 11 or greater were referred for formal polysomnogram (PSG). Other patients were reviewed by a physician certified in Sleep Medicine and a determination was made regarding their need for full PSG with monitoring of end-tidal CO₂.

Results: Review of our adult population identified 75 patients at risk for sleep disordered breathing. After 25 weeks of targeting the at risk patients in clinic or during hospital stays, 92% of the target population had been screened. Of the patients screened, 43% were recommended to have a PSG completed. Fifty-nine percent of those patients had completed a PSG within 11 months of beginning the targeted intervention. Overall, within 11 months of beginning the intervention, 26% of our target population were on treatment with either supplemental oxygen or noninvasive bilevel positive pressure ventilation as a result of the screening.

Summary: Using a brief, targeted survey that was directed toward patients at high risk for sleep disordered breathing, we were able to identify patients in need of nocturnal support and implement therapy. This method

allowed us to capture a large number of patients within a relatively short time and be sure that adequate therapy was being provided.

456★

THE CYSTIC FIBROSIS FREQUENT FLYER PROGRAMME: A PILOT 12 MONTH QUALITY IMPROVEMENT INITIATIVE FOCUSED ON INTENSIVE OUTPATIENT PHYSIOTHERAPY, EXERCISE AND DIETETIC SUPPORT

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Background: Children with moderate to severe CF require frequent hospital admissions for intensive prophylactic IV antibiotic treatment or as a response to acute events. During admissions, physiotherapy treatment includes 2 daily intensive airway clearance sessions and an exercise session. Nutritional status is also reviewed. Between admissions children are reviewed at bi-monthly outpatient appointments. An alternative model of care that provides comprehensive outpatient physiotherapy and dietetic management may be more effective. The Frequent Flyer Programme was undertaken as a quality improvement initiative within the CF Unit, in addition to the specialist services already provided.

Objectives: The primary objective was to reduce requirement for routine IV antibiotic treatment, and associated costs of healthcare. Secondary objectives were to increase exercise capacity, maintain or increase lung function, growth and body composition parameters, and improve quality of life.

Methods: Sixteen children (12 female; mean age 10.9±2.93; range 5-15 years) who required >40 days of IV antibiotics in the 12 months pre-intervention were enrolled. Physiotherapy included weekly-supervised exercise sessions that comprised aerobic and strength training components. Home physiotherapy regimens were reviewed monthly to reinforce optimal airway clearance and inhaled mucolytic techniques. VO₂ peak testing and 10m-MSWT were performed at baseline and post-intervention. Lung function was measured at clinics and during admissions. Increased dietetic input included 1-2 monthly monitoring of growth, absorption, appetite and intake. Educational sessions were delivered individually on pancreatic enzyme therapy and dosing, vitamins and energy.

Results: There was a 23% reduction (478 vs. 619 in previous year) in requirement for inpatient IV antibiotics and a 20% reduction (243 vs. 304 in previous year) in home IV antibiotic requirements during the intervention year (p=0.06). Cost analyses determined a cost saving of ±£180,858 (\$293,000) to the hospital. Exercise significantly increased VO₂ peak by 4.86mL.kg.min⁻¹ (95%CI 1.01 to 8.71; p=0.02), 10m-MSWT distances by 229m (95%CI 108.76 to 349.70; p<0.001) and incremental level achieved by 2 (95%CI 0.83 to 2.56; p<0.002). No significant differences in lung function, weight, BMI and quality of life scores were demonstrated, although there was a trend towards improvement in clinical outcomes compared to the pre-intervention year.

Conclusion: The Frequent Flyer Programme demonstrated that this alternative outpatient model of care had a positive effect on reducing IV antibiotic requirements for children with moderate to severe CF, with a cost benefit to the hospital. Exercise capacity was significantly increased and physiological outcomes trended towards improvement. An RCT has been recently funded that will aim to rigorously evaluate whether this new model of CF can produce similar clinically significant improvements in a much wider CF population with different severities of the disease.

457★

DRAWING A CYSTIC FIBROSIS (CF) ROAD MAP: IMPROVING CF INPATIENT CARE WITH AN ADMISSION PLAN

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Background: Hospital admissions for CF pulmonary exacerbations (CFPE) are an integral part of care at our CF center. Our center is part of a large children's hospital with multiple medical teams and learners; includ-

ing students, residents, and fellows. To improve our inpatient care, we focused improvement efforts on communication and the patient experience through the creation of an admission Road Map.

Global Aim: To improve the hospitalization course for CFPE, focusing on physician communication and family involvement in patient centered care.

Methods: We assembled a workgroup to evaluate our overall CF inpatient care, including physicians, nurses, respiratory therapists, and CF patients. We mapped our current process and assessed for challenges and barriers to our stated goals. Areas of specific concern included: a) multiple patient handoffs, b) inconsistent communication between care providers, c) poorly defined long term plan of care, and d) limited active patient and family participation. To address these varied concerns, we designed an admission Road Map; a standardized tool with key elements customized for each patient, resulting in an individualized admission plan. This allows for more consistent, efficient, and organized communication between various providers and the patient/family. To foster active family participation, personal goals and family centered rounds are stressed in the tool. Training on Road Map usage was given for the complete inpatient and outpatient teams, and after a trial it was implemented for all admissions.

Results: To assess the Road Map's adoption we measured compliance with usage, defined as return of a completed Road Map to the CF team. Overall compliance for the 50 total admissions over the 8 months since implementation is 86%, with an average of 1 patient monthly with a road map not used or not returned. Tool usage and usefulness is assessed through deliberate discussion with end users, including clinicians and patients. Focus groups of involved parties were utilized for specific topics. Plan-Do-Study-Act (PDSA) cycles with small tests of change are completed regularly to improve the tool and admission processes. Specific improvement steps included: 1) Design of an electronic form to aid in transfer between teams and allow easy reference to previous data. 2) Creation of a holder and designated location in the chart to provide easy access for all users. 3) Improved format and addressed duplication of work to improve end user buy-in. 4) Facilitated efficiency through the addition of check boxes and by including historical data to ease handoffs and charting.

Conclusions: To improve our patient care and communication we created and implemented a CFPE Road Map. With continuous PDSA cycles and implementation of changes, we have created a standardized form to aid in hospital care while allowing for patient centered customization and active participation. Regular tool adoption was achieved and we continue to improve the tool itself. Next steps include the transition of this tool to an electronic medical record and continued assessments of overall patient care and satisfaction.

This project is supported by a grant from the Cystic Fibrosis Foundation.

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IMPROVING PATIENT AND FAMILY EDUCATION AND QUALITY OF LIFE THROUGH AN INDIVIDUALIZED CF ACTION PLAN AND ORGANIZATIONAL TOOL

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Our pediatric CF center in Northern California cares for approximately 170 patients, one third of whom are primarily Spanish speaking. 12% of our patients and families are noncompliant with their quarterly clinic visits. In addition, 40% of our eligible patients comply with their yearly oral glucose tolerance tests (OGTT) and 45% of our patients have a body mass index (BMI) of less than the 50th percentile. Our hypothesis is that these outcomes are in part a consequence of a lack of education and knowledge of cystic fibrosis. Our CF team created a CF patient binder which includes a "CF Action Plan" with the primary objective to enhance patient/family education and organization. Our ultimate goal is to improve the quality of life for our patients and families. Secondary objectives include determining if the CF patient binder, Action Plan and appointment reminders will 1) improve clinic visit frequency; 2) improve adherence with OGTT; and 3) improve growth parameters as measured by BMI.

Methods: The project began on October 3rd, 2011 and will be completed on July 1st, 2012. The purpose of this time frame was to capture at least 2, routine quarterly visits to our CF center so that we could distribute pre- and post-project: 1) Cystic Fibrosis Questionnaire Revised (CFQR) to assess

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the quality of life of our patients and their families; as well as 2) Cystic Fibrosis Knowledge Questionnaire (CFKQ) to assess their knowledge of cystic fibrosis. The questions from the CFKQ primarily focus on pulmonary and gastrointestinal related topics. Obtaining a score of 80% or higher was the threshold we used for defining sufficient knowledge on the CFKQ. On the CFQR, we calculated the mean quality of life scores for younger and older children, adolescents, young adults and parents and compared them to previously reported scores in the CF literature. The purpose of each questionnaire was explained, distributed and collected by a member of our CF team at the same time the CF patient binder and Action Plan were explained and provided to each patient and family. Each binder contains detailed patient information as well as a section for the Action Plan which includes a current list of medications, treatment plan (signed by the patient/family and physician), recent FEV1, culture results, ideal weight, future appointments and diagnostic studies.

Results: At baseline, 80% of our patients and families received a score of <80% (mean=60%) on the CFKQ. In regards to the CFQR, our mean quality of life scores for the age groups listed above were consistent with the mean scores previously reported in the CF literature. We expect to compile the results of the post-project CFQR and CFKQ in early July at which point updated data will be presented.

Conclusions: Although the quality of life scores for each group were on par with the mean scores previously reported, the mean score on the CFKQ is of concern and suggests significant gaps in knowledge. This may help explain the suboptimal BMI, lack of adherence to clinic visits and OGTT. We hope that by providing a CF patient binder, Action Plan and appointment reminders we will enhance overall comprehension, quality of life, BMI and better adherence to clinical visits and OGTT.

NURSING ISSUES

459★

ASSESSING PARENT READINESS FOR TRANSITION FROM A PEDIATRIC TO AN ADULT CF PROGRAM

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Background: Disease specific skills assessment is a component of transition.¹ As part of an ongoing quality improvement project to facilitate transition of care from the Pediatric to the Adult CF Program, we developed a checklist for adolescents to assess their readiness for transition. We determined that we needed to assess parental readiness for transition as well.

Methods: We developed an 18 item "Parent Readiness Checklist" that mirrors our patient "Readiness Checklist" to assess their child's disease management skills and their own readiness for transition. In order to involve parents in the process, we aimed to have 100% of parents of teens (16-18 years) in transition and young adults (19-21 years) who have transitioned receive a draft of the checklist and requested feedback by mid-January 2012. Questions could be answered as follows: 1 (no way), 2 (once in a while), 3 (maybe), 4 (most of the time), 5 (yes, for sure). We asked parents to fill out the checklist and gave them a set of structured questions for feedback on clarity and adequacy of coverage of relevant issues. Free-form feedback was also accepted. We planned to evaluate the success of our process by synthesizing the feedback and creating a Parent Readiness Checklist that is acceptable to 90% of the group.

Results: Checklists were mailed to 41 families (27 patients in transition, 18 patients already transitioned). Surveys were returned by 22% (6/27) of parents of those in transition and 39% (7/18) of those already transitioned. Parents whose young adults had transitioned answered 80% of questions with a score of 4 or 5 vs. 63% of parents whose children are in transition. Questions that most frequently elicited scores of 1 or 2 in patients who had not yet transitioned pertained to the child making phone calls to the CF center (67%), parent comfort with the child making phone calls (50%), and parent perception of their child's knowledge of how to get help when sick (33%). All (100%) of parents gave the checklist a readability score of 5 and assessed content as being appropriate with a score of 4 or 5. Free-form comments of interest included: "We have not started transition at home...I feel she will transition easily." "Difficult period for parents; Parents should be involved in patient care when patient is ill. Patients should become responsible for own healthcare." "I think there should be multiple (10+) appoint-

ments with both the child and adult doctors and parents and children before the complete transition is made." "I don't know if my daughter will ever have full responsibility for her CF. Hopefully someday, but until then, she has a huge supportive family to take care of her. Don't push us away - embrace it!"

Conclusions: A readable and appropriate Parent Readiness Checklist was developed and studied. All parents who responded affirmed the applicability of the checklist. Parent comments about transition all indicated a desire for involvement in the process. Pilot data showed deficiencies in our current transition process that will be targeted for improvement. Integration of parent readiness with ongoing patient readiness may result in more successful transition from the pediatric to adult CF care team.

¹Patton SR et al. *Ped Pulmonol* 2003;36:123-130.

QUALITY IMPROVEMENT

460★

EXPLORING TRANSITION IN CYSTIC FIBROSIS CARE: PATIENT, PARENT, AND PHYSICIAN PERSPECTIVES

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Background: Cystic fibrosis (CF) is a life-threatening genetic disease affecting the lungs, pancreas, liver, and intestine with no known cure. Approximately 30,000 people in the United States are afflicted with this disease. Management of CF has improved significantly over the past several decades and life expectancy has been steadily increasing. CF is no longer a disease confined to the pediatric healthcare arena; 40 years ago, it was rare for a person with CF to live to adulthood, however today more than half of people with CF are over 18. Transitioning to adult care can be a challenge for patients with CF, the families that love them and the clinicians that treat them. Little is known about how best to effectively help people with CF transition from being "pediatric patients" to "adult partners" in the management of their disease. Understanding the nuances of transition is an important first step in promoting effective adult healthcare for persons with CF. The aim of this study was to learn and explore perspectives on this transition in healthcare from the point of view of adults with CF (primary participants; N=3) and key players in their lives (parents, pediatric providers and adult CF doctors).

Methods: Individual one-time modified life-story interviews (n=11) with three adults with CF and their identified key informants were conducted. Interview results were transcribed verbatim and analyzed for content using NVivo 9 software.

Results: Data collected from the interviews showed that while patients were all satisfied with their current care in the adult CF program, they all believed in different factors that should inform the transition process. All adult CF providers that were interviewed held similar beliefs regarding the factors that inform the decision to transition, while pediatric CF providers expressed different opinions on when transition should be decided. Parents also held varying views on factors that should inform the decision to transition a person with cystic fibrosis.

Conclusion: Data collected from this research suggests that transition be discussed and decided on by the patient, parent, pediatric and adult CF providers. Transition should be mentioned early in the care of the patient.

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ACCELERATING THE RATE OF TRANSITION FROM PEDIATRIC TO ADULT CARE

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Introduction: Transition of persons with cystic fibrosis (CF) to an adult program traditionally occurs between the ages of 18-21 years. It is optimal for patients to be well prepared for transition and taking control of CF care. There are times when pre-planning has not been ideal and patients and families are not "ready" for transition. There is also the reluctance of the physi-

cian and staff to send the patient out into the world of adult care. This seemed to be the situation at the Baylor CF Center. Transition was slow to occur; many patients were close to or a few years over the age of 21 when transition occurred. Thus, there was a need for a more organized and consistent transition procedure. Barriers included a separate location, staff, and hospital for the adult program, and a large group of pediatricians who were not synchronized in their practices regarding transition.

Method: A multidisciplinary committee was formed to develop a program for transition. The committee consisted of staff from both the pediatric and adult programs. The initial goal was to accelerate the current process. To implement this, the following steps were undertaken:

1) A list of all patients 16 years and older was reviewed at each committee meeting. Specific emphasis was placed on those over 18. This list was reviewed and updated at every meeting. Then it was distributed by the pediatric coordinator to each physician to discuss a plan, anticipated date of transition for each patient, and what barriers might be limiting this process. It was requested that the individual physicians and staff focus on overcoming these barriers for each patient to allow transition to occur at an appropriate time.

2) The coordinators from the pediatric and adult programs reviewed the patient list monthly to determine who had been seen at the adult program. Those that had been given information for making an appointment but had not followed through were contacted by the pediatric coordinator or physician's nurse, who reinforced making an appointment with the adult program.

3) The pediatric staff began to discuss reasons for transition delay with their respective physicians.

4) When the adult coordinator visited a hospitalized patient who was ready for transfer, an appointment at the adult program was given to the patient for the next clinic visit.

Results: After initiating the methods above, the number of patients transitioned to the adult program more than doubled each of the first 2 years and quadrupled in 2011.

Conclusions: Transition can occur more appropriately when a team agrees on a procedure and all "buy into" the importance of transitioning adults for adult care in a timely manner. The next phases already in development are:

1) Age specific CF self-management skills and checklists for patients, families, and staff for monitoring a patient's progress toward self-management by the time of adulthood.

2) A post-transition survey for patients to evaluate their experiences and make recommendations.

It is expected when all of these plans are implemented, there will be a complete transition process in place rather than only a process of transfer.

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UTILIZATION OF A PSYCHOEDUCATIONAL TIMELINE IN THE SUCCESSFUL TRANSITION FROM PEDIATRIC TO ADULT CARE IN CYSTIC FIBROSIS

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Introduction: The NYULMC Cystic Fibrosis Care Center is a newly established care center. At NYULMC physicians have little experience in caring for adults with cystic fibrosis. In our transition model the pediatric care team is bridging the gap between pediatric and adult care.

CF patients live longer and it has become increasingly clear that they need to transition to the care of an adult care physician to address their evolving needs. There are multiple barriers to this transition. Some of these barriers include: lack of adult physicians with experience treating cystic fibrosis, an inability to form transition support groups due to infection control issues and insurance coverage. Barriers are also present within the family system: parents' fear of letting go of their child, feeling helpless, fear of role loss and reluctance to sever the ties with their pediatrician. While the issues mentioned above are only some of the barriers to transition it is evident that by addressing these and other issues over the timeline of a patient's experience with their pediatrician, from diagnosis to transition to adult care, the family will see transition as the necessary next step in their continuum of care, organized to suit the patient's individual needs and supported and bridged by the pediatric CF center team.

Objective: To provide patients and their families with the knowledge, resources and support along a psychoeducational timeline to empower them to make the transition to adult medical care.

Methods: We are developing a psychoeducational timeline geared to transition. The materials that will be used in this project will be developed over the next year. Their success will be measured as we transition our patients to the adult care team. This program will begin with the education of the child and his/her family from their earliest visit to the CF center on the concepts of self care, medication administration and identification, cystic fibrosis, understanding insurance/entitlements and transition. Parenting groups will take place monthly that address relevant topics. As we form our parent advisory council they will be part of this process. We will add opportunities for our families to communicate via a parent support network and for our adult patients to communicate via internet discussion groups. The program is based on the premise that the process of transition should not begin at a time when the patient is most vulnerable, i.e., early adulthood. Instead a center policy of transition, coupled with a transition curriculum that is developmentally administered, parent involvement in the development of the curriculum, and the ability for communication with both the pediatrician and the adult physician will make for a successful transition experience for the patients as well as the medical team.

Conclusion: By presenting a formalized psychoeducational timeline that addresses the needs of each individual patient with a focus on center policy, self care, medication education and administration, cystic fibrosis facts, opportunities for parent involvement, access to adult physicians via pediatric CF care center team and a personalized transition plan, transition to adult care has a much better chance of being successful.

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ESTABLISHING A NEEDS ASSESSMENT TOOL AND EVALUATING RESPONSES TO IMPROVE HOME INTRAVENOUS (IV) ANTIBIOTIC THERAPY FOR PATIENTS WITH CYSTIC FIBROSIS (CF)

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Objective: To assess overall satisfaction and obtain feedback from CF patients that have completed home IV therapy, and to develop ideas for program improvement.

Background: Many CF patients choose to complete IV therapy at home to treat pulmonary exacerbations, with expected treatment duration of 2-3 weeks, weekly outpatient follow-up, and services from a home care agency. Previous concerns regarding the routine use of home IV therapy have arisen pertaining to the patient's or caregiver's ability to manage therapies, adhere to an equivalent hospital regimen in the home, and complete more airway clearance treatment (ACT).

Methods: To improve our pediatric home IV program, we developed a survey to assess patients' needs and satisfaction. An anonymous 14 question survey was emailed to patients who had completed home IV therapy in 2011 via Survey Monkey and responses were obtained from either patients or their parents. Email reminders were sent twice within 3 weeks to those who did not respond to the survey.

Results: A total of 32/67 (48%) patients responded to the survey; 28% were 0-12 years, 50% were 13-21 years, and 22% were 22 years or older. Only 3/32 patients initiated IV therapy as an outpatient with the majority initiating therapy while inpatient. 52% of the population completed IV therapy once, 29% completed twice, and 19% completed three or more times in the last year. The length of therapy was approximately 2 weeks for the majority of the patients. 97% of patients believed that completing antibiotics at home was preferable. Responses indicated that getting more sleep, having more access to preferred food choices, attending school, and having less concern about infection control made home IV therapy more preferable. Maintaining the IV schedule, going to hospital appointments, and coordinating home care were identified as difficult factors. Responses also indicated that providing a daily schedule for IV medications, assistance with ACT, follow-up telephone calls, and general education were all helpful. Suggestions were made that additional follow-up, increased education prior to discharge, more assistance with ACT, and development of a daily schedule that included all medications and ACT would be helpful.

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Conclusion: Establishing a needs assessment and patient satisfaction survey for CF patients receiving home IV therapy is the beginning of an innovative approach to addressing concerns, optimizing health care, and improving quality of life. In general, patient satisfaction with home IV therapy was high, but modifications will be needed to improve health care and guide future practice. Results of the survey will lead to improvements in communication via early identification of a home care plan and easier access to outpatient assistance. It may be beneficial to increase the amount of instructions and discharge education to patients by providing individualized daily schedules for medication administration and ACT, as well as to assess each patient's need for outpatient support to develop improved communication.

QUALITY IMPROVEMENT

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PULMONARY MEDICATION ADHERENCE AMONG INDIVIDUALS WITH CYSTIC FIBROSIS

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Background: Prior studies have found low rates of medication adherence. However, most of these studies have been conducted with small samples which limits the reliability of these estimates overall, by specific drug type and by patient subgroups.

Objective: To assess pulmonary medication adherence rates among a large sample of patients with CF and their associations with patient characteristics.

Methods: Patients ages 6+ years with a CF diagnosis and a prescription fill for a chronic pulmonary medication were identified from the MarketScan® Commercial Claims database (1/2005 to 6/2011). Medication possession ratios (MPR) for each drug were computed during a 12-month period for each patient. A composite MPR (CMPR) was calculated as the average of each pulmonary drug's MPR. If a demographic characteristic was significant in the CMPR, the association was examined by drug, except for colistin and aztreonam lysine, whose small sample sizes precluded subgroup analysis.

Results: See table for the overall CMPR and individual drug MPRs. Colistin (n=166) and aztreonam lysine (n=65) MPRs were 42.4±35.2 and 47.0±31.4, respectively. Age was significantly associated with CMPR and all age groups had lower adherence relative to the 6-10 year olds; this association held for each drug. Women had lower CMPRs than men (p<.001). There were no gender differences for dornase alpha (p=.13) or hypertonic saline (p=.49); however women had significantly lower adherence to azithromycin than men (p<.001) with a similar trend for tobramycin (p=.06). As the number of prescribed pulmonary medications increased, so did the CMPR and each individual medication's MPRs.

Conclusion: Overall rates of adherence to pulmonary medications were below 50%. Age differences found in other studies were replicated in this larger sample. Women had lower adherence than men, but only for antibiotic therapy. Our results contradict the common belief that adherence decreases with more prescribed medications and requires further evaluation. Effective strategies are need to support and improve CF medication adherence.

Supported by Novartis Pharmaceuticals Corporation.

MPR (Mean±SD) for the Composite and Each Drug

	Composite	Azithromycin	Dornase Alpha	Hypertonic Saline	Tobramycin
N	3,287	1,848	2,081	785	1,223
OVERALL	47.6±31.0	49.9±34.8	57.1±31.2	40.4±31.2	50.8±35.8
AGE(years):					
6-10	58.6±31.1	58.0±34.9	68.1±28.5	52.6±30.4	58.0±38.1
11-17	48.1±29.6	50.2±33.2	56.8±29.9	40.0±31.3	52.4±35.2
18-25	44.0±28.8	48.5±32.5	49.9±31.5	30.7±27.1	49.3±34.7
≥26	44.4±32.5	49.2±36.9	55.8±32.2	40.2±32.0	46.7±35.8
GENDER:					
Male	50.1±30.8	54.3±34.2	57.9±31.3	39.7±31.4	52.5±35.6
Female	45.0±31.1	45.1±34.8	56.1±31.1	41.1±31.0	48.9±36.0
# PULMONARY MEDICATIONS PRESCRIBED:					
1	40.7±34.7	34.7±35.2	52.4±32.5	26.6±27.1	35.1±34.6
2	50.5±28.2	53.7±33.8	54.5±31.7	37.7±30.1	47.7±35.2
3	55.5±23.9	58.2±30.9	60.9±29.3	44.0±31.5	53.9±35.3
4	59.5±21.6	65.3±28.4	66.5±27.8	46.1±31.9	62.8±34.1

PSYCHOSOCIAL/BEHAVIORAL

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ADOLESCENT AND PARENT PERCEPTIONS OF THEIR CF CARE TEAM'S ADHERENCE PROMOTION PRACTICES AND SATISFACTION WITH CARE

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Background: Little is known about CF care centers' adherence promotion practices or family satisfaction with the care received. Both are known to affect rates of adherence in other chronic illnesses.

Objective: To assess adolescent and parent perceptions of their CF care team's adherence promotion practices and satisfaction with care, and agreement between the two informants.

Methods: In all, 290 adolescents enrolled in both arms of the iCARE study (median (IQR) age=13 (12, 15) years; 52% female) and one of their parents completed a web-based survey administered by the study coordinating center. Eighteen care centers participate in iCARE. Percent Agreement and a Kappa statistic were used to determine agreement. A p>.05 for the Kappa statistic indicates poor agreement.

Results: There was a median (IQR) of 17 (7,20) responses per care center. Eighty-seven percent or more of parents and teens reported the care team asked about adherence to pulmonary medicines, enzymes and airway clearance every visit (%Agreement between teen and parent=82-87%; Kappa=.17-.33, ps<.01). Few families reported discussing barriers to adherence at every visit (Teen=37%, Parent=29%; %Agreement=53%; Kappa=.21, p<.01) or receiving advice on how to remember to do treatments (Teen=38%, Parent=31%; %Agreement=54%; Kappa=.25, p<.01). Teens were more likely than parents to say they were taught something new about airway clearance (71% vs. 61%, p<.01) and nutrition (79% vs. 63%, p<.01). Although a similar proportion agreed that nutritional information was "very helpful," (Teen=59%, Parent=72%; %Agreement=62%; Kappa=.25, p<.01), perceptions of airway clearance education was rated by parents more often as "very helpful" (Teen=67%, Parent=83%; %Agreement=62%; Kappa=.06, p=.15). Teens were less likely than parents to report they felt "completely" comfortable asking questions during a visit (Teen=56%, Parent=88%; %Agreement=55%; Kappa=.03, p=.23) or discussing treatment barriers (Teen=58%, Parent=73%; %Agreement=52%; Kappa=.05, p=.15). Adolescents (51%) and parents (69%) generally agreed that they were "very satisfied" with the help they received on following a treatment plan (%Agreement=61%; Kappa=.22, p<.01) and the majority rated their overall quality of care as "excellent" (Teen=71%, Parent=75%; %Agreement=71%; Kappa=.28, p<.01).

Conclusion: Treatment adherence appears to be assessed at every visit, while barriers to adherence are addressed less often. Only half of teens and two thirds of parents were very satisfied with the adherence support they received. While teens were more likely to report that they received education, they found this information less helpful and were more uncomfortable talking with the care team than their parents. The vast majority reported

excellent overall quality of care. These results suggest several areas that could be targeted to improve adherence promotion within care centers.

Supported by Cystic Fibrosis Foundation, Genentech, Inc. and Novartis Pharmaceuticals Corporation.

NURSING ISSUES

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IMPROVING ANTIBIOTIC TIMELINESS FOR CYSTIC FIBROSIS INPATIENT ADMISSIONS

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Background: Inpatient care for cystic fibrosis pulmonary exacerbations (CFPE) is a significant burden of CF care. The 2010 CF Foundation Registry report noted 28.9% of pediatric CF patients received intravenous antibiotics (ABx) with an average hospitalization of 12 days. As part of an ongoing comprehensive assessment of our CF admission process, we identified time to treatment as an area for focused improvement efforts.

Methods: We examined our current state by mapping our CFPE admission process and identifying potential inefficiencies. There was an abundance of patient handoffs and redundant process steps for ABx and airway clearance therapy. ABx were selected as the key quality characteristic for improvement. To establish an historic baseline, retrospective chart audit was completed of all CFPE admissions for the past 2 years (N=174). Mean time from admit to ABx order was 3.01 hours (upper confidence level (UCL)=6.21, lower confidence level (LCL)=0) and to ABx administration was 5.87 hours (UCL=8.17, LCL=3.58). Based on initial review we focused our efforts on admissions from clinic, a unique subpopulation with a more redundant process and significantly longer mean times to ABx. Key process variable of ABx order time was selected for initial intervention.

Our improvement steps involved a change in our process to allow for earlier order placement by the inpatient resident physician. A written handoff was instituted to facilitate this change. This written form ensured a more accurate hand off, eliminating the need for a redundant call back from the in-house residents. This also allowed for a more efficient initial patient encounter and team handoffs. The new process thus allowed the inpatient team to safely order the ABx more quickly. After education for involved parties and a trial period, the form was instituted for all CFPE admissions. Subsequent PDSA (Plan-Do-Study-Act) cycles have addressed delays identified during evaluation of the new process. For instance, to aid in the timely transfer of the handoff from outpatient to inpatient teams an electronic version of the form and an admission board of upcoming admits were developed.

Results: Since instituting the new process in September 2011 there has been a significant shift in our admission to ABx order and administration time, which have both remained below the baseline mean. Mean time after our intervention decreased to 1.32 hours for ABx order (UCL=3.64, p<0.001) and 4.47 hours for administration (UCL=6.17, LCL=2.78, p<0.001) (Figure).

Conclusions: Upon examining our CF admission process, we found a significant delay in ABx ordering and subsequent administration. By using a written handoff form we have seen an improvement in our order and administration times. Repeat PDSA cycles and monthly monitoring will continue. Our next steps include focusing similar interventions on timeliness to initial airway clearance therapy.

Supported by a grant from the CF Foundation.

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SURVEY OF PARENTS' EXPERIENCES OF NEBULISED ANTIBIOTICS IN PRE-SCHOOL CHILDREN WITH CYSTIC FIBROSIS

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Introduction: Early detection and aggressive treatment of first isolations of *Pseudomonas aeruginosa* (PsA) is paramount in delaying onset of chronic infection. Nebulised antibiotics are critical in eradication regimens so it is important they are used easily and effectively. However in pre-school age children, nebulisers can often be difficult to administer and lung depo-

sition is dramatically reduced in crying infants [1]. We aimed to assess the experiences of parents of pre-school children using nebulised antibiotics.

Methods: Children <5 years old who had previously had PsA isolated from a cough swab, sputum sample or bronchoalveolar lavage were identified from our database. A semi-structured questionnaire was used for telephone interviews carried out by a paediatric CF physiotherapist.

Results: From a total of 333 patients, 88 (26%) were under 5 years old. Forty of 88 (45%) had previously isolated PsA and were having nebulised antibiotics. 28/40 (70%) parents were interviewed. Mean patient age at time of interview was 2yrs 8mos (range 7 mos to 4.9 years). For 25/28 (89%) it was their first experience of nebulised antibiotics; 12/28 (43%) had been on nebulised antibiotics >12 months, 4/28 (14%) for 6-12 months and 12/28 (43%) for <6 months. Parents rated their child's 1st experience out of 10 (with 10 being the most positive) and 75% scored ≤5. Although in 64% nebuliser acceptance improved over time, 43% of parents still reported scores of ≤5. Behaviours included: screaming, crying, pulling out the power cable, biting the mask and running away. Only 5/28 (18%) parents reported they "sometimes" or "regularly" missed giving their child's nebulised antibiotics; reasons included being too busy, visiting relatives and child's behaviour. Suggestions from parents to improve their first experience split into 6 themes: using a fast nebuliser (e.g. an E-flow); having more child friendly resources; easier access to equipment; starting the first nebuliser as an inpatient; having more support at home and being given more information.

Conclusions: Nearly a quarter of parents reported having difficulties with their child's nebuliser acceptance and nearly a fifth reported missing doses of nebulised antibiotics. From the data so far, it is clear that parents' experiences need to be improved. As part of our quality improvement programme, we intend to implement the following: use of fast nebulisers; early home visit from the Homecare team; a parent information leaflet including ideas to help the child accept the nebuliser; a picture book for the children and a DVD showing other young children using a nebuliser.

[1] Geller, D. The science of aerosol delivery in cystic fibrosis. *Paediatric Pulmonology*. 2008;43 (Suppl 9):S5-S17.

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HIGH DOSE VITAMIN D FOR PREVENTION AND REPLETION OF VITAMIN D DEFICIENCY IN A PEDIATRIC CF POPULATION: RESULTS OF A QUALITY IMPROVEMENT PROJECT

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Introduction: Vitamin D deficiency is common in patients with CF. The Emory adult CF program developed a protocol for using high dose vitamin D3 and reduced the prevalence of vitamin D deficiency in their population from 76% to 32% (Enders, J.L. et al, *Pediatric Pulmonology* 33(S): 413, 2009). We adapted the adult protocol to a weight-based high dose vitamin D protocol for use in our pediatric patients.

Methods: Beginning in April 2011, we began to dispense high dose vitamin D3 at routine clinic visits, instructing patients to take 10,000 IU/ 10 kg (rounded down to the nearest 10,000 units) every week during the winter months (December 1 – February 28) and every 2 weeks during the rest of the year*, independent of their current vitamin D status. We checked 25-OH vitamin D levels along with routine annual labs. During this same time, we began checking 25-OH vitamin D levels at admission on all pediatric CF patients hospitalized for treatment of pulmonary exacerbation. If this level was >30 ng/dL, we started or continued them on the outpatient protocol. If the vitamin D level was <30 ng/dL, patients were given dose of 10,000 IU / 10 kg every other day for a total of 10 days or 5 doses, and then started on the outpatient protocol.

Results: The highest measured vitamin D level was satisfactory (30 ng/mL or over) in 40.5% of those patients who were tested in 2009. In 2010, 70.6% had at least one measurement that was 30 ng/mL or over. In 2011, the percentage increased to 78.0%. So far in 2012, 60.7% of the 56 patients in whom vitamin D levels were measured had satisfactory levels but 74.0% of those who were dispensed vitamin D3 had satisfactory levels. We will continue to track these measures and report updated results at the conference.

Conclusions: Based on the improvements we have observed in the vitamin D levels of our pediatric CF patients, we believe this strategy of using high dose vitamin D year-round is an effective method to prevent vitamin D

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deficiency in these patients. The main barrier we have found to dispensing has been our reliance on the dietitian for this task, as she does not routinely see all of the patients who need to receive it. The main barrier we have found to adherence with this protocol is patients' difficulty remembering to take the vitamin D, either every week or every two weeks depending on the season. One helpful intervention to improve adherence to this protocol has been to encourage patients and families to place reminders on their calendars at home or in their cell phones.

*Maximum D3® provided by James Grote, MD, BTR Group, Inc. Pittsfield, IL. 62363 <http://www.vitd3.com/index.php>.

QUALITY IMPROVEMENT

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DEVELOPMENT OF THE CF ALERT PATIENT CARD TO IMPROVE IN-HOSPITAL CARE FOR PATIENTS WITH CYSTIC FIBROSIS

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Background: Individuals living with cystic fibrosis (CF) typically have chronic pulmonary infections requiring regular treatment with oral, nebulized and intravenous antibiotics. In the Project on Adult Care in CF study, 35% of CF patients had taken oral antibiotics and 29% had received inhaled tobramycin on the day prior to the survey (1). The use of inhaled antibiotics was 43.1% in the Epidemiologic Study of CF Infection (2). Infection management is complex due to the polymicrobial environment of the CF lung and frequent co-infection with viral and fungal pathogens (3). Respiratory complications of CF are a frequent cause of hospitalization. An Ontario study reported that over a 10-year observation period, the mean hospitalization rate was 0.73 hospitalizations/person-year, with 75% of hospitalizations coded as respiratory-related (4). However, hospital admitting staff may not be aware that a patient has CF and has had chronic exposure to multiple antibiotics, or that particular antibiotic choices and dosages may be required. This unmet need in CF patient care was addressed by a subcommittee of the Canadian CF Nurses Interest Group (CCFNIG).

Methods: One of the authors (EG) observed that individuals treated by healthcare professionals outside the setting of a CF clinic were not always optimally managed due to a lack of awareness of the special needs of CF patients. The CCFNIG subcommittee initiated a pilot project to address this unmet need. The primary objective was to develop a means of alerting healthcare professionals about CF patients' typical bacterial colonization and an antibiotic regimen to optimize treatment in the non-CF setting.

Results: A subcommittee of the CCFNIG developed CF ALERT, an ID card containing information on the patient's respiratory pathogens and suggested antibiotic regimen, and instructions for non-CF healthcare professionals to contact the CF team if required. The card is wallet-sized so it can be carried with the patient's government-issued health card, which is required for all patients receiving treatment in Canada. The CF ALERT card was laminated with a special plastic surface that allows the CF nurse to write in the relevant information, and to update this information as needed. The goal is to distribute the cards to all nurse coordinators of CF clinics in Canada for use with their patients. CF nurse participants in the program will be surveyed at 1 year on the utility of the CF ALERT tool in practice.

Conclusions: A need to educate non-CF healthcare professionals about the special needs of CF patients was addressed by a subcommittee of the CCFNIG through the development of a CF ALERT identification card. Planned follow-ups will evaluate whether the use of this tool improves CF patient management and more rational use of antibiotics in the hospital setting.

Supported through an educational grant from Novartis Canada.

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NURSING ISSUES

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CLINICAL RESEARCH SURVEY AT A TDN SITE

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Objective: To survey patients and parents in order to learn their knowledge and attitudes regarding clinical research occurring at our Therapeutic Development Network (TDN) site.

Methods: Adult patients (n=73), pediatric patients (ages ≥13 (n=36)) and parents and legal guardians (n=166) were given surveys to complete anonymously during clinic visits from May 2011 to February 2012. Our adult center includes 93 patients; our pediatric center includes 168 patients (60 are ≥13 yrs). Logistic regression and Fisher's exact test were used to analyze the data.

Results: 87.6% of the surveys were completed. 50.7% of parents/guardians stated their children were asked to participate in a research study. When looking at the other responses, 68.6% of pediatric patients and 70.9% of adult patients stated they were asked to participate. However, actual participation in a research study was 39.4%, but we do not know if the groups counted the CF Registry as a research study.

In regards to how people felt about interest in future research, 83.3% of parents/guardians, 62.9% of pediatric patients and 66.1% of adult patients were interested. The most popular type of research study for these groups was observational or survey studies; 47.7% were interested in drug trials. The majority (62.7%) stated they preferred information provided during a clinic visit.

The results also show that odds of participating in a future study was 2.2 times higher for subjects who already participated in a study (p-value = 0.0223). The chance of a parent or guardian wanting their child to participate was 3.2 times higher compared to pediatric patients wanting to participate (p-value=0.0014). There is a trend toward healthier and female subjects wanting to participate.

The survey also explored motivations and barriers for participating in research. All three groups chose finding a cure or better treatment, and a benefit to their health as the top two reasons to participate. 40% of pediatric patients stated physician and parental influence was least important. Adult patients (45.2%) similarly stated physician influence was least important. All three groups had similar top barriers which were fear of causing harm to their health and time commitment. Adult patients also added travel and parking concerns.

Conclusions/Summary: The survey was completed by a large majority of the survey recipients. The vast majority of all groups surveyed were interested in research. To improve our TDN site's participation in clinical trials, we need to determine what motivates patients and families. When comparing the three groups:

- More than expected children (42.9%) and adult patients (33.9%) said money was most important (p-value <0.0001)
- More parents (34.7%) than expected thought physician influence was most important (p-value =0.0276)

We now have a better understanding of families' and patients' perceptions regarding clinical research at our Center. This includes exploring the benefits to health and well-being, tailoring the information provided regarding time commitments and making sure that all groups hear how important research participation is from their health care providers.

GI/NUTRITION

471★

FAMILY CENTERED ROUNDS ARE ASSOCIATED WITH HIGH FAMILY AND PHYSICIAN SATISFACTION, AND EFFICIENCY IN DISCHARGE, ON AN INPATIENT PULMONARY UNIT

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Background: Patient and family centeredness is an Institute of Medicine Goal, and the Cystic Fibrosis Foundation includes patient and family involvement as full members of the care team as one of its seven worthy goals. Implementation of family centered rounds is one method to help meet these goals.

Methods: As part of an initiative to make care more efficient on our inpatient pulmonary service, family-centered rounds were implemented in January, 2011. Patients with a cystic fibrosis diagnosis comprise 30-50% of pulmonary patients on any given day. A nurse team leader is responsible for verifying rounding time, alerting patients and families, and gathering staff and necessary equipment. The rounding team includes attending physicians, fellows, residents, medical students, nurses, a pharmacist and a case manager. Other staff members, including the social worker, attend as available. Computer workstations on wheels are used to review data with families, enter orders, and complete attending physician documentation. Surveys are completed by families and staff using a sampling methodology at least one week per month. Questions on the survey include percentage of families participating in rounds, rounding time, family and physician satisfaction, time from readiness for discharge until discharge orders are written, and time from readiness for discharge until patient actually discharged.

Results: Most parents (>90%) participated in rounds. Average time spent with each patient on rounds was 10-12 minutes during most weeks. During the first quarter of 2012, parents reported being "definitely" or "most definitely" satisfied on parameters of care coordination (92%), physician time spent with patient (100%), and concerns being heard (97%). Attending physicians were highly satisfied with rounds; residents were mostly "somewhat satisfied" but did not report being "dissatisfied." Time from identifying readiness for discharge to all orders written was around 150 minutes during the first two months of family centered rounding, then dropped to less than 50 minutes and has been sustained at that level. Time from identifying readiness for discharge to actual discharge fell more slowly, but over time decreased from 250 minutes during the first two months to less than 100 minutes during most months.

Conclusion: Implementation of family centered rounds on a pulmonary inpatient unit was associated with high family and attending physician satisfaction, and improved efficiency in discharge of patients. Further work is needed to optimize family centered rounds and improve resident satisfaction.

472★

OPTIMIZING NUTRITION IN PATIENTS WITH CYSTIC FIBROSIS: A MULTI-PHASE ALGORITHM APPROACH TO CHANGE

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Objectives: As part of the Learning and Leadership Collaborative: Adult Quality Improvement 2 program, we focused on optimizing nutrition. The primary goal was to improve each patient's body mass index (BMI) above the threshold proposed by the Cystic Fibrosis Foundation (CFF) nutrition guidelines.

Methods: All patients active in the adult Cystic Fibrosis center were included. At each outpatient clinic visit the patient's BMI was calculated and categorized as meeting the CFF BMI goal (≥ 23 for males, ≥ 22 for females) or not. Patients not meeting goal were evaluated for change using a multi-phase nutrition algorithm. Phase one included evaluation of pancreatic

enzyme replacement therapy (PERT) dosed at goal >2000 lipase units/kg body weight/meal. Phase two added an H2 blocker or PPI to suppress acid. The third and final phase, involved further assessment for nutrition failure: inadequate intake, malabsorption factors such as timing, preparation, brand or storage of PERT and/or inadequate acid suppression, CF related diabetes mellitus, non-adherence to nutrition therapies, pulmonary exacerbation, factors such as body image disturbance, financial, time management, social or psychological issues, and other causes such as uncontrolled or undiagnosed gastrointestinal complications or disease. While each member of the CF center team was empowered to discuss nutrition adherence and goals with the patient the dietitian was the driving force between discussing current BMI, goal BMI, and devising a plan acceptable to both patient and care team to achieve goal.

Results: Over a 35-week evaluation and follow-up period, 45 (51%) of the center's designated patients were identified as not meeting BMI goals. Mean gain in BMI during this period was $0.2 \pm 1.3 \text{ m}^2/\text{kg}$. Thirty of the 45 patients had an improvement in their BMI trajectory (change range $0.3\text{--}3.6 \text{ m}^2/\text{kg}$). The remaining 15 patients had worsening BMI during this period. Changes implemented included: increase in PERT regimen to goal lipase units/kg body weight/meal in 19 (42%), addition of H2 blocker or PPI in 6 (13%), addition of a high calorie/high protein supplement in 9 (20%), starting an appetite stimulant in 3 (7%) and higher caloric density tube feeding for nighttime feedings were instituted in 3 (7%). Another 6 patients (13%) were referred to either endocrine or gastroenterology for further work up. One patient expired and one received a double lung transplant during this period.

Conclusions: Using quality improvement techniques and focusing on nutrition care as a main component of the outpatient clinic visit highlighted areas of improvement to the patients and CF care team necessary to achieve BMI goals. While a positive change was noted in mean BMI, we identified areas of future behavior changes such as earlier intervention with medication or diet regimen change, feeding tube placement or referral to other medical disciplines such as gastroenterology or endocrinology. Continued discussion and mutual goal setting aimed at empowering the patient and aligning the care team efforts to influence acceptance of nutritional advice resulted in positive changes in our adult CF center.

QUALITY IMPROVEMENT

473★

UTILIZATION OF A NUTRITION AND PSYCHOSOCIAL ALGORITHM TO MAINTAIN GOAL BODY MASS INDEX IN ADULT PATIENTS WITH CF

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Background: Body mass index (BMI) has been shown to directly correlate to pulmonary function tests in CF patients. BMI at our center for 2007 measured below the national average. Previously we reported positive results from a multidisciplinary quality improvement process for a nutrition and psychosocial algorithm to assess all patients' nutritional risks and identify barriers to improving BMI. This was particularly evident in patients with BMI <20 kg/m². The aim of this analysis was to assess the effectiveness of maintaining appropriate BMI on a long-term basis.

Methods: A retrospective review was performed of all adult CF patients seen at the clinic from 12/08-7/11. Nutritional assessments reviewed the adequacy of oral supplements, enzyme dose adjustment, appetite stimulants and enteral tube feedings. Psychological assessments reviewed the presence of depression, anxiety, and body dysmorphic disorder. Medical records related to BMI reviewed: past medical history, treatment of infections, and proper care of CF related diabetes mellitus. Invariant, patient-specific parameters, such as age, gender, family income, education, and psychological assessments were controlled for with patient-specific dummy variables in the fixed effects model. Hausman test was performed to eliminate potential random bias. Paired t-test and multiple regressions were used to analyze the change in BMI before and after implementation of the algorithm in various cohorts of the sample.

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Results: This cohort analysis included 68 patients pre-algorithm implementation and followed up to 18 months after algorithm implementation. Baseline demographics are characterized by: mean age of 30 ± 8.8 yrs, 28 women (34.4%), BMI of 21.7 ± 3.6 kg/m², and hemoglobin A1c of $6 \pm 1.2\%$. When controlling for patient-specific parameters, the BMI of all 68 patients increased by an average of 0.35 kg/m² in 1 yr ($p=0.057$). There was an upward trend of 0.27 in the cohort with BMI <23 kg/m², as compared to the cohort with BMI >23 kg/m² in which there was a decrease of -0.3 ($p=0.05$). Forty-one patients had a BMI <23 kg/m². In these patients, the BMI was maintained above the CFF recommended goals after the initiation of algorithm compared to an average baseline BMI of 0.26 kg/m² before the algorithm ($p=0.05$). A significant improvement and maintenance in BMI was seen in patients with BMI of 20 kg/m² or less after the utilization of the nutrition and psychosocial algorithm.

Conclusion: With continued implementation, the nutrition and psychosocial algorithm serves as an effective tool to both increase and maintain BMI in CF patients starting with BMI of <22 kg/m² for women and <23 kg/m² for men. It is particularly useful in the subset of patients starting with a BMI ≤ 20 kg/m².

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OPTIMISATION OF THE EFFICACY OF PANCREATIC ENZYME REPLACEMENT THERAPY IN CHILDREN WITH CYSTIC FIBROSIS: DOES TIMING IN RELATION TO A MEAL PLAY A ROLE?

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Objectives: One study to date has investigated the timing of pancreatic enzyme replacement therapy (PERT) in relation to a meal in cystic fibrosis (CF). Delayed gastric emptying rate (GER) has been shown to have a negative effect on PERT efficacy. The authors investigated whether PERT given before or after a meal resulted in improved lipase activity and the effect of GER on PERT efficacy. Factors such as genotype and body mass index (BMI) percentile were also investigated.

Methods: Eighteen children with CF aged 9.8 ± 1 year (mean $\pm$ SEM, range 5-17 years) completed two ¹³C- mixed triglyceride (MTG) breath tests after administration of PERT both 10 minutes before and 10 minutes after a test meal to assess lipase activity and one ¹³C- octanoate breath test (OBT) to assess GER.

Results: There was no difference in the percentage cumulative dose recovered (PCDR) at 360 minutes of ¹³C-MTG between taking PERT 10 minutes before ($33.48 \pm 2.36\%$) or 10 minutes after ($31.20 \pm 3.23\%$) (mean $\pm$ SEM) the test meal ($p=0.751$). Seven subjects had a greater PCDR when PERT was taken 10 minutes before the test meal and eleven subjects when PERT was taken 10 minutes after the test meal. There was an improvement in 44% of patients PCDR of greater than 10%, while 28% of patients had normalised PCDR, when PERT pattern was changed (to either before or after the test meal). Six patients had a fast GER, eleven had a normal GER and one had a delayed GER. There was no relationship between GER rate and whether PERT was taken before ($r=-0.292$, $p=0.234$) or after ($r=0.0485$, $p=0.843$) the test meal and there was no relationship between GER and BMI percentile ($r=0.105$, $p=0.668$). Genotype did not play a role in whether lipase activity was greater when taking enzymes before or after the test meal.

Conclusions: This pilot study indicates that on an individual level there is a wide variation in PERT efficacy and that changing the timing can result in improved lipase activity. This gives rise to the idea that each patient should undergo objective testing to determine the best time for PERT administration. In this small study GER, BMI percentile and genotype did not seem to play a role in the efficacy of PERT.

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PARENTAL BEHAVIORS LIMITING IMPROVED GROWTH IN CF CHILDREN

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Introduction: Nutrition in the preschool child with cystic fibrosis (CF) remains a critical issue in preserving long-term respiratory function. Despite frequent monitoring and extensive education, approximately half of 2 year olds remain less than the 50th percentile for weight. As part of the CF newborn screening quality improvement (QI) consortium, we are addressing barriers to improved infant nutrition and ultimately, improved lung function and life expectancy. To identify factors contributing to sub-optimal nutrition early in life, we surveyed all parents of children aged 2-6 years with CF attending the Washington University CF Center clinic. Our questions addressed food choice, mealtime behavior, pancreatic enzyme replacement therapy (PERT) and food security to examine for potential contributors to growth delay.

Methods: A 13 question survey was prepared to address predetermined contributors to poor weight gain. Survey questions used a rating scale to address the relative mealtime frequency of high versus low fat food choices, meal behavior and PERT delivery. Additional questions addressed meal duration, food availability and awareness of the CF Foundation's nutritional classification system. All parents of children with CF ages 2 – 6 were anonymously mailed a written survey addressing their nutritional experience and education. Twenty-four completed surveys were returned either via the postal service or directly presented to unaffiliated clinical staff.

Results: The initial cohort of patients was assessed and their baseline growth determined. As with prior cohorts, both weight and weight for height z-scores were at their nadir at diagnosis (-1.1 ± 0.2). However, weight gain remained below expected levels with a weight z-score of only -0.8 ± 0.3 at one year of age. Our initial survey results revealed PERT was well established with 91% reporting never eating without supplemental enzyme administration, however ~20% report inconsistent administration of PERT with nighttime feedings. Only fifty percent of families regularly avoided low-fat foods and 64% reported a knowledge deficit regarding which foods are high in fat content. As well, 63% of respondents reported meal durations extending beyond 20' with only 80% of families providing PERT supplementation when meals extended beyond 45 minutes. Despite recommendations to the contrary, parents report meals were offered "on demand" more than 45% of the time and 50% of parents report that the majority of their mealtime is focused on child feeding. Finally, only 33% of families reported a good understanding of their children's nutritional status.

Conclusions: These data, collected as part of our QI effort on infant nutrition, support the need for improved education in the newborn period. The continued slow recovery to normal weight z-score and impaired knowledge base regarding food choice, mealtime behavior and PERT delivery, supports our premise that there are opportunities to correct these at-risk behaviors in feeding and caring for infants with CF. Our data identified educational gaps that can now be incorporated into our nutritional improvement initiative through the development of new educational tools and enhanced nutritional mentoring of this patient cohort.

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IMPROVING THE RATE OF ASSESSMENT BY A DIETITIAN IN AT-RISK CYSTIC FIBROSIS PATIENTS

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Objective: In our adult CF center, we identified that formal assessment by a dietitian was performed at a suboptimal rate in patients defined as nutritionally "at-risk" by the CF Foundation. Our aim was to increase the rate at which at-risk patients are evaluated by a dietitian. We also assessed the yearly trend in median BMI at our center.

Methods: Beginning in July 2011, we implemented a checklist at our weekly team meetings. On review of the adult CF patients to be seen in clinic in the upcoming week. We identified patients as "at-risk" if they had a BMI below CF Foundation targets (i.e., <22 in females, <23 in males) or if weight had decreased more than 3% within the prior three visits. We recorded when patients meeting these criteria had last been evaluated by a dietitian. We scheduled a formal nutritional assessment by a dietitian as part of

the clinic visit for patients who had not been seen by a dietitian in the prior 3 months or were deemed by team members to warrant more frequent follow-up. Dietitians utilized a standardized assessment form when interacting with patients, which included recorded typical dietary intake, use of nutritional supplements, calculation of estimated caloric requirements, and estimate of daily caloric intake. We assessed the proportion of “at risk” patients who were seen by a dietitian during the year in comparison to the prior two years. We also assessed median BMI at our center at the end of 2011 in comparison to prior years.

Results: The rate at which “at risk” patients were evaluated by a dietitian in our center increased from 52% in 2009 and 75% in 2010 to 94% in 2011. Chart review of patients not evaluated by a dietitian in 2011 identified that the majority of these patients had not presented to clinic at all during the six-month period after initiation of the quality improvement project. Median BMI at our increased from 22.0 in 2009 to 22.3 in 2010 and 22.4 in 2011.

Conclusions: Use of a checklist during weekly team meetings successfully increased the rate at which nutritionally at-risk patients were evaluated by a dietitian in our center. Median BMI also increased in our center during this time. Future quality improvement initiatives will focus on specific methods to increase the proportion of patients meeting nutritional goals.

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MESOSYSTEMS APPROACH TO CYSTIC FIBROSIS RELATED DIABETES (CFRD) MANAGEMENT

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Background: Patients with CF often require care from several subspecialties, which can result in fragmented and poorly coordinated care. In order to improve patient care for patients with CFRD, the pediatric CF team at Children’s of Alabama/UAB (COA/UAB) extended an invitation to the endocrine team to develop a mesosystem (the relationship between microsystems which are the front line units of healthcare delivery) to deliver CFRD care. This relationship began in 2007 and has persisted to date, despite several changes in the leadership of the endocrine team.

Goals: The goals of the CFRD mesosystem are to: 1) provide consistent and state-of-the-art care with a lead CFRD team; and 2) improve communication between the two teams, patient satisfaction and understanding of CFRD, and patient outcomes.

Methods: A lead CFRD team, consisting of an endocrinologist (ES), CF registered dietitian (RD) and a CF nurse practitioner (NP), was created in 2009. The team implemented, assessed, and reassessed guidelines for CFRD care. A CFRD clinic was created for patients with CFRD. A patient survey was administered to monitor satisfaction with this clinic. The eight-question survey assessed: the helpfulness of clinic, difficulty of diabetes management, understanding of CFRD, and understanding of the reason for the clinic visit. Finally, outcomes for the patients were reviewed, not only with the use of the CFF patient registry, but also with local databases on a quarterly basis.

Results: The COA/UAB CFRD mesosystem and the CFRD clinic have survived for four years even with frequent changes in the ES. Consistency in message and care has been maintained and improved with a monthly pre-clinic patient discussion meeting of the CFRD lead team. Consistency and communication have also been maintained by the presence of the CF NP in CFRD clinic. The two teams have collaborated on the development of a CFRD protocol based on the CFF Guidelines.

Patient surveys indicate 70% of patients had improved knowledge of CFRD following the clinic visit. The 2010 center-specific CFF registry reports a 27.9% occurrence of CFRD which is above the national average (24%), indicating more aggressive diagnostic CFRD screening. In addition HbA1c of 6 for the CFRD population at COA/UAB has been maintained below the national average of 6.1 since 2007.

Summary and Next Steps: Despite frequent changes in ES leadership in the COA/UAB mesosystem, the system has promoted patient outcomes that are above the national average. With the recent addition of an ES who has a specific interest in CFRD and is well recognized for research in CFRD, it is expected that patient satisfaction, understanding of CFRD, attendance in clinic, and outcomes will continue to improve. Change ideas include the addition of more endocrine members (RD and certified diabetes

educator) to the CFRD lead team. Plans also include a change in glucose monitoring, interventions for glucose intolerance in the inpatient population, ongoing refinement of the CFRD protocol, continued assessment of patient satisfaction, and education of both teams.

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EXERCISE TESTING AND PRESCRIPTION: A QUALITY IMPROVEMENT (QI) INITIATIVE APPLIED IN THE HOSPITAL AND CLINIC

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Background: Cystic fibrosis (CF) has a direct impact on lung function resulting in aerobic dysfunction and progressive limitation in physical activity. Exercise training has been shown to be efficacious in decreasing symptoms related to CF by improving cardiopulmonary fitness, enhancing sputum clearance, and decreasing breathlessness. Based on the importance of exercise for patients with CF, a QI project was initiated by the Boston Children’s Hospital and Brigham and Women’s Hospital CF Center for our nearly 300 adult patients to receive a graded exercise test (GXT) annually and at time of hospital discharge. As participants in the adult QI initiative (AQI2), we developed our program with the objective of launching a feasible assessment of exercise tolerance to prescribe an individualized exercise program and educate patients on the benefits of regular exercise. The gold standard for exercise testing is with measurement of peak VO₂ via expired gas analysis. The need for physician presence, cardiac monitoring and time constraints necessitated the use of a sub-maximal exercise test.

Methods: Patients were informed of our QI initiative for annual GXT and the benefits of regular exercise. Prior to the GXT, patients completed CF Questionnaire Revised (CFQ-R) and answered 5 questions to help identify barriers to exercise and current practice. Annual GXTs were scheduled to follow clinic appointments. Hospitalized patients were all tested within 24 hours of planned hospital discharge. For patients initially tested while inpatient, follow-up outpatient GXT were completed within 3 to 6 months to obtain follow-up data and to provide feedback following the initiation of a regular exercise program. Existing sub-maximal exercise tests were found to be too challenging for some patients. Our incremental treadmill test starts at a slower speed while increasing the speed during higher levels once maximal incline of our treadmill had been reached. The combination of these changes resulted in patients’ ability to complete more stages with an approximate one metabolic equivalent (METS) increase per stage. Results of the exercise test were used to prescribe both continuous and interval aerobic exercise based on peak METS and RPE (rating of perceived exertion). These data, follow-up responses to initial 5 questions, CFQ-R pre-GXT and at 1 year follow-up, were then input into the patient’s medical record and shared with staff members.

Results: Seventy patients have thus far completed GXT and given exercise prescriptions. Six of these have been during well clinic visits, 64 while hospitalized; 12 patients who were initially evaluated while inpatient have been seen for follow-up GXT.

Conclusion: Exercise testing describes patient’s exercise tolerance and enables clinicians to provide accurate exercise prescriptions. Follow-up exercise testing can illustrate improvement and optimistically these results will support patient participation in regular exercise. Future examination of data will determine impact of annual testing on patient adherence.

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STOPPING WORK DUE TO CF: TIMING IMPROVES HEALTH OUTCOMES

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Working adults with CF often leave work because of the severity of symptoms. They anticipate improvement in health after leaving work. But when should a CF adult stop work? Is there a time to stop that yields a better health outcome? This study examines CF adults who stopped working to apply for Social Security benefits. It compares FEV1% on their last day of work to their FEV1% at least six months later to determine if there is a cor-

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relation between changes in health outcomes after stopping work and when they stopped work.

Method: The study surveyed CF adults participating in the CF Social Security Project (CFSSP), a service of the CF Patient Assistance Foundation that helps adults with CF with their initial applications for Social Security benefits. The survey asked about FEV1% on their last day of work and at least six months later. A previous study of this group found 75% had improved or had stable FEV1% after ending work. In this study, the data was further examined to see if an increase in FEV1% is associated with when the adult stopped work.

Results: The study divided the respondents into Group A composed of those with an FEV1% between 40 and 49% on their last day of work; and Group B composed of those with FEV1% less than 40%. The data showed that 67% (10 of 15) of Group A had an increase in FEV1% after stopping work; 27% of Group A had a decrease in FEV1% after stopping work; and 6% had no change in FEV1%. Two thirds of Group A had an increased FEV1%. One third had a decrease or no change. Group A had an average increase in FEV1% of 8 points, which is an average increase of 19.2%.

Only 36% (10 of 28) of Group B had an increase in FEV1%; 39% (11 of 28) had a decrease in FEV1%; and 25% (7 of 28) had no change. About one third of Group B had an increase in FEV1 while two thirds had a decrease or no change in FEV1. Group B had an average change in FEV1 of only 1.5 points, which is an average increase of 3.75%.

Discussion: Adults who stopped work with FEV1% of 40% or greater are more likely to recover lung function compared to workers who stopped working with an FEV1 below 40%. An adult with CF may qualify for Social Security benefits based on low PFT when FEV1% is below 50% for at least a year. When the individual's FEV1% is below 50% there is an opportunity for the individual to leave work and receive Social Security Disability Insurance, which provides both income support and Medicare after a waiting period.

For individuals who leave the workforce with an FEV1% between 50% and 40%, they will likely recover lung function after they stop working, and lung function increases an average of 20%. Health outcomes improve for adults with CF who time their departure from the workforce when their FEV1% is 40% or more.

Yet adults who continue to work after their FEV1% is below 40% are likely not to recover substantial lung function when they stop work. The amount of lung function recovered, if any, is minimal. Health outcomes do not greatly improve for those who stop work with an FEV1% below 40%.

Conclusion: When a CF adult stops working is an individual decision made in consultation with his physician and CF care team. This study provides some guidance in assessing the improvement in health outcomes that one may expect when a person chooses to stop working.

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SUSTAINING THE FINANCIAL SUCCESS OF CLINICAL RESEARCH PROGRAMS THROUGH COLLABORATION AND TRANSPARENCY

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Background: Financial sustainability is paramount to the success of any clinical research program. UHMC has successfully developed an effective and strategic financial management system for the cystic fibrosis (CF) program. Four areas within the CF program were identified to streamline financial management and improve efficiencies: research billing, cross-functional communications, contract negotiation, and the revenue cycle. The Center for Clinical Research and Technology (CCRT) at UHMC has managed and implemented a system that provides continuity within the research billing practices of the CF program. Regulatory compliance with research billing practices is a core function to streamline processes and remains consistent with Health Care Finance Administration guidelines. These practices can also be applied across other disciplines within clinical research. By engaging the Institution's centralized research office, efficiencies in overall programmatic and administrative support to the research programs can be achieved. Through careful legal review, the CCRT executes research agreements that comply with sponsor, institutional and federal regulations and policies. The CF Operations Manager (CFOM) partners with the CCRT to

ensure that a proper budget is developed, fiscal guidelines are followed, revenue is tracked and collected, and regulatory compliance with research subject billing policies are met by maintaining up-to-date information on regulations and associated codes. Protocol review by the CCRT and CFOM helps to ensure that all costs are budgeted and a Coverage Analysis (CA) is in place.

Aims: 1) Develop collaboration between the CF Program and the CCRT; 2) Identify challenges in the current process (budgeting, coverage analysis, contract execution, internal communication, invoicing, billing); 3) Evaluate what systems work and which could be improved; 4) Develop best practices.

Methods: The CFOM and the CCRT meet weekly to identify strategies for process improvement. Process maps were developed for Budget/CA Development; Research Billing; and Pre-Award and Post-Award. Measurable objectives were identified and progress monitored periodically.

Results: Internal metrics show vast improvement in financial management and the contracting process. The process identifies strengths and weaknesses of the management of the CF program. Best practices were identified and benchmarked to track the overall success of process improvements. Improved communication led to the development of tools for shared information, including a "living document" used by the core group and updated on a daily basis.

Conclusions: Improved processes, communication and identification of best practices through collaboration between CCRT and the CFOM has enabled the CF program to continue its financial sustainability as well as creating best practices to sustain a robust and successful program.

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PROVIDER, PATIENT AND CAREGIVER PERSPECTIVES ON ESTABLISHING A CYSTIC FIBROSIS-RELATED DIABETES MANAGEMENT AND EDUCATION PROGRAM

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Background: Cystic fibrosis-related diabetes (CFRD) increases the complexity of CF care and requires additional attention to education and management. Literature on establishing a program devoted to CFRD and optimizing patient self-management is sparse. This study sought to identify provider, patient and caregiver perspectives about the best method to establish an interdisciplinary CFRD management and education program.

Methods: A focus group (FG) of health care providers and individual, in-depth, semi-structured interviews of people with CFRD and their parents/caregivers were conducted to gain perspectives on structuring the clinic for management and education. The focus group was comprised of six health care providers with a vested interest in the creation and development of a CFRD education and management program. Providers were identified by study investigators and included an endocrinologist, pulmonologist, psychiatrist, CF clinic coordinator, certified diabetes educator (CDE) nurse and dietitian. After IRB approval and assent/consent was obtained, adult and pediatric patients diagnosed with CFRD prior to August 2011 were interviewed. Parents/caregivers were included in the interview if the patient was less than 18 years of age or if the parents/caregivers provided a significant role in the patient's care.

A grounded theory approach was utilized to develop the sampling procedures to inform clinic operations. The digitally recorded FG and interviews were transcribed verbatim and independently coded by two study investigators using NVivo 9®. Following coding, general themes were identified for the FG and interviews.

Results: Three children and six adults with CFRD were interviewed and ranged in age from 6 to 33 years old. Six of the interviews also included parents. Four themes emerged from the interviews: (1) desired qualities of personnel and educators; (2) preferred style of education delivery; (3) use of technology and (4) timing of education visits. Most patients preferred to receive education from an interdisciplinary team during their quarterly CF appointments. Following initial diagnosis, education regarding insulin injection technique and carbohydrate counting were viewed as important. Use of

videos, webinars, electronic newsletters and other technology was discussed by most as a means to improve their education about CFRD.

Analysis of the focus group transcript is in progress.

Conclusion: A program focused on providing education and coordinating care for patients with CFRD would be beneficial for patient ease and follow up of recommended care. Each CF Center should consider their patient population when designing an education program. Results from this study will be utilized to develop a targeted education program for patients diagnosed with CFRD and improve the quality of care provided.

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DEVELOPING A PULMONARY EXACERBATION CLINIC TO STANDARDIZE CARE

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Background: Standardized, evidence-based treatment algorithms for the management of acute and chronic illness eliminate unnecessary variation in care, improve compliance with evidence-based treatment guidelines, decrease medication errors, decrease costs, and readily identify patients who do not respond to therapy as expected. The CF Center at Akron Children's Hospital became interested in the criteria for treatment of pulmonary exacerbations in 2004 as part of the Cystic Fibrosis Foundation's Quality Improvement (QI) Initiative. Using the available medical literature on pulmonary exacerbations, our CF center developed a clinical Pulmonary Exacerbation Score (PES) designed to identify patients experiencing a pulmonary exacerbation that has been previously described. With the assistance of a previous QI grant, we further developed a standardized approach to treating pulmonary exacerbations and developed a comprehensive, evidence-based pulmonary exacerbation treatment (PEXT) algorithm for mild (PES 5-7), moderate (PES 8-12) and severe (PES > 12) exacerbations. This algorithm includes both outpatient and inpatient components. Follow-up reassessment periods were also standardized as part of this algorithm and its implementation was associated with a dramatic reduction in variability of time to follow-up assessment.

Project Plan: One of the unintended consequences of the implementation of the PEXT program with a standardized follow-up of 2-3 weeks for those patients treated with oral/inhaled antibiotics and 1 week in patients treated with IV antibiotics was an increase in follow-up "sick" CF visits. Furthermore, some of our CF providers have not adhered to the follow-up recommendations as strictly as others. Thus, although variation in time to follow-up was drastically reduced after PEXT implementation, not all patients meet the recommended 1 to 2-3 week follow-up criteria. Our CF center is implementing a pulmonary exacerbation (PEX) clinic in our pediatric and adolescent patients to facilitate improved patient accessibility to clinic appointments, ensure real time data acquisition regarding resolution of PEX symptoms, and provide consistent care with one provider to standardize follow up and minimize variation in care. Having consistent providers (2-3) staff this clinic will minimize variation, allowing more consistent exacerbation care. Adherence to the recommendations from the PEXT will be monitored weekly and be presented to the QI team.

Experimental Design and Methods: We will continue to monitor quarterly median FEV1 in all patients > 6 years of age seen at our CF center (age 6-12, age 13-18, and > 19 years). We will monitor individual patient's FEV1 measurements pre- and post-implementation of the PEX clinic. PES and PEXT use and adherence to the treatment recommendations will be monitored weekly, with a goal of > 90% use by all providers. We will obtain information regarding exacerbation rate of our population, duration of antibiotic treatment, variation from recommended follow-up, and the percentage of patients with exacerbations that are seen in the PEX clinic and percentage of patients seen in other CF clinics. We will be adding these metrics to our current dashboard of metrics.

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EVALUATION OF PATIENT UNDERSTANDING FOLLOWED WITH EDUCATION IN EFFORTS TO IMPROVE ADHERENCE TO VITAMIN SUPPLEMENTATION AT AN OUTPATIENT CYSTIC FIBROSIS CLINIC

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Studies have shown that patient adherence to medication therapy is driven by their knowledge and understanding of treatment. The medication indication, administration, and outcome of therapy are essential factors for patients upon prescribing. Cystic fibrosis patients are a high risk population for poly-pharmacy and medication non-compliance. Although lung disease is the primary cause of mortality in cystic fibrosis, adequate nutrition is of great importance. Due to impaired pancreatic function, these patients are at high risk for malabsorption of fats and fat soluble vitamins. Therefore, supplementation of vitamins A-D-E-K is vital for growth and development in this population. We conducted a year long study at the Mountain State Cystic Fibrosis Center in which we evaluated the patients knowledge of their vitamin therapy prior to and post-education. Additionally, we measured the patient's vitamin levels pre- and post-education to assess compliance. The purpose of this study was to determine if patient education regarding their vitamin regimen directly impacted adherence.

A computer generated list via the Cystic Fibrosis Foundation website was used to identify all patients at our center currently receiving fat soluble vitamin supplementation. Two tests, patient specific and caregiver specific, were developed and validated by our team. At each clinic visit, the vitamin test was given to the patient to be completed. Tests in patients ≥ 8 years old were completed independently, while patients ≤ 7 years old were completed by a caregiver. At the end of the visit, the dietitian reviewed the test, and provided an educational hand-out highlighting the importance of each vitamin. At the next visit, the patient was re-assessed via the identical test. Vitamin levels were evaluated prior to the test, and were obtained following the post-test. Scores on the vitamin tests, and trends in vitamin levels post-education were evaluated.

78% of patients at our center participated in the evaluation of vitamin adherence. At this time, 23 patients completed the pre-test, and 108 patients (81%) completed both the pre- and post-test. We observed a significant increase in scores in both our patient and caregiver post-tests. More interestingly, this directly correlated with an increase in our patient's vitamin A, D, or E levels. Of the 108 patients that completed both pre- and post-tests, 54 had one or more sub-therapeutic vitamin level for vitamins A, D, or E. Of the 54 patients with sub-therapeutic levels, 38 had post-test labs obtained. One or more vitamin A, D, or E levels reached a therapeutic value after the post-test in 21 patients. The results showed a 55% increase of 1 or more vitamin levels from sub-therapeutic to therapeutic following education. Our study validates and supports the idea that informational interventions which educate patients about their condition will allow for the patients to have more control of their disease, and be more likely to comply.

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IMPROVING COMPLIANCE OF ANNUAL CYSTIC FIBROSIS DIABETES SCREENING IN AN ADULT CYSTIC FIBROSIS PROGRAM: A QUALITY IMPROVEMENT INITIATIVE

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Objective: To develop a sustainable program to ensure that our adult cystic fibrosis (CF) patients are being screened for cystic fibrosis related diabetes (CFRD) annually.

Poster Session Abstracts

Background: CFRD is a common complication in adults with cystic fibrosis. CFRD is found in 35% of adults aged 20 to 29 and 43% for those over 30 years old. The Cystic Fibrosis Foundation, American Diabetes Association and the Pediatric Endocrine Society recently put together guidelines with recommendations that CF patients should be screened with an oral glucose tolerance test (OGTT) once a year after age 10. A 2-hour OGTT is recommended to screen for CFRD. After reviewing our CF center data from 2011, we acknowledged a crucial need for increased vigilance with screening for CFRD.

Methods: A query of Port CF data was completed to determine the number of adult CF patients not already diagnosed with CFRD and without a documented OGTT in 2011. Of our 242 adult patients (non-transplant) over the age of 21, 58 were already diagnosed with CFRD. Of the 184 patients who did not have CFRD, only 8 had undergone an annual OGTT, approximately 4.3%. Our team then reviewed the patient and clinic barriers associated with this low rate of compliance with OGTT. These included convenience, time, and CF clinician compliance. Our clinic OGTT process was first reviewed and amended by our CF endocrinologist. In order to streamline the process and be more cost efficient and ensure patient compliance, we determined that our glucose load would be 2 cans of regular coke (or 81 grams of carbohydrate). A patient education form was developed, which included details and information on CFRD, as well as the importance of screening. An additional informational sheet was developed that outlined 2 options for obtaining an OGTT. These options included either using a local lab or physician's office near the patient's home versus having their OGTT done in our hospital lab, the previous standard for screening. Once our educational materials were ready, we sent out an email to our adult CF patients utilizing our electronic mailing list, which outlined our quality improvement (QI) initiative regarding the importance of CFRD screening with the informational sheets attached. We also put signs in each of our clinic rooms as a highly visible reminder to both patients and clinicians to discuss annual CFRD screening at each clinic visit.

Results: In 2011, eight out of 184 eligible patients underwent CFRD screening with an OGTT. Since initiating the changes in our screening process in February/March of 2012, we have surpassed the amount of patients screened with an OGTT in the first 2 months of our initiative compared to 2011. We have also scheduled an additional 20 patients.

Conclusions: There were several explanations for our poor compliance with annual OGTT screening. By reviewing the barriers, we were able to streamline the process for both the ordering clinician and for our patients who undergo an annual OGTT. Our goal is to improve our compliance rate over the next six months to 100%.

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CONNECT THE DOTS - A QUALITY IMPROVEMENT PROJECT

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Quality improvement (QI) programs are instituted in CF centers around the world to improve care for children and adults with CF. The CF center at Sick Kids in Toronto, Canada is the largest clinic for children under 18 in the country. The goal of improving clinical care for our patients led us to devise a program to identify patients at risk.

A QI theme CF retreat day was held in October 2011. The focus was an initiative to concentrate on improving both the median BMI percentile and FEV1 of a subgroup of patients. A multidisciplinary team approach was used to institute a more aggressive approach to treating pulmonary function decline and nutritional status that was suboptimal. The spotlight was to change treatment in order to improve the quality of care that our patients receive, in hope of improving health. The target was patients with an FEV1 <80% and BMI <25%.

The first goal was to identify patients that fit the criteria. At weekly pre-clinic outpatient review, patients identified at risk received a colored "dot." The color of the dots mimicked the colors of a stoplight. Patients received a red dot "L" for FEV1 <80%, a red "B" dot for BMI <10%, a yellow "B" dot for BMI between 10-25%, and green dot if lung and nutritional parameters were above set limits. To track our progress, patients identified with red or yellow dots were entered on a QI Excel spread sheet each week. Treatments and progress were also entered weekly.

Patients identified with a red or yellow dot were informed that their clinical status had been identified as below limits, and we would work together to improve their status. The action plan for red "L" patients was the physician, nurse practitioner, or physiotherapist had to propose a change in current treatment – concentrating on adding or changing inhaled drugs or antibiotics, evaluating current airway clearance technique and exercise program and/or devising strategies to increase adherence to physiotherapy. The "B" dotted patients met with a dietitian every visit for intensive counseling that included focusing on enzyme therapy compliance or adjustment, and boosting energy in meal plans. All "dot" patients were brought back to clinic more frequently than the 3 month routine. At weekly post-clinic review rounds, team members reported the plan for each patient, and accounted for the changes made.

After 6 months all patients had been tagged with a dot and results showed that we had a total of 94 patients with red or yellow dots in any category. Twenty-one patients have gotten off the list in the first 6 months, 2 have had double lung transplants and 7 have transitioned to adult care (2 having gotten off the list before transferring), with 65 remaining on the list.

In summary, this has been a team project embraced with enthusiasm. The key to sustainability is identifying one team member to champion the project and keep the records. We have been tracking changes to continue to monitor progress with periodic updates for the team. The number of patients that have been removed from the list has been most encouraging for the team and celebrated with the patient. We will report progress of the project to the families in our clinic newsletter.

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CF QUALITY IMPROVEMENT PROGRAM: A PILOT PHASE TO IMPLEMENT THE US QIP APPROACH IN FRANCE

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Background: Data from the French CF Patient Registry show variability in medical outcomes among the 49 CF Centres in France. Modelling the US-CFF Quality Improvement Initiative, the Dartmouth Institute Microsystem Academy Clinical Microsystems approach was adopted as the framework to improve the quality of care. A pilot quality improvement (QI) learning collaborative was initiated to engage CF healthcare professionals, individuals with CF and families.

Objectives: i) Ascertain the applicability and effectiveness of the Clinical Microsystems approach to improve CF care in France; ii) Work closely with French CF health care professionals, individuals with CF and families to adapt the Clinical Microsystems methodology to their local context of care delivery; and iii) Build leadership for change and national recognition of the QI Program (QIP).

Method: The Nantes CF Expertise Centre was established for steering the QIP in France. Seven centres were selected by way of an application process to participate (n=1,000 patients out of 5,700 captured in the Patient Registry). A condition of participation was transparency of centre-specific process and outcome measures and the formation of a lead team including a patient or parent. Each team was provided with a QI guide (adapted and translated from the U.S. CF Foundation QI Action Guide) and the national Patient Registry report containing outlined improvement goals and recommendations for patient education. Over a year's time, the pilot of the QIP was held, adapted from US-CFF improvement curriculum with faculty and 2 assigned quality coaches trained in TDIMA team coaching program. An evaluation of the pilot was conducted by an external researcher through focus groups and review of Patient data. An evaluation will also be conducted by the French CF Foundation (F-CFF) who funded the pilot to inform the program's next phase 2012-2013.

Results: 40 health care professionals, 3 patients and 4 parents participated in the pilot. 7 teams participated in 4 face-to-face meetings, 6 on-line learning sessions, and regular meetings with the quality coaches. The program culminated with each team creating a Storyboard of measured improvement results to share and promote learning with the entire CF centre network at a national meeting. The outside evaluation measured the following areas of interest: professional and patient representatives' satisfac-

tion; team participation rate; completion of program phases and PDSA cycles achieved; and, patient outcomes. The evaluation conducted by the F-CFF balances expenses and results of the pilot. It will be available in September 2012.

Conclusion: The Clinical Microsystems approach including coaching seems appropriate in France with a few adjustments, namely the need for a part time resource designated as the "Quality Referent" at each centre. Her role is one of a local coach, working in a close tandem with the physician-leader of the Team. She generally is a nurse or a clinical research assistant. National data on effectiveness and patient outcomes, including decisions regarding the next phase, will be available in September 2012.

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RE-EDUCATING PATIENTS AND STAFF WITH AIRWAY CLEARANCE TECHNIQUES (ACT) TO IMPROVE ADHERENCE

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Background: Using the Clinical Microsystem process, we focused on adherence to pulmonary therapies in our adult CF population. We derived a fishbone identifying barriers to adherence. Knowledge deficits of the non-respiratory therapy team members were identified. There was no targeted assessment of knowledge and compliance of ACT among our patients. Integrating standardized educational materials into a personalized self management plan was also lacking in our program.

Objectives: To improve CF center staff knowledge of ACT and to improve adherence to ACT in 16-50 year old adults with CF by education.

Methods: The CF center staff (13 members) completed a 20 question knowledge assessment test on ACT. This was followed by an educational session (Re-Educating Airway Clearance Techniques, REACT) by respiratory therapy (RT). A post-test survey was conducted. Surveys targeting knowledge and compliance with airway clearance therapy were mailed to patients and obtained during clinic visits. Personalized review of lung functions and the impact of ACT was discussed. A mutually agreed upon plan was prepared by RT at each clinic visit. Lung function data and a post-REACT survey were collected and analyzed.

Results: The center staff had a pre-test score of 10/20 and their post-test score was 20/20. Post-session they almost universally felt more comfortable conversing with patients about ACT. Out of our target population of 32 patients, we obtained surveys from 24. Our pre-REACT patient responses for compliance and knowledge assessment are summarized in the Table. We recognized equipment deficiencies and recommended changes to existing equipment. Vest settings were revised in 17, equipment upgrades were required (i.e., revision of Vest garment size) in 20 patients and new compressors in 13 patients.

Comparison of % predicted FEV1 pre- and post-REACT (1-3 months) were not significantly different by paired t test.

Conclusion: Educating all members of the CF center consolidates a team approach towards improving adherence towards airway clearance therapy. Equipment factors do affect adherence. A detailed review of ACTs with targeted education by RT and other staff members may reinforce compliance to therapy.

This project was supported by the Second Learning and Leadership Collaborative Adult Quality Improvement of Cystic Fibrosis Foundation.

Pre-REACT Knowledge and Compliance Responses

How often are you doing your airway clearance	None-4, once - 10, twice - 8, three times - 2
Time spent doing airway clearance	None -4, 15 mins -2, 20 mins - 8, 30 mins-10
correct cleaning method	No - 7 Yes - 17
exercise as primary mode of ACT	No - 9 Yes - 15
Type of ACT	CPT-0% Flutter - 0% Acapella - 13% Vest - 88%
Recommended frequency of use	Daily - 62.5% only when sick - 12.5% only when I feel like it - 12.5% other - 12.5%
Prescribed length of therapy	20 mins - 62.5%, 20-30 mins - 12.5%, 45 mins - 12.5%, no answer - 12.5%
Recommended daily use	Once - 50% twice -50% three - 0%
Should you increase frequency when sick	Yes - 100% No - 0%
Perception of effectiveness of ACT	Yes - 87.5 % No - 12.5%
Issues that prevent ACT compliance	Yes - 75% No - 12.5% No answer - 12.5 %

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PATIENT-PERCEIVED BARRIERS TO RECOMMENDED CARE AT THE ADULT INTERMOUNTAIN CF CENTER: A CROSS-SECTIONAL STUDY

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Improving the delivery of quality care in the U.S. is a national priority. As more Americans live with chronic diseases the need for quality health care intensifies to promote health, preserve quality of life, and reduce economic burden. The CF care center network is regarded as an efficient and effective model of care delivery; however the adherence to care guidelines remains well below the national goal.

Background: CF is a complex and challenging disease to treat from diagnosis through end of life care. Each stage of life presents new obstacles. Known barriers specific to CF care include a high degree of non-compliance, cost of care, limitations of insurance coverage, treatment burden, and patient perceptions. The identification of obstacles is the first step to overcoming barriers and improving adherence to care.

Objective: Identify patient-perceived barriers to recommended care at the Adult Intermountain CF Center.

Methods: An anonymous questionnaire was distributed to 195 patients that received care at the Intermountain CF Center in 2010 or 2011. Questionnaires were collected from February to March 2012.

Findings: Eighty-four (43%) patients responded. Respondent demographics included 53.1% female and 46.9% male, 36.6% were older than 36 years, and 3.7% had an FEV1 <25%. Cost and travel were found to be the greatest barriers. Travel findings included: 38.7% travel over 50 miles, 12% over 250 miles; 25.9% agreed that travel costs keep them from attending quarterly visits. Coverage responses included: 78.2% reported private coverage, 36.4% had at least two policies, 7.7% reported no coverage, and 38.2% did not have in-network coverage. Additional cost responses: 84.3% reach their deductible, 23.4% agreed that co-pays were too expensive to attend quarterly, and 14% mentioned coverage and costs as greatest barrier to clinic visit.

Additional barriers to quarterly visits were reported: 19.5% agreed they only need visits when sick, 17.1% agreed they do not attend quarterly because they are too healthy, 41.5% agreed that the visit takes too much time, and 26% mentioned difficulty taking time off work/schedule as greatest barrier. In free response questions respondents expressed dissatisfaction with overall length of the visit, discussing identical issues with multiple team members, billing and insurance, and appointment availability. Most respondents included unsolicited expressions of appreciation and satisfaction.

Conclusions: The identification of barriers is the first step to improving the delivery of quality care. The most commonly cited barriers, travel and costs, are difficult to change on the provider level. However, five feasible provider-focused recommendations were developed. Each recommendation addressed the value of the patient-provider/team relationship. Improving the quality of care is ultimately about improving outcomes by helping patients navigate responsible choices. Although this questionnaire focused on the Intermountain CF Center, many commonalities likely exist with centers throughout the nation.

Co-Investigators: Holly Carveth, MD and Katie McDonald, PhD, RD.

Acknowledgments: Theodore Liou, MD, Teresa Kreger, RT, Jennifer Kinsfather, RN, and Cathy Loring.

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USING SURVEYS OF PATIENTS' EXPERIENCES AND SATISFACTION WITH CF CARE FOR QUALITY IMPROVEMENT: A SINGLE CENTRE EXPERIENCE

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Quality management programmes were implemented at the CF centre in Innsbruck (IBK) more than ten years ago, including regular ISO 9001:2000 certificates since 2006. To account for the patients' opinion on our services, we prospectively evaluated the experience and satisfaction of parents, CF adults, and adolescents, and used the results for quality improvement.

Poster Session Abstracts

Methods: In 2009, we participated in a pilot study (1) to develop a CF specific patient's experience and satisfaction questionnaire. Two years later, a nationwide postal survey was performed in Germany using the same instrument. The centre in IBK also took part in that survey, so that we were able to compare our results of 2011 and 2009 with those of the German study in 2011 with 2104 participating adults and parents. For adolescents, an established questionnaire (2) was used to evaluate their health care preferences with a focus on physician-patient communication.

Results: In 2009 and 2011, 63 and 76 participants from IBK (both parents and adult patients) responded to the surveys (response rate in 2011: 78%). Ideal problem scores of 0%, indicating no problems for the respective topic, were reported in 2009 for 15% and 19% of all items by adults and parents, respectively. The thirteen adolescents reported more problems (mean PS: 28.0%) than adults or parents (mean PS 12.1% each). After quality improvement measures had been implemented, the percentage of ideal scores increased to 32% and 48% (adults and parents) in 2011. Some areas that still rated low in performance were "inadequate support to meet other patients/ families" (PS 62%, reflecting the segregation policy of our centre), "inadequate information on side effects of drugs" (PS 48%), or "delayed information about diagnostic findings" (PS 46%). For adolescents, several steps to improve physician-patient communication were taken after the first survey, leading to considerably better follow-up results in 2011 (mean PS: 22.0%). Compared to Germany (response rate 74%), results from IBK were within the top 16% range for 31% (adults) and 41% (parents) of items, while only 3% of items had problem scores in the worst 16%. When items were grouped into factors by regression analysis, results from 9 out of 10 factors had lower problem scores in IBK than in Germany. Participants from IBK gave particularly favourable feed-back for treatment and services on the ward, psychosocial staff, organisation of the outpatient clinic, and nurse-patient-interaction.

Conclusion: The CF specific questionnaire identified strengths and weaknesses of patient care "through the patients' eyes." After improving procedures, lower problem scores in the 2011 IBK survey indicated the success of certain measures. The generally positive feedback might be due to the systematic approach and structured processes developed at our centre through quality management. We will continue to use patients' views to assess our performance.

Supported by CF-TEAM Forschung, Innsbruck, Austria, and Mukoviszidose e.V., Bonn, Germany.

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PATIENT ACCEPTANCE AND SATISFACTION WITH COMPLETE SEGREGATION WHILE HOSPITALIZED

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Introduction: Complete segregation of inpatients with cystic fibrosis became the infection control policy (IC) at Children's of Alabama and the University of Alabama at Birmingham (COA/UAB) pediatric CF center in July of 2010. The old guidelines restricted only patients with *B. cepacia* to their room. All other patients were allowed access to the cafeteria, physical therapy, and child life rooms with limitations. The CF team anticipated that the change in IC to completely segregate all patients to their room would be met with resistance by patients.

Purpose: To determine patient satisfaction with hospitalization after the change in IC and to determine patient acceptance of complete segregation while hospitalized.

Methods: A 14 item multiple-choice and open-ended question survey was developed and distributed in electronic form to 36 patients over a 6 month period. The section of the survey pertaining to the acceptance of segregation was adapted from a survey by Kate Russo. Patients chosen to complete the survey had been hospitalized before the change in IC in 2010 and were currently hospitalized at time of completion.

Results: A total of 44% (16) of 36 patients completed the survey. All patients were older than 6 years of age with 67% of patients ranging in age from 14 to 21 years. Sixty-three percent of patients had been hospitalized less than 3 times in the past year. A majority (56%) of patients reported that hospitalization since initiation of the segregation experience had improved. Patients also reported no change or an improvement in services from the various disciplines involved in care delivery. Eighty-eight percent of patients

reported understanding the need for the change in IC and 50% reported better protection from infections since the change. Sixty-nine percent reported that the change added little to no stress to their hospitalizations. Despite this reported stress level, comments from 75% (12) of patients indicated a high degree of frustration with segregation to the room. Fifty percent (8) of patients made suggestions for improvement which included changes in food services, increase in physical therapy services and increased availability of activities for use in the patient room.

Discussion: The results from the multiple choice portion of the survey were contrary to the assumption of the team with patients reporting high levels of satisfaction with hospitalizations and low stress levels secondary to segregation to their rooms. However, the open-ended questions revealed a high degree of patient frustration with limited suggestions for improvement. The discrepancy in the results may be related to the test design. Patients may have chosen a multiple choice answer which indicated satisfaction with the change in IC while taking the opportunity to express their feelings in the more subjective open ended questions. Nonetheless, these results are the basis for development and implementation of change ideas to improve the experience of patients with CF during their hospitalization. We plan to continue soliciting patient and family feedback as new ideas are tested.

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USING THE NEW VITAMIN D RECOMMENDATIONS TO IMPROVE VITAMIN D STATUS IN PATIENTS WITH CF

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Background: Vitamin D deficiency is common in individuals with cystic fibrosis (CF). The CF Foundation established recommendations for vitamin D supplementation in 2002 (1) which were revised in 2005 (2) and again in 2012 (3). We report the results of a quality improvement project with intervention based partly on previous recommendations and partly on the 2012 CFF recommendations.

Methods: In 2011 we changed our CF center policy to recommend cholecalciferol (D3), either 1,000 or 2,000 units/day in addition to CF-specific vitamins for all patients. Our CF center is located at latitude 42° 53' 11". In this quality improvement project, our specific aim was to check 25-OH-vitamin D levels on 100% of patients followed at our CF center in the first quarter of 2012. If 25-OH-vitamin D was < 30 ng/mL we increased vitamin D3 supplementation per the new CFF vitamin D guidelines. Our second specific aim was to recheck 25-OH-vitamin D levels in 100% of patients who needed additional supplementation three months after the new dose was started.

Results: Of the 92 pediatric patients at our CF Center only 4 were not taking supplemental D3; of the 73 adult patients, 8 were not taking supplemental D3. One hundred thirty-two of 165 (80%) of patients had a 25-OH-vitamin D level checked in the first quarter of 2012; of these, 100% were taking their CF vitamin plus step 1 of the new treatment recommendation (3). Fifty-five of 132 (42%) had 25-OH-vitamin D levels < 30 ng/mL and were started on either a higher Step 1 dose or a Step 2 dose of D3. At the time of this abstract submission, 11 of these 55 (20%) had their blood rechecked approximately 3 months after dose increase; others have not yet been on treatment for three months. Of the patients whose 25-OH-vitamin D level was re-checked, 6 (55%) had 25-OH vitamin D levels > 30 ng/mL. Those whose levels did not reach this goal were increased to Step 2 supplementation. Updated results will be presented at the meeting.

Conclusion: Despite recommending supplemental vitamin D3 (1,000 or 2,000 IU/day) in addition to CF-specific vitamins, vitamin D status at our CF Center is suboptimal. This may be because patients do not adhere to prescribed recommendations or because this amount of vitamin D supplementation is inadequate. We came close to but did not meet our quality improvement goals. Based on our initial data we conclude that: 1) most patients need at least Step 1 supplementation with vitamin D3; 2) it is important to check 25-OH-vitamin D levels to assure the routine doses are adequate; and 3) it is important to re-check levels after dose escalation to assess whether or not further intervention is required.

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New Vitamin D3 Recommendations

Age	Routine Dosing with CF-specific vitamins (IU)	Step 1: Dose Increases (IU)	Step 2: Dose titration maximum (IU)	Step 3
Birth-12 months	400-500	800-1,000	Not >2,000	Refer
> 12 mo to 10 yrs	800-1,000	1,600-3,000	Not > 4,000	Refer
>10-18 years	800-2,000	1,600-6,000	Not >10,000	Refer
>18 years	800-2,000	1,600-6,000	Not >10,000	Refer

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CF CARE THROUGH THE PATIENTS' EYES – A NATIONWIDE SURVEY ON PATIENTS' EXPERIENCE AND SATISFACTION WITH SERVICES USING A CF-SPECIFIC QUESTIONNAIRE

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Finding out how patients and families experience the care provided by CF staff is important for improving the quality of services. In 2009, we developed a CF-specific patients' experience questionnaire from interviews with focus groups of patients and parents (1). After a pilot study in four centres (2), we used a revised form with 110 items for a nationwide survey in Germany.

Methods: Ninety CF centres in Germany were invited to participate. Centre staff informed their patients about the survey, and collected patient consent forms. The patients' addresses were sent to the Picker Institute Germany, the non-for-profit organisation which conducted the investigation. Items had between 3 and 6 response categories, which were dichotomised to a "problem score" (PS), indicating the presence or absence of a reported problem. A score of zero percent would be ideal. Adolescents aged 12 to 17 years reported their health care preferences and experiences using an established questionnaire (3).

Results: Fifty-six CF centres took part in the survey. Of 1642 adults and 1205 parents recruited, 74% in each group responded to the questionnaire. Regression analysis revealed 10 different factors covering 70 of the 110 items. Adults and parents reported the best results for the factors "physiotherapists" (PS 6%), and "physician-patient relationship" (PS 9%). The highest problem scores were reported for inpatient and outpatient "facilities and services." Items with the highest problem scores were "not enough information on possible side effects of drugs" (PS 49%) and "inadequate support to meet other patients/ families" (PS 46%). Adolescents (n=359) reported good experiences concerning "My doctor..." "is always completely honest with me" (PS 5%), "has a lot of experience taking care of people with my condition" (PS 6%), or "always does what is best for me" (PS 9%). Low satisfaction was reported for items like "My doctor..." "always lets me decide on treatment issues when I want to" (PS 81%), or "always lets me decide what to talk about in a visit" (PS 57%). The problem scores from participating institutions differed considerably. Centers received reports showing both their individual results and the mean problem scores of all participating centres. A priority matrix allowed identifying areas that rated low in performance, but high in importance. After reviewing the survey results together with patient representatives, staff will be able to implement measures that should improve the quality of centre care.

Conclusions: The survey with CF-specific items identified problems with care as well as areas with high patient satisfaction. Results can be used as a tool for quality improvement.

Supported by: Mukoviszidose e.V. (the German CF Trust).

We thank patients and staff from the 56 centres for participating in the survey.

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SUPERHERO OR VILLAIN: CHANGING PERCEPTIONS ABOUT GASTROSTOMY TUBES IN PATIENTS WITH CYSTIC FIBROSIS

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Background: Optimal nutrition is critical in CF as it is intimately related to lung function. A gastrostomy tube (g-tube) can be a useful tool for gaining and maintaining weight. Despite its known utility in improving nutrition, our center had only 5/98 patients with g-tubes as of 5/2011 (5.1%). In addition, a pilot survey identified that many of our patients had misconceptions about g-tubes and viewed them as a "last ditch effort." The purpose of this quality improvement (QI) project was to educate our patients about g-tubes and to change the culture surrounding this intervention at our center.

Methods: We developed a survey querying our patients' and parents' knowledge of and perceptions about g-tubes. It contained demographic information and eight 10-point Likert scale or multiple choice items about indications for a g-tube, self-reported knowledge level about g-tubes, and perceptions about the procedure. We administered the survey for two three-month periods surrounding our six-month educational intervention. After reviewing available educational materials about g-tubes, our multidisciplinary team developed two handouts. Handout #1 provided general information for all patients about g-tubes, regardless of nutritional status. Handout #2 was more detailed, and it focused on frequently asked questions about g-tubes for those with BMI <25%ile. We also developed relevant talking points about g-tubes and nutrition for each provider (MD, dietitian, RT, PT, counselor, RN), which were used at one visit for each patient during our six-month intervention period.

Results: Forty-five and 27 patients and families returned the pre- and post-education surveys, respectively. Self-reported knowledge about g-tubes increased from 3.7/10 before the intervention to 5.8/10 after. In response to the question "If a g-tube were a movie character, would it be a 'good guy' (10) or a 'bad guy' (1)?" pediatric patients' score increased from 6.5 to 8.4. Sixty-two of 98 patients received g-tube education during the study period. Of 19 patients with BMI <25%ile without g-tubes who came to clinic, 9 were offered g-tubes; 3 of those had g-tubes placed. Of our 8 patients with g-tubes by the end of the study period, 7 saw an increase in their BMI (mean 14.9 pre, 15.5 post). The eighth patient, whose BMI decreased from 19.8 to 16.8 during the study period, had a g-tube placed and then removed within one month due to bleeding and discomfort.

Conclusions: Face-to-face education coupled with targeted educational materials was modestly effective in improving self-reported knowledge about g-tubes at our center. It also had a positive effect on perceptions about the procedure, especially for children. Our patients with g-tubes generally saw a modest improvement in their BMI, but our numbers were small and our study period short. We will consider adopting this educational method to address other topics. We plan to explore further the reasons for hesitating to offer and accept a g-tube at our center.

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SYSTEMATIC IMPLEMENTATION OF BEST PRACTICES IMPROVED PHYSICIAN ADHERENCE TO CLINICAL CARE GUIDELINES

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Background: There is significant variability in clinical outcomes, including growth and lung function, between the various cystic fibrosis (CF) centers. No specific or unique therapeutic practices have been found to account for these differences. However, more uniform care at centers is associated with better outcomes. Therefore consistent use of best practices within a CF center may improve outcomes.

Poster Session Abstracts

Objective: To implement best practices for diagnosis and treatment of nutritional failure and lung inflammation in order to achieve better health care provider adherence to the Cystic Fibrosis Clinical Practice Guidelines.

Methods: Best practice treatment plans for both nutrition and lower airway inflammation (LAI) were implemented on January 1, 2010. "Best practices" were based on CF Foundation guidelines, evidence-based medicine, as well as feedback from the local CF Family Advisory Board. Best practices were developed and agreed on by all members of the health care team at the CF center at the University of Iowa. Diagnoses for nutritional status and lung inflammation were based on objective criteria and each diagnosis was associated with specific best practices. The main outcome measure was if patients' medications/treatments were consistent with the agreed upon best practices for their diagnosis. Two tools were utilized to help increase adherence to best practices and were tracked as secondary outcome measures: (1) use of the correct diagnoses; and (2) if issues pertinent to the best practice plans were discussed in the "Plan" section of the clinic note. Health care provider adherence to utilizing diagnoses, treatment plans consistent with these best practices, and discussing this in the clinic note were measured by reviewing medical charts from July 2009 – June 2011. The proportion of clinic encounters consistent with the best practice plans was calculated and compared over quarterly time-points. Periodic feedback with provider-specific data was used to help increase adherence to best practices.

Results: The proportion of clinic visits where the provider completely followed nutrition best practices increased from 60.11% to 79.19% ($p=0.09$), and LAI best practices increased from 65.62% to 85.36% ($p=0.02$), from baseline to 18 months post-intervention. The use of nutritional diagnosis and its discussion in the clinical plan increased from 14.86% to 65.17% and 63.14% to 91.93%, respectively. Similarly diagnosis of LAI and its discussion increased from 1.29% to 50.24% and 27.06% to 85.33%, respectively.

Conclusion: Implementation of best practice plans for nutritional and pulmonary issues in CF resulted in more uniform care of CF patients. Having objective criteria for diagnoses and agreed-on treatment plans for each of those diagnoses allowed for monitoring and individual feedback. Increases in utilization of correct diagnoses and discussion of the best practice in the clinic note were associated with improvements in physician best practice adherence.

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INDIVIDUALIZED SELF-MANAGEMENT ACTION PLAN USING A STEPWISE APPROACH TO AIRWAY CLEARANCE TECHNIQUES AND HOME SPIROMETRY MONITORING IN CYSTIC FIBROSIS: STEP-UP TO BETTER BREATHING (SUBB)

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Background: Some chronic disease states use monitoring devices to help manage the disease process: one example would be a glucometer for daily glucose measurement. In cystic fibrosis (CF), the forced expiratory volume in the first second (FEV1) is an important value in the management of CF. Treatment and disease management decisions are made as a result of these values. Individuals with CF do not know their FEV1 value between center visits or how it is impacted by exacerbation or airway clearance (ACT) activities.

Objective: This study will provide FEV1 and exacerbation data to the individual on a daily basis. This information provides a foundation to make daily, self-management decisions on how to plan the course of the day in terms of stepping-up ACT and treatment needs.

Method: This is an observational study performed over a 2 to 3 month time period between center visits. Forty-four patients were given a PIKO 6 spirometer as well as exacerbation score (based on the Akron Children's Hospital exacerbation score). The patients were instructed to perform and measure FEV1 daily as well as a self-assessment of exacerbation. These values were documented on their daily log. As a result, if the patient noted a decline in FEV1 or a rise in their exacerbation score, they would step-up

their ACT activities. If their exacerbation score reached 3 on a 10 point scale, for more than 3 days or reached 5 on the 10 point scale, they were instructed to call the center and report their data. CFQ-R scores were obtained at first visit and then at the end of the study. A six question post-study questionnaire was given at the end of the study.

Results: The pre- and post-CFQ-R overall was: 73.34 pre-study, 72.01 post-study which does not appear to be a significant reduction in CFQ-R. The mean FEV1 pre-study was 2.39 L and post-study was 2.44 L. Minimal change was noted. The mean exacerbation score pre-study was 2.66 and post-study was 1.87 on a 10 point scale. This represents a 30% reduction in exacerbation score post study. When asked, "I feel the SUBB study helped me to understand my disease better," 20 of the respondents "agreed" and 13 "strongly agreed." When asked, "I understand and I am able to identify CF pulmonary exacerbation better since I participated in the SUBB study," 19 of the 38 respondents "agreed" and 14 "strongly agreed." When asked, "The SUBB Study helped me to identify the times I needed to step-up my airway clearance activities," 16 "agreed" and 16 "strongly agreed."

Conclusion: Using home spirometry may help to identify early exacerbation in CF. Home spirometry and daily assessment of exacerbation helped patients to see directly the impact of pulmonary exacerbation in their lives. While the results were positive overall, further studies such as a blinded, multi-center study should be accomplished to further investigate results.

*This study was a part of the CF Foundation AQI2 Program.

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THE RELATIONSHIP BETWEEN PATIENT KNOWLEDGE AND HEALTH MAINTENANCE COMPLIANCE IN ADOLESCENT PATIENTS WITH CYSTIC FIBROSIS

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Background: As the number of adults with cystic fibrosis (CF) continues to rise, optimizing transition of care from pediatric to adult providers has become a priority. Little is known about the factors that predict a successful transition of care. Transition can be hindered by inadequate education leading to poor self-management; improving patient understanding of disease is therefore an important component to address. Several self-management programs have focused on improving participants' disease knowledge. To better understand the relationship between compliance, health status and knowledge in our adolescent CF population, we assessed knowledge of disease in our CF center and examined the correlations with our adolescents' overall health and compliance.

Materials and Methods: Patients with cystic fibrosis age 13-20 years completed a knowledge survey about their disease ($n=38$). Scores were generated in four domains: Lung health, Nutrition health, CF health, and Treatment. Overall score and scores in each domain were then correlated to patient data including BMI, FEV1, use of nutrition supplements, and presence of gastrostomy tube. Scores were also correlated to compliance with obtaining sputum cultures, pulmonary function testing (PFTs), and dietary visits at the appropriate intervals. Results were analyzed using both T test and linear regression using the STATA program.

Results: BMI had no significant correlation with either the overall score or Nutrition health score on the knowledge survey. A higher overall score correlated with decreased use of nutritional supplements at a level approaching significance ($OR=0.94$, $Z=1.7$, $p=0.086$). Higher overall knowledge scores significantly correlated with regular attendance at dietary visits ($OR=1.1$, $Z=1.95$, $p=0.05$). The presence of a gastrostomy tube, supplement use, and attendance at dietary visits had no significant correlation with Nutrition health score. FEV1 had no significant correlation with overall score, CF health score, or Lung health score. Higher overall and Treatment knowledge scores significantly correlated with timely completion of PFTs (overall- $OR=1.1$, $Z=2.3$, $p=0.02$; treatment- $OR=1.1$, $Z=2.3$, $p=0.02$). Higher overall and Treatment knowledge scores also significantly correlated with timely completion of routine cultures (overall- $OR=1.1$, $Z=2.3$, $p=0.024$; treatment- $OR=1.1$, $Z=2.2$, $p=0.026$).

Discussion: Patients who are compliant with attendance at routine clinic appointments (surrogates being attendance at dietary visits and completion of routine PFTs and sputum cultures) have better knowledge of their disease. Unfortunately, this does not translate into better controlled disease as FEV1 or BMI are not affected. Targeting improvement of patient education and knowledge may help improve compliance of clinic attendance and

adherence of routine screening evaluations. Our hope is that the knowledge survey can allow us to plan our educational goals for our teens and improve their self-management and success of transition of care.

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IMPROVING LUNG FUNCTION IN CF CHILDREN

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Background: In 2001 we began a long-term project to improve the pulmonary status of the children in our center by emulating the practice pattern characteristic of centers with median percent predicted FEV1 (MPPF) in the highest quartile. This association between lung function and practice pattern was first described in an analysis of participants in the Epidemiologic Study of Cystic Fibrosis in the late 1990s that subsequently was published in a 2003 article. Since the development of the Center-Specific Report (CSR) of the Cystic Fibrosis Patient Registry, each CF center has been able to compare its practice patterns and key clinical outcomes with two important benchmarks: the national average data and, for relevant variables, the ten highest centers' data. In 2001 our MPPF for 6 to 12 year olds was 8 below the national average and 19 below the ten highest centers, and for 13 to 17 year olds it was 6.3 and 15 below. Here we report the impact of efforts to improve lung function over a decade in 6 to 12 and 13 to 17 year olds in our center.

Methods: Since 2001 our CF team has met annually to review our CSR data, and based on that review of outcomes and practice pattern data, the team has agreed to specific changes in practice that might improve MPPF. The standards adopted over this decade included: a 10 week interval between visits, treatment of loss of FEV1 of 10% or greater, eradication of early *Pseudomonas* infection, early adoption of new preventive treatments, lung function testing at every visit, culture of airway secretions at every visit, minimum of 14 days for inpatient treatment and increased correction of nutritional deficiencies.

Results: Lung function of children in our center increased more rapidly than national data ($p < 0.0001$ in both age groups). MPPF increased linearly over the decade in two age groups (6-12 and 13-17) by linear regression analysis. The variables listed include the slope m , the intercept b and R^2 . MPPF in 6 to 12 years old UCSF patients ($m=2.45$, $b=79.2$, $R^2=0.92$) increased 2.5 more rapidly than the national average ($m=0.983$, $b=88.1$, $R^2=0.94$) and 5 times more rapidly than in the ten highest centers ($m=0.510$, $b=100.9$, $R^2=0.82$). In the 13 to 17 year olds, the MPPF in UCSF patients ($m=1.51$, $b=71.2$, $R^2=0.82$) increased 1.6 times more rapidly than the national average ($m=0.947$, $b=80.9$, $R^2=0.97$) and 1.7 times more rapidly than in the 10 highest centers ($m=0.875$, $b=92.0$, $R^2=0.93$). In 2010 MPPF for our 6 to 12 year olds ranked high in the upper quartile for all CF centers, and MPPF for our 13 to 17 year olds exceeded the national average.

Conclusions: These data strongly support the hypothesis that patterning care after the highest performing CF centers results in improved pulmonary outcomes in a single center. Improving lung function is seen first in younger patients, and observation times must be long to demonstrate significant sustained improvement.

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IMPROVING NUTRITION FOR PEDIATRIC CYSTIC FIBROSIS PATIENTS

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Objective: To improve the nutritional status of patients between 3 and 20 years of age in our cystic fibrosis (CF) center. We used median body mass index percentile (BMI%) as a marker of nutritional status, as it has been shown that being at or above the 50th percentile correlates with better pulmonary health. Our specific aim was to increase the median BMI of those patients below the 25th percentile by 20 percent in one year.

Methods: 1. We reviewed our center's CF Foundation patient registry data. 2. We chose to work on improving the nutritional status of our CF patients, with BMI less than 25th percentile as our primary outcome. 3. We instituted the use of a standardized care plan and an algorithm was developed. Daily caloric needs and weight gain goals were calculated by our dietitian and discussed with the team, patients and families. If there was difficulty in meeting weight gain goals, we escalated nutritional support in a

stepwise fashion starting with a high calorie diet and oral supplements. That was followed by appetite stimulants, additional social work and psychology intervention, more frequent visits, and the placement of G-tubes. Data from October 2009 through December 2010 were reviewed.

Results: The median BMI% for our center at baseline was 54 and remained stable over the evaluation period. The median BMI% for those below the 25th percentile at the start of the study was 13.5. At the end of the evaluation period, this increased to 18.9. This is a 40 percent increase in the median BMI% for this group of patients.

Conclusions: By teaming up with patients and families, standardizing care, providing a written plan with clear goals and developing and implementing a nutritional care algorithm, we were able to successfully improve nutritional outcomes in our highest risk patients.

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IMPROVING CYSTIC FIBROSIS CARE: USING A PATIENT AND FAMILY EXPERIENCE OF CARE SURVEY TO UNDERSTAND IMPORTANT CARE PROCESSES AT CF CENTERS

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Objective: In 2012, the Cystic Fibrosis Foundation in partnership with the Dartmouth Institute for Health Policy and Clinical Practice completed development of a national patient and family experience of care survey. The purpose is to provide CF Foundation accredited care centers with additional information beyond process and medical outcome measures to inform local improvement efforts to meet patient and family needs.

Methods: Twenty-five care centers across the United States recruited patients and family members to complete a survey. A pediatric version was completed by parents on behalf of individuals with CF under age 18 years and an adult version was completed by individuals with CF age 18 years or older. Series of survey codes were assigned in such a manner as to identify a CF center. Within a center each patient/family was assigned a unique code to protect their privacy. Respondents accessed the survey using a web-site or calling a toll-free number and using voice recognition software. Center-specific reports were generated that showed their positive response ratings for each question compared to overall and also across center comparison for those that had more than 10 respondents. The reports were distributed to all participating centers and they were asked to complete a follow-up survey to collect feedback on the report format and usefulness.

Results: Almost 500 patient and family responses showed differences across centers. The question of how often did the health care team treat you with courtesy and respect (adult survey), the overall rate for "always" or "almost always" was 96%; however one center had a significantly lower rate of 73%. Sixty-two percent of the adult respondents said their airway clearance treatments worked "always" or "almost always," which varied across centers from 14% to 86%. The center that had 14% was significantly lower. Center-specific reports highlighted significant results, which centers could consider as possible areas to target their improvement efforts. Across the 25 centers, the report feedback survey was completed by 45 individuals; 63% health care professionals and 37% patients and families. Areas most often cited for improvement include infection control, inpatient hospital care, and improving patient self-care education. The reports validated perceptions of the care at the center and generated hypotheses regarding burden of care and differences between patients seen frequently versus those seen less often.

Conclusion: A patient and family experience of care survey with center specific reports and comparisons provides additional feedback to health care professionals, individuals with CF, and families to continue to accelerate the rate of improvement in CF care.

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AN OUNCE OF PREVENTION – WHAT IS THE PREVALENCE OF FLU VACCINATION IN A LARGE ADULT CYSTIC FIBROSIS CLINIC

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The flu is a contagious respiratory illness caused by the influenza virus. People with CF are at high risk for serious flu-related complications. The best way to prevent the flu is to receive an annual vaccination during the flu season. People with CF are among those recipients on the highest priority list as recommended by the Canadian National Advisory Committee on Immunization. However, not all people with CF receive the flu vaccination each year.

Purpose: The objectives of this quality improvement (QI) initiative were to develop a strategy to ensure patients attending our ambulatory CF clinic were offered the flu vaccination; to track the proportion of individuals who received the vaccination; and to identify barriers to vaccination.

Methods: The Toronto Adult Cystic Fibrosis Program cares for 400 adults with CF; on average 32 patients attend the ambulatory clinic each week. A standardized form was developed to track flu vaccinations in our clinic during the flu season (October to March). The form was completed by medical staff; data included whether the flu vaccine was offered to the patient, if it was received and where (CF clinic vs. other site); and where applicable, the reasons for not receiving the flu vaccination. The form was printed on distinct purple paper and was inserted into the charts prior to clinic. At the end of each clinic, the forms were collected and results were tabulated weekly.

Results: Baseline data from the 2010 flu season showed that 68% of the patients seen were offered the flu vaccine; and a total of 192 patients received the flu vaccine during the 2010 flu season. During the 2011 flu season, the number of patients offered the flu vaccine increased from 78% in the first month of the flu season to 91% in the last month, with an overall average of 86%. During the 2011 flu season, 280 patients attended the clinic; 207 of them received the flu vaccine. Of those, 53% received the vaccination during a CF clinic visit; 45% received the flu vaccine from their family physician; and 2% at work. Six patients had no record of whether they received the flu vaccine or not. Among the 67 people who did not receive the vaccination, 61% refused; 3% did not have time to receive the vaccination; 3% reported an allergy to eggs and/or to flu vaccination; and 33% selected "other" as the reason, which primarily was due to active pulmonary infection. Of the 41 people who refused the vaccine, 37% did not provide a detailed explanation; 32% stated they felt worse after the vaccination in previous years; 22% did not believe in the benefit of the flu vaccination; 2% were advised by another physician not to receive the flu vaccination; and 7% did not provide any comment.

Conclusions: The number of individuals in our clinic who were offered and received the flu vaccine increased over the study period. This project allowed us to implement a systematic process for evaluating whether or not patients received the flu vaccination. Several knowledge gaps about the benefits of the flu vaccination were identified; future QI projects will focus on education around the flu vaccine and thereby maximizing flu vaccinations in our patient population.

GI/NUTRITION

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BOTH CFTR-DEPENDENT AND -INDEPENDENT HCO₃⁻ SECRETION REDUCE MUCUS VISCOSITY IN WT MOUSE INTESTINE

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Background and Aim: The normal mucus layer coating the gastrointestinal (GI) tract is one of the first lines of innate host defense. However, in cystic fibrosis (CF), mucus obstruction always occurs in the distal small

intestine, not in the colon, even though the colon has more goblet cells and a thicker mucus layer, which indicates that the HCO₃⁻ secretion critical for mucus formation in the colon may not be limited to CFTR-dependent transport. Therefore, we hypothesize that both CFTR-dependent and -independent HCO₃⁻ secretion can function similar to CFTR-dependent HCO₃⁻ secretion in regulating mucus viscosity.

Methods: pH-stat and Ussing chamber techniques were used to measure HCO₃⁻ secretion. Segments of distal small intestine (ileum) and colon from C57BL/6 WT mice were incubated with stimulants in vitro in either NaCl (no HCO₃⁻) or NaHCO₃ (with HCO₃⁻) Ringer's solution. Mucus samples were collected from the incubated intestines and analyzed by a spot tracking method for mucus viscosity.

Results: Serotonin (5-HT) and prostaglandin E₂ (PGE₂) were used to stimulate HCO₃⁻ and mucus secretion. 5-HT (10 μM) and PGE₂ (1 μM) induced significant HCO₃⁻ secretion in the WT mouse ileum and distal colon, 0.79 ± 0.26 mM/cm²/h (n=3) and 0.76 ± 0.22 mM/cm²/h (n=3), respectively. However, CFTR inhibitor, glyH-101 (20 μM) significantly inhibited 5-HT and PGE₂-induced HCO₃⁻ secretion in the ileum (0.13 ± 0.12 mM/cm²/h, n=3; p < 0.05), but not in the distal colon (0.56 ± 0.11 mM/cm²/h, n=3; p > 0.05), suggesting that CFTR-independent HCO₃⁻ secretion is present in the distal colon, while HCO₃⁻ secretion in the ileum is CFTR-dependent. Furthermore, we tested the effect of secreted HCO₃⁻ on the viscosity of mucus secreted from the mouse ileum and distal colon, respectively. The viscosity of mucus from both ileal and distal colonic sacs incubated in NaHCO₃ Ringer's solution was significantly lower than the mucus from sacs incubated in NaCl Ringer's solution (ileum: decreased 77.5%, n=4, p < 0.05; distal colon: decreased 83.6%, n=4, p < 0.05), indicating that HCO₃⁻ secretion decreases the mucus viscosity in both the ileum and distal colon.

Conclusions: Both CFTR-dependent and -independent HCO₃⁻ secretion exist in the intestinal tract. Similar to CFTR-dependent HCO₃⁻ secretion, CFTR-independent HCO₃⁻ secretion also decreases mucus viscosity in the WT mouse intestine.

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NEWBORN CYSTIC FIBROSIS PIGS AND FERRETS HAVE FATTY ACID ALTERATIONS SIMILAR TO HUMANS

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Altered fatty acid composition is well-described in humans with cystic fibrosis (CF). Prominently, linoleic acid (LA, 18:2ω6) levels are diminished in plasma and tissues that express cystic fibrosis transmembrane conductance regulator (CFTR). Other fatty acid alterations include low plasma docosahexaenoic acid (DHA, 22:6ω3) and high eicosatrienoic acid (20:3ω9). Arachidonic acid (AA, 20:4ω6) is usually not elevated in plasma, but is so in cells and tissues where its release is increased, suggesting a role in the excessive inflammatory response in CF. To determine whether plasma and tissue lipid profiles were altered in CF pigs and ferrets as in humans with CF, the plasma of newborn CF (CFTR^{-/-}, n=6) and non-CF ferrets (CFTR^{+/+} and CFTR^{+/-}, n=6) and the plasma and livers of CF (CFTR^{-/-} and CFTRΔF508/ΔF508 n=18) and non-CF (CFTR^{+/+} and CFTR^{+/-}, n=13) pigs were analyzed by electrospray ionization tandem mass spectrometry phospholipid profiling (ESI-MS/MS) and ESI-MS/MS plus capillary gas-liquid chromatography fatty acid composition, respectively. Just as in humans, plasma LA was significantly decreased in CF pigs and lysophosphatidylcholine containing LA was decreased in CF ferrets. Plasma eicosatrienoic acid and palmitoleic acid (16:1ω7) were significantly increased and AA was unchanged in newborn CF pigs compared to non-CF.

Hepatic LA was significantly lower and eicosatrienoic acid and AA were significantly higher in CF, compared to non-CF pigs. Likewise, LA content was significantly lower and AA content was significantly higher in the hepatic lysophosphatidylcholine and lysophosphatidylethanolamine of CF pigs. The hepatic expression of $\Delta 6$ desaturase, $\Delta 9$ desaturase, elongase 6 were unchanged between the genotypes, but the $\Delta 5$ desaturase expression was increased ($p=0.08$) and the $\Delta 5/\Delta 6$ desaturase ratio was significantly higher in CF pigs ($p=0.004$). These results indicate that fatty acid composition is altered in CF pig and ferret plasma and tissue phospholipids, even in the newborn period, in a similar fashion to humans. These changes may play a role in disease pathogenesis as may altered fatty acid desaturase expression.

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EARLY EXOCRINE PANCREATIC DAMAGE DETERMINED BY SERUM IMMUNOREACTIVE TRYPSINOGEN IS A SIGNIFICANT PREDICTOR OF CF-RELATED DIABETES AT A LATER AGE

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Introduction: It has been posited that decline of serum immunoreactive cationic trypsinogen concentration (IRT) with age acts as a surrogate marker of exocrine pancreatic damage in CF patients. Exocrine pancreatic damage in turn contributes to the development of CF-related diabetes (CFRD) later in life. We set out to use longitudinal serum IRT measurements to estimate trypsinogen at birth and its rate of decline, and then test the hypothesis that IRT at birth and its rate of decline can predict CFRD.

Methods: We used serial IRT levels collected from CF diagnosis on 129 CF patients with severe CFTR genotypes known to be associated with pancreatic insufficiency. CF diagnosis was determined by characteristic clinical manifestations coupled with at least two elevated sweat chloride measurements. A baseline measurement of IRT within the first 24 months of life was required for inclusion; however the number of measurements per person ranged from 1 to 16, up to 23 years of age. Serum IRT levels were determined by the double-antibody radioimmunoassay technique in subjects with CF. To determine whether the risk of CFRD was associated with IRT, we used both longitudinal linear mixed models that simultaneously account for the limits of detection of the assay (values less than 3 ng/mL), and Cox proportional hazards regression. After natural log transformation of IRT and age to satisfy linear model assumptions, we estimated subject-specific IRT measurements at birth (intercept) and rate of decline, and used these as predictors of CFRD in a Cox proportional hazards model to account for the time-to-event nature of CFRD.

Results: Thirty-seven of 129 subjects had been diagnosed with CFRD. The subject specific IRT intercept was a significant predictor of CFRD ($p=0.004$), where the hazard ratio for a one unit change in the estimated log(IRT) at birth is 0.534. Therefore, it is estimated that there is a 46.7% decrease in the risk of CFRD for every one unit increase in log(IRT), where the interquartile range of log(IRT) is 2.28 to 4.38.

Conclusion: An individual's estimated IRT level at birth, but not its rate of decline, is a significant predictor of later-onset CFRD. Future work will determine the predictive ability of IRT values from newborn-screened measurements.

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RESTING ENERGY EXPENDITURE AND RESPIRATORY QUOTIENT IN CHILDREN WITH CF AND PI RECEIVING HIGH CALORIE AND FAT DIETARY INTERVENTION

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Background: The treatment of fat malabsorption and growth failure in children with cystic fibrosis (CF) and pancreatic insufficiency (PI) is a challenge for the care team. A readily absorbable lipid matrix (LYM-X-SORB™ [LYX]) has been shown to improve growth and nutritional status in children with CF in a preliminary study. Resting energy expenditure (REE), known to be elevated in subjects with CF, is hypothesized to decrease as the nutritional status improves.

Objective: To determine the 12-month effect of a novel organized lipid matrix compared with a placebo with similar high calorie and fat content on REE and RQ.

Method: Subjects (5 to 18 yrs) participated in a 12-month double-blind randomized placebo-controlled supplementation trial of LYX. Both supplements had similar calorie (303 or 456 kcal/d) and fat content (11 or 18 g/d) but different choline (LYX: 591 or 887mg/d v. placebo: 78 or 117mg/d); dose depended on age. REE was evaluated by indirect calorimetry at baseline, 3 and 12 months and analyzed as a percentage of Schofield predicted REE. Height and weight were measured and BMI and Z-scores calculated. Fat mass (FM) and fat free mass (FFM) were assessed by a whole body DXA scan. A longitudinal mixed effect model was used to analyze changes in REE kcal/day over time adjusted for FFM and FM.

Results: In 63 children (57% males, age [mean±SD] 10.6±2.9, FEV₁% 96±22), REE measurements were performed at baseline, 3 and 12 months. Mean baseline REE was 109±8% of predicted REE for all subjects. No significant difference in REE or RQ between the LYX (n=27) and placebo (n=36) groups was observed over time so data were pooled. In the total sample REE kcal/day significantly decreased at 12 months ([mean±SE] -32±12 kcal, $p=0.009$) after adjustment for FFM and FM. The decrease in REE was significant in males (-49±16 kcal, $p=0.002$) but not females (-8±19 kcal). Growth significantly improved and RQ significantly increased in both sexes with both supplements over time.

Conclusion: REE decreased in males and RQ increased in all children after 12 months of supplementation. These changes were accompanied by improvement in growth status and balanced increases in FFM and FM. These data suggest that better nutrition status may have decreased inflammation, work of breathing or changed other contributors to daily energy needs.

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Mean±SD	Males (n=36)			Females (n=27)		
	Baseline	3 mo	12 mo	Baseline	3 mo	12 mo
HAZ	-0.4±0.7	-0.3±0.7**	-0.3±0.8**	-0.6±1.1	-0.5±1.1*	-0.5±1.0*
WAZ	-0.4±0.7	-0.3±0.7**	-0.4±0.9	-0.5±0.7	-0.3±0.8**	-0.3±0.8*
BMIZ	-0.2±0.6	-0.1±0.7*	-0.2±0.9	-0.2±0.8	0.0±0.8**	-0.0±0.8
% pred. REE	109±8	108±8	106±8*	108±8	110±7	107±10
RQ	0.86±0.07	0.88±0.05	0.90±0.06*	0.86±0.06	0.88±0.07*	0.89±0.05*

Significantly different than baseline by t-test * $p<0.05$, ** $p<0.01$

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FAT SOLUBLE VITAMIN STATUS IN CHILDREN WITH CF AND PI RECEIVING A CALORIE AND FAT INTERVENTION

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Background: Subjects with CF and PI are at risk for fat soluble vitamin deficiency. A unique lipid matrix that facilitates fat absorption may impact fat soluble vitamin absorption.

Objective: To assess the changes in fat soluble vitamin status in children with CF and PI receiving 12 months of daily supplementation with LYX-SORBTM (LYX) compared to a placebo with similar calorie and fat content.

Methods: Subjects with CF and PI participated in a 12-month double-blind randomized placebo-controlled supplementation trial of LYX vs. placebo. Both supplements had similar calorie (303 or 456 kcal/d) and total fat content (11 or 18g fat/d) with the same 5:1 ω6: ω3 fat ratio, and no fat soluble vitamins except for α-tocopherol which was higher in LYX than placebo (4.8 and 1.6 mg/d, respectively). Serum vitA (retinol), vitE (α-tocopherol, γ-tocopherol, α-tocopherol:cholesterol ratio), vitD (25OHD), vitK (undercarboxylated osteocalcin [%ucOC] and plasma proteins induced by vit K absence-factor II (PIVKA II)) levels were assessed at baseline, 3 and 12 months. Dietary and supplemental intakes of fat soluble vitamins were assessed by a 3 day weighed food record and DRIs were calculated as %RDA and %AI.

Results: 70 subjects (30 females, mean 10.4±2.9 years) completed the 12 month LXS (n=31) or placebo (n=39) trial. At baseline 8% were vitK deficient based on serum %ucOC >50%, 19% were deficient based on PIVKA II levels >2ng/mL. 4.5% were vitD deficient with serum levels <20ng/mL. No one was vitA deficient (<20 ng/dL). 17% had low α-tocopherol levels based on an α-tocopherol:cholesterol ratio <5.4 mg/g. Dietary (food) and supplemental (vitamin pills) intakes were respectively 186%+602% RDA for vitA, 50%+263% RDA for vitD, 124%+1661%RDA for vitE, 112%+1225% AI for vitK. Overall retinol levels increased significantly by 3 and 12 months after adjusting for dietary and supplemental intake of vitA and %ucOC decreased significantly at 12 months after adjusting for intake. No significant changes were evident in 25OHD, PIVKA II, vitE levels (not shown). When run separately by group these results were the same in LYX and placebo except for α-tocopherol which increased significantly at 3 months in LXS only (p=0.018).

Conclusions: In children with CF and PI on a high fat nutritional intervention, vitA and vitK status improved after 12 months (both LYX and placebo), while vitD status remained unchanged. VitE status only improved in subjects receiving LYX. The improvements in vitA and K may be related to the intervention and to the placebo or possibly to improved adherence to CF care.

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Mean ± SD	Retinol ng/dL	α-tocopherol mg/L	α-toco:chol mg/g	25OHD ng/mL	%ucOC	PIVKA II ng/mL
Baseline	38±11	10.5±3.3	0.07±0.02	33±9	30±15	1.4±0.7
3 months	40±12*	10.6±3.8	0.07±0.02	32±11	31±14	1.4±0.7
12 months	40±11*	10.1±3.8	0.07±0.02	31±11	22±14**	1.7±2.3

Significantly different than baseline *p≤0.05, **p≤0.01

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COMPARISON OF GROWTH BETWEEN CDC AND WHO GROWTH CHARTS DURING THE FIRST TWO YEARS OF LIFE IN BREASTFED AND FORMULA-FED INFANTS WITH CF

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Background: We recently reported that CF infants exclusively breastfed for longer than two months experienced reduced weight gain compared to those exclusively breastfed for a shorter duration or formula-fed (AJCN 2011;93:1038). It is unclear whether these differences remain if the 2006 WHO growth charts are used instead of the 2000 CDC growth charts. This is important to assess because the WHO reference population weighs more before six months of age, but less thereafter until age 2 compared to the CDC reference population, while the WHO reference population is taller than the CDC reference population through age 2 years. More importantly, the 2012 AAP policy endorses WHO growth charts for monitoring growth between 0-2 years and this recommendation may be adopted in the care of CF infants.

Methods: Growth of the study population examined in our previous report was re-evaluated using the WHO growth charts as the standard to calculate weight and length percentiles. Briefly, the study population included 103 children born during 1994-2006 and enrolled in the Wisconsin Routine CF Newborn Screening Study. Of the 103 infants, 24 had MI, 9 had pancreatic sufficiency (PS, defined as having at least one PS mutation and not taking pancreatic enzymes), and 70 had pancreatic insufficiency (PI) but not MI. The 70 PI infants were classified into 5 feeding groups: exclusively breastfed longer than 2 months (n=10), exclusively breastfed 1-1.9 mo (n=8), exclusively breastfed < 1 mo (n=16), exclusively fed formula containing standard caloric density of 20 kcal/oz (n=22), and exclusively fed formula with caloric density >22 kcal/oz for some duration during infancy (n=14).

Results: Overall, length percentiles did not differ significantly with age during the first 2 years but WHO percentiles (42.9 ± 30.3) were consistently lower than CDC percentiles (47.1 ± 28.5), p < 0.0001. On the other hand, WHO weight percentiles were higher at birth (47.3 ± 28.5), lower between 2-6 months of age (30.1 ± 25.0), and higher between 9-24 months (53.5 ± 25.7) compared to CDC percentiles (38.9 ± 26.5, 35.9 ± 25.9, and 37.8 ± 26.8 at birth, 2-6, and 9-24 months, respectively), p < 0.0001. These trends were observed within each feeding group, and the magnitude of differences between WHO and CDC weight percentiles did not differ significantly among the 5 feeding groups, p=0.1. In other words, use of WHO instead of CDC growth standards shifted percentiles systematically and consistently for all feeding groups. Therefore, even though the percentile values changed using WHO instead of CDC standards, our previous observation that infants exclusively breastfed longer than 2 months had reduced weight gain compared to the other 4 feeding groups remained unchanged.

Conclusions: In our study population, regardless of the use of WHO or CDC growth charts, CF infants exclusively breastfed longer than 2 months had reduced weight gain compared to those exclusively breastfed for a shorter duration or formula-fed. Nevertheless, use of WHO growth charts resulted in lower weight percentiles during the first 6 months and lower length percentiles throughout the first two years of life, which may affect treatment decisions for nutrition intervention. (Supported by NIH-R01DK072126.)

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OCCURRENCE OF CYSTIC FIBROSIS-RELATED DIABETES BY AGE 20 YEARS IS ASSOCIATED WITH IMPAIRED PUBERTAL LINEAR GROWTH

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Background: Cystic fibrosis-related diabetes (CFRD) increases during adolescence, a critical period for pubertal linear growth. Our previous analyses showed that about 40% of adolescents experienced impaired pubertal linear growth, i.e., delayed and/or attenuated peak height velocity (PHV). We hypothesize that impaired pubertal growth predisposes adolescents to a greater risk of developing CFRD by age 20 years.

Methods: Data from the 1986-2008 CF Foundation Patient Registry were utilized. Patients born 1984-1987 (age 18-21 by 2008) with at least one height measurement per year were included in analyses (n=2372). Age and

magnitude of PHV were determined using a semi-parametric shape-invariant model. Those with no identifiable PHV ($n=135$) were excluded. Of the remaining 2237, delayed PHV was defined as the age at PHV 2 SD later than the average maturers (i.e., later than age 15.3 in boys and 13.3 in girls), and attenuated PHV was defined by the magnitude of PHV below the 5th percentile (i.e., in average maturers, < 7.5 cm/year in boys and 6.5 cm/year in girls), based on Tanner reference. CFRD by age 20 years was used to define the occurrence of CFRD because CFRD was not widely screened prior to early 2000, therefore $<50\%$ were screened for it before age 15. However, by age 16, 95% were screened and 99% were screened by age 20. Few pancreatic sufficient patients had CFRD (5%, 5 out of 90) and were also excluded from analysis.

Results: Of the 2147 pancreatic insufficient patients, mean PHV was 8.4 ± 1.6 cm at age 14.0 ± 1.3 years in boys, and 6.9 ± 1.4 cm at age 12.1 ± 1.3 years in girls. Sixty-two percent ($n=1337$) had normal PHV, 204 (10%) had delayed PHV, 484 (23%) had attenuated PHV and 122 (6%) had both delayed and attenuated PHV. Overall, 540 (25%) developed CFRD by age 20. The occurrence of CFRD was lowest in patients with normal PHV (21%), followed by delayed (25%) or attenuated PHV (28%), and highest among those with delayed and attenuated PHV (37%), $p=0.0002$. In addition, sex and pre-pubertal growth were significant predictors of CFRD. More females (29%) had CFRD than males (19%), $p<0.001$. Patients who were underweight (BMI z-score <-1) and short (height z-score <-1) at age 7 also had higher percent of CFRD (underweight: 24%, short: 26%, both: 34%) than those with better height and BMI z-scores (21%), $p=0.0003$. After adjusting for sex and pre-pubertal growth, the PHV and CFRD association remained statistically significant, $p=0.007$. In patients with good nutritional status at age 7 (both height and BMI z-scores >-1) and normal PHV, 18% (136 out of 748) developed CFRD by age 20, while 79% (11 out of 14) of those who were underweight and short at age 7, and had delayed and attenuated pubertal PHV, developed CFRD, $p<0.0001$.

Conclusion: CFRD at age 20 and impaired pubertal linear growth during adolescence were prevalent. Impaired pubertal growth was a significant predictor of CFRD. Further, CFRD was 4 times higher in those with poor nutritional status during childhood, and both delayed and attenuated pubertal growth, compared to those with good nutritional status during both time periods. Prospective studies are needed to determine whether optimizing childhood and pubertal growth lowers the risk of CFRD. (Supported by NIH-R01DK072126.)

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IMPROVED NUTRITION-RELATED OUTCOMES THROUGH 24 MONTHS OF AGE IN CHILDREN WITH CYSTIC FIBROSIS MAY BE ATTRIBUTABLE TO INCREASED CLINICAL FOCUS ON NUTRITION

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Background: In recent years, there has been greater focus on nutrition as a critical component of cystic fibrosis (CF) management. Of particular interest is the CF Foundation's 2005 increase in recommended weight-for-length and BMI from the 25th to 50th percentile. During the same year, comparison of CF center outcomes and consequent benchmarking visits led to inclusion of growth standards in return-to-clinic algorithms.

Objective: To assess the impact of these changes, we compared growth outcomes, feeding practices, and clinic visit frequency through age 24 months between cohorts from two Wisconsin CF centers born from 2000-2004 (older cohort) and 2006-2010 (younger cohort).

Design: Retrospective medical record review of 53 infants with CF born in 2000-2004 and 37 infants with CF born in 2006-2010 was completed. Growth percentiles were computed using CDC growth charts.

Results: The 2 cohorts did not differ by gender, birth weight or age at first clinic visit. A similar percent had meconium ileus (MI) [younger cohort: 13% ($n=5$); older cohort: 15% ($n=8$)], but pancreatic sufficiency (PS) was more prevalent in the younger (30%, $n=11$) compared to the older (11%, $n=6$) cohort, and pancreatic insufficiency without MI (PI) was lower in the younger (57%, $n=21$) versus older (74%, $n=39$) cohort, $p=0.088$. Over 0-24 months of age and adjusting for gender, birth weight, pancreatic status and CF center, weight-for-length percentile was significantly higher in the

younger cohort (54.1 ± 28.8 versus 44.5 ± 29.0 in the older cohort, $p=0.04$). However, length-for-age was lower in the younger (39.4 ± 27.2) compared to the older cohort (51.6 ± 27.4), $p=0.007$. Results were similar when analyses were restricted to PI patients. More infants in the younger cohort were ever breastfed (78%) compared to the older cohort (53%, $p=0.01$). Exclusive breastfeeding duration in the younger cohort (2.6 months) was twice that in the older cohort (1.3 months), $p=0.01$. A greater percentage of children in the younger cohort received high calorie supplementation (78%) than in the older cohort (34%), $p<0.0001$. Age at start of supplementation did not differ between younger and older cohorts (5.5 versus 5.1 months, $p=0.79$). Ever breastfeeding and supplementation rates were similarly distributed among MI, PS and PI in both cohorts. Children in the younger cohort cumulatively visited their CF providers more often by 6 months (4.5 visits) and 24 months of age (13.7 visits), compared to 3.3 visits ($p=0.001$) and 10.2 visits ($p<0.0001$), respectively, in the older cohort.

Conclusion: A greater focus on nutrition management in children with CF aged 0-24 months born after 2005 was confirmed by more frequent clinic visits and a greater prevalence of calorie supplementation. The impact of the shift in clinical practice on growth is less clear because of higher weight-for-length but lower length-for-age percentile in the younger cohort. (Supported by MCHB Pediatric Pulmonary Center grant T72 MC 00008 and NIH-R01DK072126)

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NOVEL METHOD TO MEASURE FAT AND PROTEIN DIGESTION RATE AND THE ACUTE RESPONSE TO PANCREATIC ENZYME INTAKE IN PEDIATRIC AND ADULT PATIENTS WITH CYSTIC FIBROSIS

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Background: An impaired intestinal digestion capacity is one of the main factors responsible for malnutrition in CF. Currently no methods are available to acutely measure the digestion rate of protein and fat and the effectiveness of pancreatic enzyme preparations in CF.

Methods: We developed a stable isotope method to quantify fat and protein digestion rate in healthy subjects ($n=8$) and patients with CF ($n=19$) requiring pancreatic enzyme replacement therapy. We tested this method in the CF group by examining the acute response in digestion rate to intake of pancreatic enzymes. Fat and protein digestion rate were measured during standardized continuous feeding during 6hr (via sip feeding every 20 min or feeding tube if present) of Ensure Plus (amount based on 1/3 of daily energy requirement of protein). We added to the nutrition the stable isotopes of 1,1,1-¹³C3-tripalmitin and 2,2-H2-palmitic acid to measure fat digestion rate (= ratio [¹³C]palmitic acid to [²H2]palmitic acid), and 15N-spirulina protein and 2H5-phenylalanine (PHE) to measure protein digestion rate (= ratio [¹⁵N]PHE to [²H5]PHE). Pancreatic enzyme (Creon®) intake in CF took place at 2h into feeding and the dose was based on the fat content of the meal (4000u/g fat intake). Blood sampling was done every 20 min until the end of the study. Tracer-tracee ratios of the stable isotopes were analyzed by GC-MS and LC-MS/MS. Statistics was done using unpaired t-test and two-way ANOVA.

Results: Compared to healthy subjects, protein and fat digestion rate during feeding were both reduced to 48% of normal in the CF group. After intake of the pancreatic enzymes, 100 min were needed before protein digestion rate reached its maximal value of 97% of normal. Protein digestion started to decrease again 160 min after enzyme intake. Intake of the pancreatic enzymes gradually increased fat digestion to 80% of normal at 4h after the enzyme intake. Stratification of the CF group in children ($n=10$, age: 14.9 ± 0.2 y, FEV1: $82 \pm 1\%$ pred) and adults ($n=9$, age: 28.6 ± 0.8 y, FEV1: $64 \pm 3\%$ pred.) showed a comparable protein but lower fat digestion rate in CF children during feeding (34 vs. 63% resp., $P<0.05$). The increase in protein digestion rate after enzyme intake was not different between children and adults with CF. We observed for fat digestion rate a significant group ($P<0.001$) and time effect ($P<0.01$) but no interaction, indicating that the increase in fat digestion rate after pancreatic enzyme intake was compa-

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table in children and adults with CF but the absolute fat digestion rate remained lower in the CF children even at 4 hours after enzyme intake (67 vs. 94% resp., $P < 0.05$).

Conclusion: We have developed a method that is able to acutely measure fat and protein digestion rate during feeding and their acute response to pancreatic enzyme preparations in patients with CF.

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CFTR KNOCKDOWN AND/OR INHIBITION INDUCES PRO-INFLAMMATORY CHANGES IN INTESTINAL CELLS

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Background/Objectives: Besides maldigestion, malabsorption and mucus accumulation cystic fibrosis patients also exhibit moderate intestinal inflammation, which etiology is still ill defined. Given the role of the CFTR in modulating pro-inflammatory responses in several tissues and its high expression in the intestinal epithelium, we hypothesize that CFTR dysfunction may play a role in the onset of intestinal inflammation. We carried out studies aimed at investigating the role of CFTR in intestinal inflammation using the human intestinal cell line Caco-2/15.

Methods: Levels of CFTR expression were genetically depleted using shRNAi in the presence or absence of the pharmacological inhibitor, CFTR inh-172 (5 or 20 μ M for 72 hours). Cells treated with the inhibitor alone were also studied. Inflammatory conditions were induced by the addition of human recombinant tumor necrosis factor- α (TNF- α ; 25 and 50 ng/mL) or Interleukin-1 β (IL-1 β ; 5 and 25 ng/mL) for various periods of time. IL-8 secretion in the cell supernatants was assessed by ELISA and pro-inflammatory signaling pathways (p38 mitogen-activated protein kinase (p38MAPK), extracellular signal-regulated kinase (ERK1/2) and c-jun N-terminal kinase (JNK)) were investigated by Western blot.

Results: Using shRNAi, CFTR knockdown in Caco-2/15 cells resulted in the reduction of gene (74%) and protein (50%) expression compared to cells infected with the scrambled shRNA. Compared to control cells, basal secretion of IL-8 was increased only when CFTR was genetically depleted whereas TNF- α or IL-1 β -stimulated secretion of IL-8 was greater when CFTR was either genetically depleted or pharmacologically inhibited. However, the combination of both manipulations resulted in a much further increase in IL-8 secretion in response to both cytokines. Consistently, IL-1 β or TNF- α treatment of CFTR-inhibited Caco-2 cells caused an activation of the p38 and p44/p42 (ERK1/2) MAPK pathways as indicated by the enhanced expression of phospho-p38 and phospho-ERK1/2 compared to non-inhibited cells whereas phosphorylation of JNK remained less affected. In contrast, CFTR activation by forskolin and IBMX led to a blunted TNF- α or IL-1 β -induced secretion of IL-8 as well as reduced levels of phosphorylated p38 and ERK1/2 MAPK upon IL-1 β or TNF- α stimulation.

Conclusion: These studies demonstrated that reduced CFTR expression and/or pharmacological inhibition of CFTR activity in intestinal absorptive cells induced a pro-inflammatory state. Whether this state could affect intestinal functions or be modulated by anti-inflammatory compounds or nutritional factors will be explored in future studies.

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PREEMPTIVE TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT (TIPSS) FOR PORTAL HYPERTENSION FROM CF LIVER DISEASE: 5 YEARS EXPERIENCE

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Background and Aim: Liver disease is the second cause of death in CF. Liver insufficiency mostly occurs late and is preceded by fibrosis (LF) causing portal hypertension (PH) with splenomegaly and a cascade of complications. Only the latter prompt to (mostly vascular) interventions. Aiming to

prevent them, the value of preemptive TIPSS, before variceal haemorrhage, was investigated. Our experience since 2007 is reported.

Discussion:

* first signs of LF were detected at a young age thanks to systematic abdominal palpation and annual ultrasound and fibroscan.

* TIPSS could not reverse established signs of PH complications but slowed progression or stabilised them for some years as they were at the time of TIPSS placement. This persisted only for 2 to 3 years after which progression of PH resumed.

* Except for 2 revisions of TIPSS for stenosis no other complications occurred. No encephalopathy developed.

Conclusions: Placement of TIPSS when signs of progressive PH are detected can temporarily slow further progression and development of complications depending on the timing of the procedure. It is therefore mandatory to detect development of PH early and confirm its progressive character.

Patients and Results:

	1	2	3	4	5	6
Age at detection of LF (y)	4	4.9	6.5	4.5	7.5	4.5
Age at TIPSS (y)	8.5	9	9	6.4	8.8	7
Spleen size*	3+/2+	3+/OLTx	0/+	0/0	0/+	0/0
Platelets* (10E3/ μ L)	55/72	93/50(OLTx2010)	299/177	224/157	154/137	247/258
WBC* (10E3/ μ L)	2.7/3.6	3.7/4.96	9.5/6.2	9.2/7.6	7.4/7.9	6.6/7.4
RBC* (10E6/ μ L)	4.3/4.0	3.9/4.1	4.5/4.9	4.6/5.4	4.6/4.3	5.4/4.4

* at placement TIPSS/ at present

+ Patient 2 underwent liver transplantation 2 y after TIPSS placement for unrelated reasons.

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SUCCESSFUL CORRECTION OF VITAMIN D LEVELS WITH HIGH-DOSE SUPPLEMENTATION: A ONE YEAR EXPERIENCE FROM A LARGE ADULT CYSTIC FIBROSIS CENTRE

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Background: Vitamin D (VitD) is implicated in many biological systems including bone metabolism, diabetes and infection. VitD-deficiency is well described in CF with guidelines on adequate levels, but with little agreement on optimal high-dose supplementation. We developed a high-dose correction protocol for use in our adult CF unit and prospectively studied its efficacy in achieving adequate levels.

Methods: From 2011, patients were enrolled consecutively with serum 25-hydroxyvitamin D (25OHD) classified as either optimal (>30 ng/mL), adequate (>20 ng/mL), insufficient (10-20 ng/mL) or deficient (<10 ng/mL). Those with adequate or optimal levels continued existing low-dose supplements with standard combination vitamin A and D and calcium supplements. High-dose oral cholecalciferol was given at either 50,000IU (25OHD level 10-14 ng/mL) or 100,000IU (<10 ng/mL) weekly until adequate levels were achieved, up to a maximum of 12 weeks. Those with levels insufficient but >14 ng/mL were given an increased dose of standard combination supplements and only high-dose cholecalciferol if they failed to increment. Those who failed to increment on oral cholecalciferol were given intramuscular doses at 300,000IU every 10 weeks. Once adequate levels were achieved, maintenance dosing was given at 50,000IU alternate weeks.

Results: Thirty-four patients have commenced cholecalciferol with median baseline 25OHD levels of 7 ng/mL (range <1 -14 ng/mL). Twenty-three patients have been analysed in detail with treatment ongoing for the remainder. Twelve of 23 patients (52%) were female, the median age was 26 years (17-45) and 19/23 (83%) were pancreatic insufficient. Of the 23 patients, 22 (96%) achieved adequate levels as per protocol. Only 1 patient (with severe liver disease) required intramuscular dosing. Eleven of 23 (48%) patients achieved adequate levels within 4 weeks of starting high dose therapy, 17/23 (74%) by 6 weeks, 18/23 (78%) by 9 weeks and 21/23 (91%) by 12 weeks. Those 3 patients who only apparently corrected between 9-12 weeks all had levels >40 ng/mL at this first measurement, suggesting that adequate levels may have been achieved before this. Seven of 8 patients (88%) given maintenance dosing were successfully able to maintain adequate levels when measured over the subsequent 12-week period with a median level of 21 ng/mL (range 20-35 ng/mL). No side effects or toxicity was observed with

only 1 patient demonstrating high serum corrected calcium (2.76nmol/L) who was otherwise asymptomatic; levels corrected with reduction in associated calcium supplements.

Conclusion: High-dose vitamin D supplementation given as cholecalciferol 50-100,000IU weekly is safe, well tolerated and effective in correcting low levels in adult CF patients. By our protocol adequate levels were achieved in 96% of the cohort and have been successfully maintained by the vast majority thereafter.

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ASSESSMENT OF GASTRIC EMPTYING IN CYSTIC FIBROSIS USING A NOVEL BEDSIDE ULTRASONOGRAPHIC TECHNIQUE

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Objectives: Delay in the gastric emptying rate (GER) in patients with CF can have important clinical consequences. However, GER assessment is problematic: previous studies have employed expensive scintigraphic techniques, which are unsuitable for routine use and have produced conflicting results because of the complexity and variability of meals used. We have assessed the use of a novel ultrasonographic technique to assess GER in subjects with CF and healthy controls with a 3.5-MHz abdominal transducer probe measuring gastric antral size¹, using liquid and mixed meals.

Methods: After an overnight fast, 6 healthy controls (mean age 28 ± 3 years, 50% male) and 19 non-diabetic CF subjects (mean age 27 ± 6 years, 3 pancreatic sufficient, 84% male) consumed 113 mLs (made up to 200 mLs with water) of Polycal[®], a liquid energy supplement and on a separate day, a standard mixed meal (75 g carbohydrate), within 10 minutes. Regular ultrasound measurements were made in the supine position for 2 hours. GER ≥63% at 90 minutes is considered normal.

Results: Healthy controls had normal GER at 90 minutes for both types of meal, but compared to controls, there was a decrease in GER at 90 (p<0.001) and 120 minutes (p<0.0008) in CF subjects following both types of meal (see table). Only 6 CF patients (2 pancreatic sufficient) had GER ≥63% at 90 minutes.

Conclusions: This study shows that this novel bedside ultrasound technique provides a simple method of assessing GER in CF patients and confirms that those without known cystic-fibrosis related diabetes have delayed gastric emptying for both liquid and mixed meals. This investigation will aid the improved nutritional management of these patients.

Reference: 1. Darwiche G et al. J Ultrasound Med. 1999;18:673-682.

Comparison of % GER (Mean [SEM]) in healthy controls and CF subjects

Meal	Group	30 minutes	60 minutes	90 minutes	120 minutes
Liquid	Controls	36 [2]	52 [3]	70 [2]	75 [1]
	CF	30 [2]	45 [2]	56 [2]	65 [2]
Mixed	Controls	40 [3]	53 [3]	74 [1]	80 [1]
	CF	34 [2]	43 [3]	56 [2]	64 [2]

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THE CONTRIBUTION OF GASTRIC EMPTYING TO THE DIURNAL VARIATION OF GLUCOSE IN CYSTIC FIBROSIS

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Objectives: In normal subjects, plasma glucose levels (both fasting and after an oral glucose challenge) increase throughout the day¹, and these changes are influenced by gastric emptying, which is in turn slowed by hyperglycemia². Although increased glucose levels are common in CF patients without known CF related diabetes (CFRD), there have been no

studies looking at the diurnal variation of gastric emptying and its interaction with blood glucose levels in this population. To study this further, using ultrasonography³ we looked at the diurnal variation in gastric emptying rate (GER) in adult CF patients and correlated this with blood glucose levels.

Method: Following a fast of at least 10 hours, 19 CF subjects (mean age 27 ± 5 years) without known CFRD consumed a standard mixed meal at 0800, 1300 and 1800 hours, on the same day. GER was assessed with a 3.5-MHz abdominal transducer probe measuring gastric antral size, where a GER of ≥63% at 90 minutes is considered normal. Plasma glucose measurements were carried out at baseline, 30, 60, 90 and 120 minutes following completion of each meal, and the area under the curve (AUC_{Glucose}) calculated for the 120 minute duration.

Results: See table: GER at 90 minutes was reduced throughout the day. Following the morning meal, GER at 90 minutes and the AUC_{Glucose} were inversely correlated (r=-0.47, p=0.04). The AUC_{Glucose} increased in the evening but there was no correlation with the 90 minute GER.

Conclusions: This study demonstrates altered gastric emptying in CF subjects. However, there does not appear to be a decrease in GER as the day progresses. The inverse relationship in the morning between glucose and GER suggests hyperglycaemia is related to a decreased GER. Further studies are needed to evaluate the causative and contributory factors for the diurnal variation in blood glucose.

References:

1. Jarrett, R. J. et al. (1972). Br Med J 1(5794): 199-201.
2. Samsom M, et al. Gut 40:641-646, 1997
3. Darwiche G et al. J Ultrasound Med. 1999;18:673-682.

Gastric Emptying (%) and AUC_{Glucose}

OGTT	Morning (mean ± SEM)	Afternoon (mean ± SEM)	Evening (mean ± SEM)
AUC _{Glucose} (mmol/L)	821 ± 35	701 ± 21	838 ± 36
90 mins GER (%)	56 ± 2	57 ± 3	59 ± 1

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A RANDOMIZED PLACEBO-CONTROLLED TRIAL OF CHOLECALCIFEROL FOR VITAMIN D DEFICIENCY IN ADULTS WITH CYSTIC FIBROSIS

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Background: Vitamin D deficiency has been reported in up to 80% of patients with cystic fibrosis (CF). Vitamin D deficiency is associated with worse health outcomes in the general population including reduced bone mineral density, increased risk of diabetes, cancer and worse lung function. Vitamin D supplementation is standard practice in CF; however, the dose needed to normalize serum levels is unclear.

Purpose: To assess whether 5000 IU daily of cholecalciferol in addition to standard vitamin D supplementation increases serum 25-hydroxyvitamin D (25OHD) levels in vitamin D deficient CF patients.

Methods: Subjects were recruited from the Toronto Adult CF program between September and March (2009 - 2012) to participate in a double-blind randomized placebo-controlled trial of cholecalciferol. Inclusion criteria included CF patients older than 18 years of age with vitamin D deficiency defined as serum 25OHD level <75 nmol/L. Exclusion criteria included individuals with a history of hypercalcemia, renal stones, psychiatric history, use of tanning beds/travel to sunny locations within the study period, transplantation, pregnancy or lactation. Participants received a daily dose of 5000 IU of cholecalciferol or placebo in addition to their standard vitamin D supplementation for 12 weeks. Participants and study investigators were blinded to the treatment groups. The primary outcome measure was the change in mean serum 25OHD levels. Other variables collected were genotype, pancreatic status, age, gender, microbiology, body mass index (BMI), and lung function. Descriptive statistics were conducted for continuous variables (mean ± standard deviation) and proportions for categorical variables. Independent sample t-test or chi square test was used to test for differences as appropriate.

Results: Interim results are reported. To date, 67 patients were randomized to the treatment arm (n=33) or placebo (n=34). Preliminary data analyses are available on 40 subjects (18 cholecalciferol, 22 placebo). Baseline demographics of the study population (n=40): mean age 34.4 ± 1.4 yrs,

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56.7% males, 23.3% had diabetes, 32.4% homozygous delta F, 64.9% *Pseudomonas aeruginosa* positive, BMI 22.7 ± 3.4 kg/m², FEV₁ 2.3 ± 1.1 L. Mean serum 25OHD increased significantly from 48.2 ± 18.9 nmol/L to 73.5 ± 27.4 nmol/L in the cholecalciferol group whereas there was no significant change in the placebo group ($p < 0.01$). In the treatment arm, 44.4% reached a 25OHD level higher than 75 nmol/L. There was no difference in routine oral vitamin D supplementation outside of the study drug. No adverse events were identified during the study period.

Conclusions: Preliminary analysis on approximately one-half of the anticipated sample size shows that serum 25OHD levels significantly increased with 5000 IU of cholecalciferol. However, less than half of the study subjects achieved a serum 25OHD level above the recommended target of 75 nmol/L suggesting that a higher daily dose may be required to optimize levels. This interim analysis reflects small sample sizes and analysis with the entire cohort is required prior to drawing firm conclusions.

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FOLIC ACID STATUS AND PREDICTORS IN CHILDREN WITH CYSTIC FIBROSIS AND PANCREATIC INSUFFICIENCY

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Background: Folic acid is involved in 1-methyl metabolism and red blood cell (RBC) metabolism; determinants of RBC folate status have not been well described in children with CF. Folate status is especially important in adolescent females of childbearing age.

Objective: To describe RBC folate status and explore predictors in a current cohort of children with CF and PI following current clinical care practices and vitamin supplementation.

Methods: Baseline measurements were obtained from subjects with CF and PI (aged 5-17 yrs) with FEV₁ $> 40\%$ predicted and no known liver or metabolic disease participating in a multicenter nutrition intervention study. RBC folate was analyzed from fasting whole blood samples by immunoassay. Dietary folic acid intake was assessed from 3-day weighed food records and supplemental folic acid from CF-specific and over-the-counter vitamins was reported. Daily intakes of both dietary and supplemental folic acid intake were compared to dietary reference intakes (DRIs) for age and gender groups and expressed as percent recommended dietary allowance (%RDA). Descriptive statistics (means, standard deviations, medians and ranges) are presented for serum RBC folate and dietary and supplemental folic acid. Predictors of serum RBC folate including age, gender, dietary and supplemental intake of folic acid as both actual intake and %RDA were explored in multiple regression models.

Results: For 100 subjects (59% male, 10.4 ± 3.1 yrs), RBC folate concentration was 520 ± 168 ng/mL (median 494; range 231 to 1045 ng/mL): four subjects had values below and two subjects had values above the laboratory reference range for age and gender. There were no age and gender related serum RBC folate trends. Dietary folic acid intake was 468 ± 238 µg/d (median 416, range 140 to 1472 µg/d) representing 173 ± 90 %RDA (median 159, range 39 to 491 %RDA). Supplement based intake was 353 ± 233 µg/d (median 400; range 0 to 1200 µg/d) representing 127 ± 84 %RDA (median 100; range 0-400 %RDA). The best predictor of RBC folate status was dietary folic acid intake ($R^2 = 0.12$, $p = 0.004$). Supplemental folic acid intake, age and gender did not predict serum folate status in these children with CF and PI.

Conclusions: Folate deficiency is uncommon in children with CF under current care models, with 4% of the current cohort with RBC folate below reference ranges. Dietary intake remains the most important predictor of serum status.

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VITAMIN B₆ STATUS AND PREDICTORS IN CHILDREN WITH CYSTIC FIBROSIS AND PANCREATIC INSUFFICIENCY

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Background: Serum B₆ vitamin status has not been well described in children with CF, and is involved in 1-methyl and intersecting phospholipid metabolic pathways.

Objective: To describe serum vitamin B₆ status and explore predictors of B₆ status in a cohort of children with CF and PI following current clinical care practices for vitamin supplementation.

Methods: Baseline measurements were obtained from subjects with CF and PI (aged 5-17 yrs) with FEV₁ $> 40\%$ predicted and no known liver or metabolic disease participating in a multicenter nutrition intervention study. Fasting serum B₆ concentrations were analyzed using high performance liquid chromatography. Dietary B₆ intake was assessed from 3-day weighed food records and B₆ intake from CF-specific and over-the-counter vitamin supplements were collected. Daily intakes of both dietary and supplemental B₆ were compared to dietary reference intakes (DRIs) for age and gender groups and expressed as percent recommended dietary allowance (%RDA). Means, standard deviations, median and ranges are reported for serum and dietary and supplemental B₆ intake values. Predictors of serum B₆ levels including age, gender, dietary and supplemental intake of B₆ as both actual intake and %RDA were explored using multiple regression models.

Results: For 106 subjects (57% male, 10.5 ± 3.0 yrs), serum B₆ was 20.1 ± 13.5 ng/mL (median 17.1, range 5.1 to 91.7 ng/mL): 16 subjects (15%) had values above the laboratory reference range; none were below. Dietary intake was 2.3 ± 1.5 mg/d (median 1.9, range 0.5 to 8.8 mg/d), representing 277 ± 187 %RDA (median 217, range 51 to 931% RDA). Daily supplemental intake of B₆ was 3.6 ± 7.6 mg/d (median 2.4 mg/d, range 0-75 mg/d) representing 394 ± 735 %RDA (median 311, range 0 to 7500 %RDA). The best predictors of serum B₆ concentration were dietary B₆ intake ($p = 0.007$) and supplemental B₆ intake ($p = 0.04$); age and gender did not contribute further to the overall model ($R^2 = 0.15$, $p = 0.006$).

Conclusions: Serum vitamin B₆ levels were within or greater than (15%) reference values under current dietary and supplemental intake practices in children with CF and PI. Both dietary and supplemental B₆ intake contributed to serum B₆ status in these children.

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TRENDS IN FAT-SOLUBLE VITAMIN INTAKES AND BLOOD CONCENTRATIONS IN ADULT PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Pancreatic insufficiency is common in patients with cystic fibrosis (CF) leading to malabsorption of fat-soluble vitamins. Multivitamins including vitamins A, D, E and K have been routinely prescribed to patients with CF to prevent vitamin deficiencies. There has been increased attention to prevention of vitamin deficiencies in patients with CF over the past several years. The objective of our study was to examine whether increased supplementation in adults with CF resulted in improved serum concentrations of fat-soluble vitamins.

Methods: We conducted a retrospective chart review of patients with CF who have been treated at Emory Clinic and Emory Hospital during 2008-2012. We collected demographic information, fat-soluble vitamin concentrations in blood and amount of fat-soluble vitamins prescribed during each year between 2008-2012.

Results: One hundred and eighteen charts were reviewed. Mean age (SD) was 31.2 (10.4) years. Mean (SD) body weight and BMI were 61.8 (14.3) kg and 21.9 (4.4) kg/m², respectively. Eighty-nine percent of patients used pancreatic enzyme replacement for malabsorption. Prescription for fat-

soluble vitamins increased in the past 5 years ($p < 0.001$). There was, however, no increase in blood concentrations of these fat-soluble vitamins (Table 1).

Conclusions: Although fat-soluble vitamins are prescribed more frequently and at higher dosages in our clinic, there was no parallel increase in blood concentrations of these vitamins within the past 5 years. The findings suggest sub-optimal dosages of these supplements, low adherence to these vitamins, or ongoing issues with malabsorption.

Table 1 Fat-soluble vitamins intake and serum concentrations

Intake	Mean (SD)	p value	Serum level	Mean (SD)	p value
Vitamin A (IU) 2008 (n=53) 2009 (n=71) 2010 (n=87) 2011 (n=97) 2012 (n=89)	10,904 (10,790) ^a	<0.001	Retinol (mg/L) 2008 (n=42) 2009 (n=61) 2010 (n=80) 2011 (n=89) 2012 (n=30)	0.44 (0.20) ^{ab} 0.37 (0.13) ^b 0.41 (0.17) ^{ab} 0.43 (0.21) ^a 0.37 (0.18) ^{ab}	0.01 0.38
	14,930 (12,274) ^{ab}		Retinyl Palmitate (mg/L) 2008 (n=42) 2009 (n=61) 2010 (n=80) 2011 (n=86) 2012 (n=30)	0.03 (0.04) 0.03 (0.04) 0.02 (0.01) 0.03 (0.03) 0.02 (0.00)	
	17,541 (12,338) ^{bc}				
	18,970 (13,812) ^c				
	19,483 (14,027) ^c				
Vitamin D (IU) 2008 (n=54) 2009 (n=75) 2010 (n=92) 2011 (n=103) 2012 (n=92)	961 (696) ^a	<0.001	25(OH)D (ng/mL) 2008 (n=59) 2009 (n=74) 2010 (n=87) 2011 (n=94) 2012 (n=30)	29.7 (14.3) 28.7 (11.9) 31.5 (13.0) 32.0 (14.2) 29.4 (13.7)	0.06
	1,628 (2,776) ^a				
	1,668 (1,112) ^b				
	1,888 (1,296) ^b				
	1,903 (1,281) ^b				
Vitamin E (IU) 2008 (n=53) 2009 (n=70) 2010 (n=88) 2011 (n=98) 2012 (n=89)	236 (236) ^a	<0.001	α Tocopherol (mg/L) 2008 (n=54) 2009 (n=66) 2010 (n=83) 2011 (n=90) 2012 (n=28)	8.3 (4.2) 8.6 (4.2) 7.7 (3.8) 8.1 (3.8) 7.7 (3.3)	0.47 0.01
	339 (237) ^{ab}		β γ Tocopherol (mg/L) 2008 (n=54) 2009 (n=65) 2010 (n=84) 2011 (n=86) 2012 (n=27)	1.0 (0.7) ^{ab} 1.1 (0.6) ^a 0.8 (0.6) ^b 0.9 (0.6) ^{ab} 0.9 (0.7) ^{ab}	
	330 (270) ^b				
	345 (253) ^b				
	336 (251) ^b				
Vitamin K (IU) 2008 (n=52) 2009 (n=69) 2010 (n=87) 2011 (n=95) 2012 (n=87)	422 (572) ^a	<0.001	Vitamin K (ng/mL) 2008 (n=40) 2009 (n=30) 2010 (n=10) 2011 (n=2) 2012 (n=1)	0.77 (0.91) 0.69 (0.67) 0.66 (0.50) 0.55 (0.58) 0.06	0.94
	631 (620) ^{ab}				
	792 (644) ^{bc}				
	823 (661) ^c				
	859 (670) ^c				

Repeated measures ANOVA used to determine differences between the years. Values not connected by the same letter are significantly different (Tukey's Post-Hoc analyses).

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THE BONE MINERAL DENSITY OF CHILDREN WITH CF

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Introduction: Low bone mineral density (BMD) is reported frequently in adult cystic fibrosis (CF) patients but the data is less consistent for children and adolescents. The aim of our study is to describe bone mineral density (BMD) in a group of children over a period of 10 years and to determine if BMD is related to vitamin D level, calcium intake, lung function, height and age.

Methods: A retrospective review of 123 dxa scans conducted in 50 children with CF was evaluated (Lunar, DPXL/ PED, Winconsin, USA). To adjust for body size we calculated apparent BMD (BMAD) of dxa reports using the Dutch reference database¹ as recommended by the International Society for Clinical Densitometry (ISCD)². Subsequent dxa scans were repeated at 1, 2 or 3 years depending on severity. Nutritional status (BMI), pulmonary status (FEV1%), vitamin D, calcium intake (5 day food diary) and age were evaluated as potential correlates of BMAD. Minitab statistical package (version 14) was used to analyse the data.

Results: Fifty children (24 girls) with mean age 12.8±2.1(SD) years at time of first dxa scan were included. BMAD was normal (dxa z score of >-1) in 64% of children while 30% had dxa Z score between -1 to -2 and 6% had low BMD with dxa Z score of <-2. Follow-up dxa scans at 1, 2 and 3 year intervals revealed deterioration rates of 2%, 43% and 75% respectively. The BMAD correlated significantly with FEV1 ($r=0.33$, $p=0.00$), BMI ($r=0.36$, $p=0.00$) and height ($r=0.37$, $p=0.00$). Calcium intake ($r=0.10$, $p=$

0.34), vitamin D level ($r=0.10$, $p=0.37$) or age ($r=0.07$, $p=0.41$) did not influence BMAD.

Conclusion: Low BMAD was present in a significant proportion of children and adolescents with CF independent of age or sex. BMAD correlated with pulmonary function, height and BMI. This study highlights the importance of earlier and more regular monitoring of BMD in children with CF to help prevent deterioration of their bones.

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THE EFFECT OF HIGH DOSE VITAMIN D SUPPLEMENTATION ON VITAMIN D LEVELS IN CHILDREN WITH CYSTIC FIBROSIS (CF)

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Introduction: Children with CF are at risk of vitamin D deficiency due to malabsorption, decreased sun exposure due to hospitalisation, immobility and photosensitivity from antibiotic therapy. Vitamin D is important in CF especially for bone health but the complete impact is yet to be determined e.g. innate immunity, diabetes, muscle function etc. Vitamin D supplementation is required to ensure adequate vitamin D levels but the exact dose or duration remains to be established. Thus we developed our own local CF departmental guidelines for the treatment of low vitamin D in children with CF and we set out to evaluate these.

Methods: Children with vitamin D levels less than 75nmol/ L were recruited into the study. All patients received 6080IU vitamin D (cholecalciferol) daily for a period of 6 weeks (42560IU D3 per week). Vitamin D was given in the form of a small capsule; D Pearls (Pharma Nord, Denmark). Each capsule contains 1520IU of vitamin D3 and children took 4 per day. Vitamin D levels were analysed using HPLC mass spectrometry, pre- and post-supplementation. Minitab statistical package (version 14) was used to analyse the data.

Results: Twenty-two children (14 males) with mean age of 14 ± 3.3 (SD) years of age were included in study. Twenty-one (95%) of vitamin D levels increased following supplementation. The mean vitamin D pre-supplementation was 46nmol/ L compared with 87nmol/ L post-supplementation ($p=0.000$).

Conclusion: This study shows that high dose supplementation with 6080IU daily for a 6 week period is effective in increasing vitamin D levels in children with CF. Further research is needed to investigate if the improvement is maintained following discontinuation of supplementation.

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A WEIGHTY PROBLEM: THE PREVALENCE OF OVERWEIGHT AND OBESE ADULTS WITH CYSTIC FIBROSIS OVER TIME

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Background: Nutritional therapy for individuals with cystic fibrosis (CF) includes a high-fat, high-energy diet. Variability in disease severity creates the potential for excessive weight gain in individuals with lower energy requirements. Obesity is associated with increased health risks in the general population, but whether this applies to CF patients is unknown.

Purpose: 1) To determine the prevalence of being overweight or obese over time; 2) To describe clinical characteristics of overweight or obese adults with CF.

Methods: Adults attending the CF Clinic at St. Michael's Hospital with ≥ 1 clinical measurement in the Toronto CF database between 1986-2011

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were included. Post-transplant clinical data and data from women during pregnancy and 6 months post-partum were excluded from the analysis. Body mass index (BMI) was calculated using weight (kg)/height (m)². Subjects were categorized into BMI groups: underweight (BMI<20), adequate weight (BMI 20-24.9), overweight (BMI 25-29.9), obese (BMI≥30) by selecting the maximum BMI per patient per year. Age, lung function, pancreatic status, CF-related diabetes (CFRD), genotype, and lipid profile were recorded. Descriptive statistics were performed for each BMI category. Mixed modeling with repeated measures was used to assess changes in BMI over time.

Results: Between 1986 and 2011, 909 adults with CF were included in the study. Mean BMI increased over time from 21.1 to 23.1 kg/m² (p<0.001). Females had a lower BMI at all time points compared to males. No sex interaction was identified. Pancreatic sufficient (PS) subjects had a higher mean BMI compared to pancreatic insufficient (PI) subjects (23.8 vs. 21.2 respectively). PS subjects gained more weight over time compared to PI subjects (p<0.001). The proportion of overweight subjects increased from 7.1% in 1986 to 21.7% in 2011 while those with a BMI ≥ 30 kg/m² increased from 0% in 1986 to 5.3% in 2011. Between 2000 to 2011, 651 adults were evaluated. Using the last recorded measurement, 187 (29%) were underweight, 319 (49%) had adequate weight, 120 (18%) were overweight, and 25 (4%) were obese. Individuals with a BMI ≥ 30 kg/m² were older (age 41.9±11 vs 33.2±10 yrs) and had higher lung function (FEV1 76.6±19.4 vs 56.5 ± 24.8 % pred) compared to those with an adequate BMI. PS was more common in the obese group (88% vs. 21% in the adequate BMI group). CFRD was identified in 4% of the obese cohort vs 25% of the adequate BMI group. Thirteen obese subjects had available lipid profiles and 39% had a cholesterol ≥ 5.2 mmol/L and TG ≥ 1.7 mmol/L compared to only 9% of the adequate weight group (n=129).

Conclusions: The prevalence of overweight and obese individuals has increased over time in our adult CF population. Compared to those with adequate nutrition, obese individuals have milder CF disease overall and PS individuals are at highest risk for obesity and hyperlipidemia. Further study is required to better understand the long-term consequences of being overweight or obese in the CF population. Nutritional recommendations may need to be re-evaluated, particularly for PS individuals who are obese.

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DOES PANCREATIC ENZYME REPLACEMENT THERAPY CAUSE INTESTINAL INFLAMMATION?

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Background and Aim: The cause of intestinal inflammation in cystic fibrosis (CF) is unclear. Pancreatic enzyme replacement therapy (PERT) has been proposed as a potential cause. Large doses of PERT have been associated with fibrosing colonopathy in individuals with and without CF. Increased intestinal permeability has also been reported with PERT, unrelated to the cause of pancreatic insufficiency (PI). Therefore, the aim of this study was to evaluate the relationship between intestinal inflammation (based on stool calprotectin) and PERT in CF.

Methods: Stools were collected in children with CF for calprotectin measurement (normal <50 mg/kg). Comparisons were made between PI patients on PERT and pancreatic sufficient (PS) patients not on PERT. Pancreatic function status was determined based on faecal elastase-1 or 72 hour faecal fat. PERT dose was ascertained in all patients.

Results: In all, 35 PI (mean [SD] = 9.3 [3.5] years) and 4 PS (11.5 [1.0] years) CF patients were recruited (p=0.23). Higher stool calprotectin levels were seen in PI (n=35) than in those with PS (n=4) (median [IQR] = 92.2 [47.4-149.2] vs. 30.8 [24.0-39.6] mg/kg; p=0.037). The median (IQR) PERT dose used was 5173 (3198-6480) unit/kg/day. There was no difference between stool calprotectin levels among patients with PERT <5173 vs. >5173 unit/kg/day (median [IQR] = 92.2 [36.2-184.8] vs. 61.3 [43.9-121.2]; p=0.73). Among PI CF patients, there was no correlation between stool calprotectin levels and PERT dose (r = -0.08; p=0.68).

Conclusion: PI CF patients have elevated stool calprotectin indicating intestinal inflammation. In contrast, PS CF patients have normal stool calprotectin levels. There was no association between PERT dose and intestinal inflammation. These findings suggest that PERT, given at recommended

doses, is unlikely to be the primary cause of intestinal inflammation in CF. Intestinal inflammation in PI may reflect altered intestinal milieu due to severity of CFTR dysfunction.

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LOW SERUM LINOLEIC ACID (THE PRINCIPAL ESSENTIAL FATTY ACID) IS PREVALENT IN THE FIRST TWO YEARS OF LIFE AND EARLY ADOLESCENCE

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Background: Many studies have demonstrated essential fatty acid (EFA) deficiency, particularly low serum level of linoleic acid (LA, 18:2n-6), and its negative impact on growth and pulmonary outcomes in patients with CF. However, it is unclear whether these findings apply to young children diagnosed through newborn screening followed by more aggressive nutritional care emphasized in recent years. In addition, little is known on EFA status in adolescents with CF.

Methods: In the Wisconsin Routine CF NBS study which followed 152 children born during 1994-2010 in the Madison and Milwaukee Pediatric CF Centers, serum fatty acids were measured in the annual blood specimens (non-fasting) obtained as part of routine clinical care beginning in 2006. Of the 152 patients, a total of 356 fatty acid measurements were available from 136 (89.5%) patients ranging from age 1.5 months to 16.0 years [28 (21%), 37 (27%), 42 (31%), 17 (13%) and 12 (9%) patients had 1-5 yearly measurements, respectively]. Fatty acid levels were expressed as molar percentage of total fatty acids. Poor EFA status was defined by serum LA below 26% (Pediatr Res 1985;19:104) or triene/tetraene ratio above 0.02, 0.04 or 0.2 (JPGN 2008;47:635; AJCN;1969:32:2390). Of the 136 patients included in the study, 19 (14%) were pancreatic sufficient [PS, defined as having a PS mutation (JCF 2008;7:179) and not taking pancreatic enzymes], 27 (20%) had meconium ileus (MI) and 90 (66%) had no MI but were pancreatic insufficient (PI).

Results: Overall, the prevalence of low serum LA was 8.1% at all ages and was highest in MI patients (16.4%), followed by 6.6% in PI patients, and 2.4% in PS patients, p = 0.010. Consistently, MI patients had significantly higher prevalence of elevated triene/tetraene ratio (39.7% > 0.02; 5.5% > 0.04) compared to PI patients (19.8% > 0.02; 1.2% > 0.04) and PS patients (14.6% > 0.02; 0% > 0.04), p = 0.0008 and 0.082 respectively. In addition, MI and PI patients also had significantly lower levels of linolenic (18:3n-3), docosahexanoic (DHA, 22:6n-3) and total fatty acids than PS patients (p = 0.034, 0.028 and 0.012, respectively). Arachidonic acid (20:4n-6) was lower but not significantly comparing MI and PI to PS patients (p = 0.105).

Among MI and PI patients, the prevalence of low serum LA was highest in infancy (37.5%, 3 out of 8), declined to 14.3% (2 out of 14) in the second year of life, further lowered during ages 2-9 years to an average of 8.1% (197 measurements, 15-39 per year), but increased again to 11.5% during ages 10-12 years (61 measurements, 17-23 per year), yet decreased to 0% during ages 13-15 years (35 measurements), p=0.015. On the other hand, the prevalence of elevated triene/tetraene ratio did not differ significantly with age, p > 0.2.

Conclusion: This study provides evidence that low serum linoleic acid remains prevalent in the first two years of life in CF children despite early diagnosis and recent treatment during 2006-2010. In addition, a new finding that early adolescence is associated with an increased prevalence of low serum linoleic acid was found. Further analyses are needed to determine the clinical significance of these observations. (Supported by NIH-R01DK072126).

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ELEVATED SUBSTRATE OXIDATION AND RESTING ENERGY EXPENDITURE IN CF MOUSE MODELS

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Introduction: CF patients typically experience reduced growth; both body weight and height are low for age, resulting in lower BMI. Despite nutritional intervention (high-fat high-energy diets) to improve body composition and support growth, children and young adults with CF still have

impaired growth and low adiposity. This suggests that simply increasing caloric/lipid intakes may not readily improve adiposity and BMI. The reasons for low rate of adipose tissue growth are not clear. The few existing studies in CF patients indicate adipose tissue energy imbalance via decreased lipid uptake and increased breakdown. Using CF mouse models, we previously showed that CF mice have low lipogenesis and adipose tissue lipid uptake contributing to retarded lean phenotype. Feeding CF mice high-fat diet to overcome low lipogenesis rates did not result in weight gain indicating defect of lipid uptake and/or elevated lipid oxidation. The increased lipid breakdown in CF patients is likely driven by the reported elevated resting energy expenditure (REE) strongly associated with severe CF genotypes, i.e. F508del. In some studies, REE was elevated in spite of good nutritional status, normal lung function, and good athletic level indicating basic metabolic dysfunction in substrate utilization. Concomitant with elevated REE, studies reported elevated respiratory quotient (RQ) indicating shift in substrate utilization towards higher reliance on carbohydrates. We hypothesized that similar to CF patients, CF mice have elevated resting energy expenditure and substrate utilization contributing to the poor growth phenotype.

Methods: We tested our hypothesis by using indirect calorimetry to measure energy expenditure and substrate utilization in wildtype (WT) and CF mice (severe (F508del) and mild (R117H) genotypes) fed either low-fat (9% fat) or high-fat (58% fat) diet. Stable isotopes were used to determine lipid oxidation.

Results: F508del mice fed low-fat diet had elevated 24-hr energy expenditure (3939 ± 89 vs. 5169 ± 92 kcal/kg hr, WT vs. F508del), but similar respiratory quotient (RQ), (0.86 ± 0.04 vs. 0.85 ± 0.02 , WT vs. F508del). R117H mice fed low-fat diet had similar values for energy expenditure (3619 ± 130 vs. 3676 ± 116 kcal/kg hr, WT vs. R117H) and respiratory quotient (0.88). However, when mice were fed high-fat diet, WT mice had significantly lower energy expenditure consistent with other reports, while R117H mice had elevated values (2806 ± 51 vs. 3612 ± 147 kcal/kg hr, WT vs. R117H). WT mice fed high-fat diet had lower RQ values, as compared with R117H mice (0.75 ± 0.01 vs. 0.77 ± 0.01 , $p < 0.001$).

Conclusions: We found that F508del mice have elevated basal energy expenditure, consistent with CF human findings. We found no difference in substrate oxidation in CF mice fed low-fat diet. Interestingly, mice with mild CF mutation (R117H) had similar energy expenditure values. When challenged with high-fat diet, WT mice had lower energy expenditure and became obese, while R117H mice had elevated energy expenditure and resisted weight gain. In summary, we found that in addition to low growth, CF mice have elevated energy expenditure contributing to retarded growth phenotype. These studies are supported by National Institutes of Health and Cystic Fibrosis Foundation.

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THE PREVALENCE OF LIVER CIRRHOSIS IN CYSTIC FIBROSIS: CLINICAL AND GENETIC CHARACTERISTICS OF PATIENTS

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Objectives: The liver disease associated with cystic fibrosis (CF) ranges from mild subclinical liver involvement to severe clinical symptoms related to cirrhosis with portal hypertension and hypersplenism. The severity of chronic liver disease in CF may determine the prognosis of CF individuals. The aim of study was to analyze the prevalence of liver cirrhosis in our CF population and the clinical and genetic characteristics of these patients.

Methods: All patients older than 2 years ($n=96$) were screened for liver disease through clinical, biochemical and ultrasound assessment. Liver biopsy was not included in the study protocol for liver cirrhosis; however, 5 patients with ultrasound defined cirrhosis underwent this procedure. Liver cirrhosis was defined by ultrasonographic findings of distinct heterogeneity of liver parenchyma and nodular liver surface and/or by liver biopsy findings. Portal hypertension was suggested by enlarged spleen, distended portal vein and abnormal portal venous flow. Clinical and genotype data were analyzed.

Results: Sixteen patients (nine males and seven female) were found to have liver cirrhosis, three of them with portal hypertension. Average age of patients with cirrhosis was 17.7 years (5-26). Average age of diagnosis of liver cirrhosis was 8.5 ± 3.8 years. In two patients the diagnosis of CF was

established at the age of 14, after the development of portal hypertension. All patients had pancreatic insufficiency. Nutritional status expressed as standard deviation score (Z score) for weight, height, and body mass index was as follows: $zW = -0.40 \pm 1.24$, $zH = -0.83 \pm 1.02$, and $BMI = 20.1 \pm 2.3$. CF patients with liver cirrhosis generally had mild-to-moderate lung disease, with average FVC and FEV1 values of $97.1 \pm 16.5\%$ predicted and $87.9 \pm 23.5\%$ predicted, respectively. Genetic analysis showed much higher frequency of F508del mutation in the group with cirrhosis (90.6%) in comparison with the frequency of mutation in general CF population in Macedonia (69.2%). All patients in the group with cirrhosis were either F508del homozygous (13) or compound heterozygotes for F508del and other severe mutation (3).

Conclusion: The prevalence of liver cirrhosis in the Macedonian CF population older than 2 years is 16.6%. It's usually develops during the first decade of life. This suggests that, for CF patients who will develop liver cirrhosis, the mechanism and risk factors for liver damage are already present in early childhood. Patients with pancreatic insufficiency and with severe CFTR mutations, especially F508del, are exposed to higher risk for developing liver cirrhosis. Liver cirrhosis does not have significant impact on pulmonary function and nutritional status, until end-stage liver disease.

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THE ASSOCIATION OF LEAN BODY MASS AND PULMONARY FUNCTION IN CYSTIC FIBROSIS

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Objective: Pulmonary function is a vital indicator of health in patients with CF, and is positively correlated with body mass index (BMI). BMI alone, however, does not entirely reflect nutritional health. We hypothesized that lean body mass (LBM) may be a better indicator of nutrition and its impact on lung function, and tested whether LBM would be more strongly associated with pulmonary function than BMI in children and young adults with CF.

Methods: Anthropometrics, pubertal status, DXA-determined body composition, and spirometry were assessed in 208 individuals, ages 5-21 years with CF and pancreatic insufficiency. Lean body mass index ($LBMI = LBM/height^2$) and fat mass index ($FMI = FM/height^2$) were calculated and then converted to sex- and age-adjusted Z-scores using contemporary reference data from 479 healthy volunteers for growth, and body composition (Thayu, et al., *Gastroenterology*, 2010). Linear regression models were developed for each sex to test the associations of BMI-Z, LBMI-Z, FMI-Z adjusted for age, with FEV1%-predicted. These associations were tested individually and in combined models, and were compared using R^2 . Subjects were also categorized as having acceptable BMI by current standard of care ($BMI > 50\%$ tile) vs. suboptimal ($BMI < 50\%$ tile) to examine for effect modification.

Results: Among females, BMI-Z (β -coefficient=7.5, $p=0.001$, CI: 3.3, 11.7, $R^2=0.35$) and LBMI-Z ($\beta=8.3$, CI: 4.3, 12.3, $p<0.0001$, $R^2=0.4$) were positively associated with FEV1 whereas FMI-Z was not associated with FEV1 ($p=0.06$). The model using LBMI-Z accounted for a greater variability in the FEV1 than did BMI-Z model (R^2 0.4 vs. 0.35). The relationship between LBMI-Z and FEV1 was modified by nutritional status: the association of LBMI-Z on FEV1%-predicted is stronger in the setting of CF Foundation defined sub-optimal BMI ($p=0.013$). The association between FEV1%-predicted and LBMI-Z becomes weaker in individuals with "acceptable" body weight.

In males, BMI-Z (β -coefficient=7.7, CI: 3.4, 11.9, $p=0.001$, $R^2=0.16$) and LBMI-Z ($\beta=8.5$, CI: 4.9, 12.1, $p<0.0001$, $R^2=0.25$) were positively associated with FEV1%-predicted but FMI-Z ($p=0.19$) was not associated. LBMI-Z model accounted for a greater variability in FEV1%-predicted than the model evaluating BMI-Z alone (R^2 0.25 vs. 0.16). No interaction was found between LBMI-Z and BMI% category in males.

Pubertal status did not alter the relationship of body composition with FEV1 in this CF population.

Conclusion: We confirmed that BMI-Z is positively associated with lung function (FEV1%-predicted) in children and adolescents with CF and pancreatic insufficiency. However, LBMI-Z was better associated with FEV1%-predicted in both sexes, and these associations were irrespective of age and pubertal status. LBMI may be especially relevant to pulmonary function in underweight females. These data support the hypothesis that LBM is an important marker of nutritional status and is correlated with

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FEV1 in CF. Further, these data support the notion that nutritional intervention in CF might better target improved LBMI rather than merely BMI, especially in young females.

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CFTR-RELATED CHRONIC OR ACUTE RECURRENT PANCREATITIS: A SINGLE-CENTRE EXPERIENCE

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Objectives: We assessed the genotype/phenotype relationship in CFTR-related chronic (CP) or acute recurrent pancreatitis (ARP) at a single Italian pediatric Cystic Fibrosis (CF) Centre during the 2003-2012 period.

Methods: We studied, retrospectively, all consecutive pediatric patients affected by CP or ARP of undefined etiology. All patients underwent chloride sweat testing and CFTR genetic analysis by sequencing of the entire gene.

Results: A total of 102 young patients (52 male, mean age at diagnosis 10.1±7 yrs) were enrolled. Forty of them (39.2%) tested positive for CFTR gene mutations. The genotype/phenotype relationship in these patients showed: a) Six of 40 (15%) patients had classic CF, previously diagnosed, and one of them had pancreatic insufficiency; b) Eighteen of 40 (45%) patients showed 2 CFTR gene mutations. In this group 7/18 patients had pancreatitis as the first manifestation of CF: 4 patients had a positive sweat test, 3 patients had borderline values. Eleven of 40 (27.5%) patients with 2 CFTR mutations had a negative (n=9) or borderline (n=2) sweat test. They did not have more than one CF causing mutation and CF diagnosis was clinically excluded. c) Sixteen of 40 patients (40%), finally, did not have CF but they carried one CFTR mutation and showed negative sweat test, except one patient having borderline values.

Conclusions: Our study confirms a high percentage of CFTR gene mutations in pediatric patients affected by CP or ARP. Particularly, in 17.5% of patients we diagnosed CF with pancreatitis as the first manifestation of the disease. Furthermore, in 27.5% of patients we found 2 CFTR gene mutations that were not clearly CF causing and CF was ultimately excluded by clinical follow-up. Further studies are needed to assess the clinical impact of this class of mutations.

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INFLUENCE OF VITAMIN D STATUS, BODY MASS INDEX AND LUNG FUNCTION ON CF EXACERBATIONS

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Introduction: Vitamin D deficiency is frequently encountered among patients with CF due to impaired absorption of fat-soluble vitamins. Low vitamin D status has been linked to decreased bone mass, reduced lung function and impaired immune response. Previous research has linked vitamin D deficiency with increased frequency of CF exacerbations. The primary objective of this study was to identify the relationship between vitamin D and CF exacerbations among adult patients.

Methods: The retrospective sample was obtained from adult patients (≥18 years) seen in the CF clinic who had a vitamin D level measured. Vitamin D level and concurrent height (meters(m)), weight (kilograms(kg)), and FEV1 were recorded; body mass index (BMI, kg/m²) and percent FEV1 were calculated. Frequency of CF pulmonary exacerbations within one year of the vitamin D level was also documented. A CF pulmonary exacerbation was defined by Akron Pulmonary Exacerbation Score ≥ 5 and/or provision of oral or parenteral antibiotic therapy. Data were categorized for bivariate analysis, CF exacerbations were categorized as none versus one or more, vitamin D was categorized as low (< 30 ng/mL) versus normal (≥30 ng/mL), BMI was categorized as nutrition risk (≤22.5 kg/m²) versus not nutrition risk (>22.5 kg/m²), and percent FEV1 was categorized as mild (>70%), moderate (50-70%), severe (30-50%) and very severe (<30%). Chi-square analy-

sis was performed to determine difference in proportions of CF exacerbations, a p-value of 0.05 was utilized to indicate significance. A binary logistic regression was performed to determine predictors of CF exacerbations.

Results: A total of 75 vitamin D levels were available for analysis. Vitamin D level was not related to CF exacerbations, the proportion of patients who experienced one or more exacerbations was equal between the 2 groups (low vitamin D: 63% versus normal vitamin D: 69%). However, percent FEV1 was related to exacerbations; the proportion of patients who experienced one or more exacerbations doubled across percent FEV1 categories (mild: 41%, moderate: 83%, severe: 87%, very severe: 86%; p<0.001). Exacerbations occurred in a higher proportion of patients with a low BMI (low BMI: 71% versus normal BMI: 54%), though the difference was not significant (p=0.163). Patients with a low vitamin D tended to have a low BMI and moderate, severe and very severe percent FEV1. Severe/very severe percent FEV1 was the only predictor of CF exacerbations (OR 5.73, 95% CI 1.72-19.10). Controlling for BMI and vitamin D status did not dampen the effect of percent FEV1 on CF exacerbations (OR 5.51, 95% CI 1.60-18.98).

Conclusions: In this study vitamin D level was not related to the presence or absence of CF pulmonary exacerbations. Lung function appears to be a better indicator of exacerbation risk. Low vitamin D status may be linked to decreased percent FEV1; patients with impaired lung function may have limited sun exposure and decreased intake of vitamin D foods. More research is needed to determine the relationship between vitamin D, lung function and BMI on CF exacerbations.

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ADULT G-TUBE INTERVENTIONS; LESSONS LEARNED?

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Background: Nutrition support is an essential component of CF care. The correlation between BMI, lung function and outcomes in CF patients is well documented. Many CF adults need to increase their energy intake significantly in order to achieve their BMI goal. Tube feedings (TF) are one way to accomplish this. The decision to place a gastrostomy tube (GT) is difficult and tends to be delayed with CF adults due to cost, fear of surgery, treatment burden and body image issues. In our center we noticed that many patients with GT were not able to achieve adequate weight gain. This prompted a retrospective review of our adult CF patients' GT outcomes to determine why enteral nutrition did not always meet expectations.

Method: Data from GTs placed at our center from 2000 to present were reviewed. Subject characteristics were recorded, including age of GT placement, gender, weight, BMI and FEV1% 2 years pre- and 2 years post-GT intervention. Patients were classified with a severity score (low, moderate and high) based on a point system depending on their nutritional and pulmonary status. Nutritionally patients were categorized as high risk (BMI<18.5), at risk (BMI 18.5 – 21.9♀ or 22.9♂) or acceptable (BMI≥ 22♀ or 23♂) and severe (FEV1< 40%), moderate (FEV1 40-79%) or normal (FEV1>80%) pulmonary status. A GT was considered to be a successful intervention if the patient reached their CF BMI goal and/or received a lung transplant. A TF questionnaire was sent out via mail and e-mail to 12 patients to provide feedback to our CF team regarding their TF experience.

Results: There were 20 patients with GT identified from 2000-2012; 7 males/13 females born from 1957-1987. The age range at GT placement was 18-51. A quarter of the patients received a lung transplant. Fifteen percent no longer have their GT due to multiple reasons (GI issues, anorexia, early satiety, poor adherence or psychosocial issues). Twenty percent are still using their GT to maintain/gain weight. Forty percent have died; 45% including one post-transplant death. Four patients died within the year of getting their GT. Patients with the highest severity score (75%) had a 7% success rate nutritionally (BMI @ goal) and 27% success rate via transplant. The remaining 25% were classified as moderately severe and had a 60% success rate nutritionally (BMI @ goal) and 20% success rate via transplant. Overall 45% was successful due to reaching their nutritional goal or receiving a lung transplant while 55% were unsuccessful. Only 20% of those successes were due to reaching their nutritional goal. Our patients with poor pulmonary status (FEV1 < 40%) at time of GT intervention were unable to meet their nutritional goal with TF. Patients with poor nutritional status (BMI < 18.5) at time of GT intervention result in only a 14% success rate.

Conclusion: We found that the moderately severe patient population had better GT outcomes. We believe that this is because TFs were initiated before advanced lung disease was irreversibly established. We recommend early education on use and benefits of enteral nutrition to help change the mind set of patients/families that GTs are a last resort. GT placement should be recommended and started at the earliest of point of intervention possible. Evidence-based care guidelines for TF in CF are needed.

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DELIVERY OF ADENO-ASSOCIATED-9 VECTOR TO NEWBORN PIGS TRANSDUCES GENES IN THE PANCREAS

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Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The pancreas is universally involved in CF with progression to pancreatic insufficiency in most cases within the first few years of life. Pancreatic insufficiency correlates with the severity of lung disease and CF-related diabetes (CFRD), therefore preserving pancreatic function in CF may have an important impact on disease morbidity and mortality. Currently, there are no treatments to prevent the pancreatic disease progression in CF. Gene therapy has the potential to cure CF, as it addresses the root cause of the disease, but gene transfer studies have not been done in humans with CF and other pancreatic diseases, because the current methods are ineffective and unsafe in rodent models. As CF mice do not develop pancreatic disease similar to humans, it is difficult to develop a gene transfer approach in this model. Pancreatic and airway histopathology and physiology are very similar between humans and pigs with CF, therefore the CF pig model creates an excellent opportunity for studying gene therapy in this model. Currently, gene therapy cannot be fully explored in the CF pig model because there are no studies showing the transduction efficiency of pig pancreas with gene transfer vectors. Using a novel and minimally invasive transumbilical catheterization technique, we delivered a vector with a known affinity to pancreas, adeno-associated virus 9 (AAV9) to deliver the green fluorescent protein (self-complementary (sc) AAV9-eGFP) or sham vehicle control to the artery that supplies major branches to the pancreas (celiac artery) of wild-type newborn pigs (n=10). We selected AAV9 as studies in other models have documented its remarkable ability to transduce a variety of organs. Three different doses were given (2.5×10^{11} , 1.2×10^{12} or 2.5×10^{12} vector genomes (vg)/animal) and results were evaluated at two time points (30 and 90 days). The pancreatic eGFP expression was determined with immunofluorescence, and morphometry was performed using ImageJ. At 90 days, eGFP expression was dose dependently and stably expressed in the centroacinar cells, intercalated, and intralobular ducts, co-localizing with CFTR expression, as well as in small and large interlobular pancreatic ducts. Ten random pancreatic fields (20x magnification) were assessed per animal with an average eGFP transduction of 20% (2.5×10^{12} vg dose). The histopathological assessment of tissues showed normal architecture with no inflammatory response. These results show the successful transduction of pancreatic cells by AAV9sc-eGFP and offer a novel and minimally invasive method for gene delivery that has the potential to be translated to humans with CF. Future studies will evaluate the efficacy of this approach using a porcine CFTR vector in the newborn CF pig model.

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LONG-TERM SAFETY ASSESSMENT OF ZENPEP® (PANCRELIPASE DELAYED-RELEASE CAPSULES) IN INFANTS AGED 1-12 MONTHS WITH EXOCRINE PANCREATIC INSUFFICIENCY ASSOCIATED WITH CYSTIC FIBROSIS

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Introduction: Exocrine pancreatic insufficiency (EPI) is commonly reported in subjects with cystic fibrosis (CF). Treatment of EPI with porcine-derived pancreatic enzyme products improves malabsorption signs and symptoms. The efficacy and safety of ZENPEP® (pancrelipase delayed-release capsules) have been previously established in subjects with EPI and CF aged <7 years.¹ A capsule containing 3,000 USP lipase units was developed for infants and small children to provide a more age- and weight-appropriate dosing option and reduce the need to divide contents of higher-dose capsules.

Methods: The primary objective of this study was to evaluate safety (treatment-emergent adverse events [TEAEs]) during long-term treatment with ZENPEP® 3,000 lipase units. The secondary objective was to evaluate subjects' ability to thrive (weight, length, percentiles and Z-scores for weight-for-age, length-for-age and weight-for-length on CDC growth charts). Eligible subjects who completed two, 10 day treatment periods in an open-label, crossover study continued receiving ZENPEP® 3,000 lipase units for up to 12 months. Study assessments were conducted at 3-month intervals.

Results: Fifteen subjects (9 male) aged 3-14 months (median 9 months) constituted the study population; 12 completed the study. The mean duration of therapy was 315 [± 100] days. At the study end, median body weight and length increased from 7.8 (range 6.0-10.6) to 11 (9.8-14) kg and 68.5 (59-79) to 81 (74-91) cm, respectively. Median weight-for-age, length-for-age and weight-for-length percentiles increased from 22 (2-91) to 49 (8-93)%, 36.5 (1-74) to 42 (2-91)% and 41.5 (8-98) to 55.5 (24-95)%, respectively. Five subjects experienced 15 TEAEs judged possibly (n=13) or probably (n=2) related to treatment. Most common treatment-related TEAEs were diarrhea, vomiting and constipation. Except for one possible treatment-related TEAE (severe diarrhea), all of the other treatment-related TEAEs were of mild or moderate severity. No deaths were reported.

Conclusion: The results indicate that ZENPEP® 3,000 lipase units is safe for infants with EPI associated with CF. These findings demonstrate consistent improvement in individual growth percentiles. Promotion of normal growth has been associated with better pulmonary function and survival in CF subjects.² To the extent of the authors' knowledge, this is the first long-term study reported in this pediatric population.

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THE ROLE OF ENDOSCOPY AND BIOPSY IN THE MANAGEMENT OF SEVERE GASTROINTESTINAL DISEASE IN CYSTIC FIBROSIS PATIENTS

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There is increasing evidence to suggest the presence of chronic inflammation in the gastrointestinal (GI) tract of cystic fibrosis (CF) patients. Some CF patients continue to have very severe gastrointestinal symptoms despite conventional CF treatment. In our centre, these patients are managed in a CF gastroenterology clinic, jointly with a paediatric gastroenterologist. A number have required GI endoscopy and biopsy. The aim of our study was to characterise these patients and determine whether endoscopy and biopsy changed their management. We reviewed all the patients seen in the CF gastroenterology clinic from 2004-2009, who had GI endoscopies performed. The GI symptoms these patients were experiencing included abdominal

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pain, nausea and vomiting, rectal bleeding, failure to thrive, loose stools and constipation. Twelve patients had GI endoscopies with mucosal biopsies performed. The median [Interquartile range (IQR)] age at referral to the CF gastroenterology clinic was 4[0.9-8]years. Their body mass index (BMI) was 15.2[13.7-15.5]. Twenty-five percent were homozygous delta F508. Two patients had previously had meconium ileus as neonates requiring surgical intervention. One other patient had needed abdominal surgery for intussusception. Ninety-two percent were pancreatic insufficient, 25% were chronically infected with *Pseudomonas aeruginosa* and 17% were on regularly 3 monthly intravenous antibiotics. Of the 10 patients who were able to perform spirometry, FEV1 was 101[67-125]% predicted. Nine of the 12 patients had evidence of mucosal inflammation in their biopsies, including duodenitis with eosinophilic infiltrate, chronic non-specific inactive gastritis, enteropathy with partial villous atrophy, and non-specific colitis. Immunosuppressive and anti-inflammatory therapies were commenced in these 9 patients, including prednisolone, azathioprine, methotrexate, ketotifen, mesalazine and sulphasalazine as well as the use of parenteral nutrition and elemental feeds. All the patients clinically responded to therapy. Five of the patients commenced on anti-inflammatory therapy had repeat biopsies 1-5 years following commencement of treatment and all showed histological improvement of the mucosal inflammation. GI endoscopy with mucosal biopsy has a significant role to play in the management of CF children with severe GI disease. In our study, it influenced the management in 75% of patients with severe GI symptoms. Furthermore, if GI mucosal inflammation is identified on biopsy, management with immunomodulatory agents may be clinically beneficial.

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EFFECT OF PROTON PUMP INHIBITORS ON ABSORPTION AND SERUM LEVELS OF VITAMIN D IN CYSTIC FIBROSIS PATIENTS

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Introduction: A common problem associated with cystic fibrosis (CF) is malabsorption of fat-soluble vitamins A, D, E, and K. Approximately 90% of CF patients require additional supplementation of these vitamins. Furthermore, 81% of patients with CF may be vitamin D insufficient. CF patients often receive proton pump inhibitors (PPI) to facilitate absorption of enzymes in the intestinal tract as well as treatment of gastrointestinal reflux disease (GERD). Recent studies have linked PPI therapy to an increased risk of fractures in healthy patients due to impaired calcium absorption; an important consideration given the high risk of osteoporosis (25%) and osteopenia (40%) in CF. This retrospective analysis investigated the association between PPI use and vitamin D level in CF.

Hypothesis: Increasing doses of PPI therapy are associated with decreased serum levels of vitamin D without significant adverse effects on bone density.

Methods: This is a retrospective chart review that included CF patients who were vitamin D insufficient (less than 30 mg/L) with concomitant PPI therapy less than 1 year. IRB approval was granted by the University of Alabama and Samford University. There were a total of 53 patients included in this study. A comparison of vitamin D levels after a PPI dose change using paired t-test analysis, serum vitamin D levels and PPI dose (high vs. low dose (<1 mg/kg)) by unpaired t-test analysis and correlation between PPI dose (mg/kg) and DEXA z-score using Pearson's r calculation was performed. A p value ≤ 0.05 was considered statistically significant.

Results: The average baseline serum vitamin D level for CF patients concomitantly on a PPI was 33.25 mg/L. Serum vitamin D levels pre- and post-addition of a PPI showed no significant difference (33.1 mg/L vs 33.2 mg/L; $p=0.9$) with the average change of serum vitamin D levels after initiation of PPI therapy being minimal. Patients on high-dose PPI therapy (>1 mg/kg/day) were younger (5 vs 10 years; $p=0.001$) and smaller (20 vs 36kg; $p=0.001$), but serum vitamin D levels remained similar (33.1 vs 33.5; $p=0.9$) between groups. No correlation was present between PPI dose (mg/kg) and DEXA z-scores, although DEXA scans were only performed in 39% of patients (21/53). DEXA z-scores also did not correlate with total PPI dose, duration of PPI therapy, age or serum vitamin D level.

Conclusions: The addition of a PPI did not alter serum vitamin D level and increasing PPI dose based on mg/kg did not have a measurable effect on serum vitamin D in our population. Although this cross-sectional retrospective study did not identify an association between PPI and DEXA z-score, a longitudinal randomized control trial would be necessary to ascertain the safety of PPI therapy on bone health in CF patients.

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OVERWEIGHT AND OBESITY IN CYSTIC FIBROSIS PATIENTS

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Cystic fibrosis (CF) is often characterized by growth failure, which can be due to malabsorption, increased energy needs, anorexia, and CF-related diabetes. The nutritional status of patients with CF is continuously monitored in hope of addressing the comorbidities associated with inadequate nutrition, including accelerated loss of lung function and bone disease. Despite the challenges many patients face in achieving adequate weight gain, overweight and obesity have been observed in patients with CF. The aim of this study is to describe the prevalence of overweight and obesity as well as factors associated with over-nutrition.

Methods: The Cystic Fibrosis Patient Registry was queried for patients aged 2-18 who were seen the prior year at the Children's Hospital of Pittsburgh. Patients with a BMI over the 85th but less than the 95th percentile were considered overweight while patients with a BMI greater than the 95th percentile were considered obese. We abstracted data including pancreatic status, lung function, and genotype. Spearman's rank correlation coefficient was used to determine correlations of continuous variables and a Mann-Whitney test was performed to explore relations of BMI to pancreatic status and caloric supplementation. Data was analyzed using MATLAB.

Results: Of the 233 CF patients aged 2-18, 46 (20%) were overweight or obese. Thirty (13%) were overweight, with an average BMI percentile of 90 and an FEV1 of 98 percent predicted. Of these patients, 7 (23%) were prescribed dietary supplements. Twenty-three (77%) were prescribed pancrelipase with a mean of 1976 units lipase/kg/meal. Sixteen (7%) were obese with an average BMI percentile of 97 and an FEV1 of 92 percent predicted. One (6%) of the obese patients was receiving dietary supplements and seven (44%) were prescribed pancrelipase with a mean of 1997 units/kg/meal. No significant correlation between BMI percentile and FEV1 percent predicted ($r=-0.25$, $p=0.11$) or BMI percentile and lipase units per kilogram per meal ($r=-0.09$, $p=0.64$) were found in overweight and obese CF patients. While the mean BMI percentile of those who received caloric supplementation and those who did not were similar (91 vs. 93, respectively), the mean BMI percentile of patients who were pancreatic sufficient was higher than in those that were insufficient (94 vs. 92, $p=0.02$).

Conclusion: The prevalence of overweight and obesity in our CF center is noteworthy. In this population, lung function was excellent but not correlated with BMI. The majority of overweight patients were pancreatic insufficient. Nutritional approaches may need to include counseling about the adverse effects of overweight and obesity.

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NUTRITIONAL STATUS AND DISEASE SEVERITY IN PRE-ADOLESCENT AND ADOLESCENT PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Optimizing nutritional status in patients with cystic fibrosis (CF) during pre-adolescence and adolescence contributes to maintaining lung function and extending the median lifespan. By examining the factors that contribute to overall nutrition status, medical nutrition therapy can be tailored to promote optimal clinical outcomes and improve quality of life in CF.

Objective: The aim of this study was to examine and compare commonly-measured nutrition related health metrics in pre-adolescent and adoles-

cent patients with CF with BMI for age percentile (BMI%), a clinical marker of disease severity.

Methods: IRB approval was obtained. We completed a retrospective review of 114 medical records of patients with CF ages 8-19 years during 2011. Data collected included patient BMI%, serum fat-soluble vitamin levels, lung function as measured by forced expiratory volume in one second percent predicted (FEV1%), glucose tolerance as measured by oral glucose tolerance test (OGTT) and hemoglobin A1C (HgbA1C), and bone density as measured by dual energy x-ray absorptiometry (DEXA) scans. Records were also reviewed for commonly-prescribed, nutrition-related pharmaceutical interventions. Fischer's chi-square was used to examine associations between DEXA, OGTT, pharmaceutical interventions, and BMI%. T-tests were used to examine associations between continuous variables, such as vitamin levels, HgbA1C, FEV1%, and BMI%.

Results: Compared to patients in nutrition failure (defined at our Center as a BMI% $\leq$ 25%), patients in optimal nutrition (defined as a BMI% $\geq$ 50%) had higher mean levels of vitamins A (40.42 μ g/dL vs. 33.03 μ g/dL, $p=0.003$) and E (10.67 mg/dL vs. 7.99 mg/dL, $p=0.0008$). Patients in optimal nutrition were more likely to have normal bone density as patients in failure (mean 72.4% vs. 34.8%, $p=0.0111$). Patients in optimal nutrition had significantly higher FEV1% when compared to those in failure (mean 95.52 vs. 72.19, $p<0.0001$). Patients in nutrition failure were found to be much more likely to be prescribed: appetite stimulants (47.2% vs. 24.4%, $p=0.0181$), vitamin A supplements (19.4% vs. 2.6%, $p=0.042$), vitamin K supplements (26.1% vs. 16.7%, $p=0.0303$), and oral supplements (58.3% vs. 37.2%, $p=0.0430$).

Conclusions: Our patients in nutrition failure were found to have significantly lower mean serum vitamin A and E levels, bone density, and lung function compared to patients in optimal nutrition. Despite being more commonly prescribed oral supplements and appetite stimulants, patients in nutrition failure did not demonstrate clear positive clinical impact of these therapies. We observed a lack of efficacy despite aggressive nutritional intervention that may have resulted from inadequate or delayed therapy or be relative to poor health status. Based on the results of this study, we feel further research is warranted to determine if current nutritional interventions in CF are adequate and timely or if they should be modified to promote better clinical outcomes in the population.

ENDOCRINE

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CONTINUOUS GLUCOSE MONITORING (CGM) FOR CYSTIC FIBROSIS RELATED DIABETES (CFRD): SWEET SUCCESS OR BITTER DISAPPOINTMENT?

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Introduction: Although current guidance suggests an oral glucose tolerance test (OGTT) to diagnose CFRD, this uses glucose thresholds derived from a non-CF population to prevent micro-vascular complications. However, in CF, glucose handling is variable and a single OGTT based on these thresholds may be misleading. CGM has been validated for use in CF¹, allows evaluation of a glycemic profile up to 120 hours, is a stronger predictor for the development of CFRD than OGTT², and a glucose $\geq$ 7.8 mmol/L for $\geq$ 4.5% CGM time is associated with declining weight and lung function³.

We routinely use CGM for CFRD diagnosis and optimisation of diabetic control at our large CF center (n=270; 40% CFRD), and we have explored its utility in the management of this CF complication.

Method: We looked at CGM traces in 45 patients over a 12 month period, along with any subsequent changes in weight, pulmonary function, HBA₁C and antibiotic treatment. Food and exercise diaries completed during CGM were also evaluated.

Results See Table: Of the 27 who underwent CGM for diagnostic purposes, 17 (63%) had CFRD (mean time glucose $\geq$ 7.8 mmol/L, 23%) despite previous normal HBA₁C and serial glucose monitoring. Following insulin initiation in these patients, there was improvement in weight in 82% ($p=0.003$), lung function in 59%, and HBA₁C in 41%.

Of the 18 with CFRD who underwent CGM to aid glucose control [management group] (mean time glucose $\geq$ 7.8 mmol/L, 42%), 72% gained

weight, 78% improved their lung function ($p=0.006$) and 72% improved HBA₁C ($p=0.002$).

Overall, 24 patients (53%) required less IV antibiotic therapy [$p=0.008$ in the management group] and 20 (38%) required fewer oral antibiotic courses.

Review of their food and exercise diaries and subsequent dietary alteration achieved better glycemic control in 12 diagnostic group patients (44%) and in 11 management group patients.

Conclusion: This study demonstrates the utility of CGM as a diagnostic tool, especially since HBA₁C, OGTT and serial blood glucose monitoring have significant limitations. Furthermore, it is useful in treatment optimization including the dosage and type of insulin therapy, where hypoglycaemia is not uncommon due to endogenous insulin production. Additionally, the concurrent mapping of food and exercise diaries to CGM results is of educational benefit allowing the fine tuning of any adjustments of treatment.

References:

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2. Schiaffini R, et al. Eur J Endocrinol 2010 Apr;162(4):705-10.
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Clinical Parameters Pre and Post CGM

Group	FEV ₁ (%)		Weight (Kg)		HBA ₁ C (IFCC mmol/mol)	Oral Antibiotics (courses)		IV Antibiotics (courses)	
	Pre	Post	Pre	Post		Pre	Post	Pre	Post
Diagnostic (CFRD - Insulin commenced)	60 [26]	63 [26]	54 [8]	56 [9]	40 [4]	0.65 [0.70]	0.50 [0.73]	1.35 [0.86]	1.06 [0.97]
Management	59 [30]	64 [29]	59 [13]	60 [13]	64 [30]	0.78 [0.94]	0.50 [0.71]	1.18 [1.01]	0.59 [0.71]

Results: Mean [SD]

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IMPAIRED AFTERNOON AND EVENING GLUCOSE HANDLING – IMPLICATIONS FOR THE DIAGNOSIS AND SCREENING OF DIABETES IN CYSTIC FIBROSIS

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Introduction: In the normal population, blood sugar levels after an oral glucose load increase throughout the day¹ and it has been suggested that impaired glucose tolerance later in the day may be an early sign of diabetes: if current oral glucose tolerance test (OGTT) diagnostic criteria were applied to patients tested in the afternoon, half of all cases of undiagnosed diabetes would be captured². This is particularly important in CF, where glucose handling is known to be deficient, even in those without established CF-related diabetes (CFRD). To look at this further, we compared the diurnal response to an OGTT in the CF population with healthy controls.

Method: We studied 20 CF patients (17 pancreatic insufficient) not known to have CFRD and compared them with 6 healthy (age, BMI matched) controls. Following an overnight fast subjects consumed, within 10 minutes, a standardized mixed meal (the gold standard measure of endogenous insulin secretion³) providing an equivalent glucose load to a standard OGTT, at 0800, 1300 and 1800 hours on the same day. Blood glucose levels were measured at 0, 30, 60, 90 and 120 minutes for each test meal. The area under the curve was calculated for the initial 30 minutes (AUC₃₀) and the total 120 minutes (AUC₁₂₀) of each test.

Results: The table summarises glucose levels (mean mmol/L $\pm$ SEM) during the 3 tests. CF subjects had greater overall glucose levels throughout the day than controls [AUC₁₂₀ morning: CF 820 $\pm$ 33 vs controls 500 $\pm$ 27; afternoon: 697 $\pm$ 20 vs 560 $\pm$ 9; evening: 831 $\pm$ 35 vs 571 $\pm$ 22 respectively, all $p<0.005$]. Furthermore, CF subjects had higher early glucose levels which increased throughout the day [AUC₃₀ morning CF 184 $\pm$ 6 vs controls 138 $\pm$ 12; afternoon: 168 $\pm$ 4 vs 145 $\pm$ 5; evening: 186 $\pm$ 4 vs 146.7 respectively, all $p<0.0001$], suggesting a reduction in early glucose control. There was a trend ($p=0.02$) to increasing AUC₃₀ with progression of the day, the differences between the afternoon and morning ($p=0.008$) and evening ($p=0.005$) being significant.

Conclusions: This study demonstrates an increase in glucose intolerance as the day progresses in the CF population with a greater 30 minute glucose excursion in the afternoon and evening, attributed to a diminished pancreatic response to a glycemic stimulus. The clinical implications of this

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study are important for the diagnosis and management of CFRD, questioning whether an OGTT should be done later in the day to provide a more accurate screening method that allows for an earlier diagnosis of CFRD.

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	Controls					CF				
	0 mins	30 mins	60 mins	90 mins	120 mins	0 mins	30 mins	60 mins	90 mins	120 mins
Morning	4.5 ± 0.1	4.7 ± 0.7	3.3 ± 0.2	4.3 ± 0.2	4.3 ± 0.2	4.8 ± 0.1	7.4 ± 0.3 Ω	7.8 ± 0.4 *	6.7 ± 0.4 *	5.7 ± 0.4 Ω
Afternoon	4.5 ± 0.1	5.2 ± 0.4	4.2 ± 0.2	4.5 ± 0.2	5.0 ± 0.1	4.5 ± 0.1	6.6 ± 0.2 Ω#	6.0 ± 0.3 ΩS	5.5 ± 0.2 µE	5.5 ± 0.2 #
Evening	4.6 ± 0.2	5.0 ± 0.4	4.6 ± 0.1	4.7 ± 0.2	4.8 ± 0.2	5.0 ± 0.2	7.3 ± 0.3 *B	7.3 ± 0.4 *B	6.9 ± 0.4 *x	6.6 ± 0.3 *EB

Comparison between groups: *p<0.0001, ^ap=0.003, ^bp=0.02; Within a group: Comparison with morning: ^Sp<0.0001, [#]p=0.00, ^Ep=0.03; Comparison with afternoon: ^Bp=0.003, ^p=0.0007

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REDOX IMBALANCE IN THE DEVELOPMENT OF CFRD
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The pathogenesis of CFRD is complex and incompletely understood. Oxidative stress is a key factor in the development of type 2 diabetes. Redox imbalance to a more oxidizing state is the precursor of oxidative stress but also is a regulator of a variety of cell functions - the most relevant to diabetes being the effect on insulin secretion and glucose uptake. Since CF is a disease of oxidative stress, the goal of this study was to elucidate whether redox balance is altered with the development of CFRD. We hypothesized that measuring redox potentials (Eh) at baseline and following glucose challenge in CF subjects with varying degrees of glucose intolerance would uncover a biochemical phenotype that could directly contribute to the pathogenesis of CFRD. Blood was drawn for measurement of redox status of the major redox couple in blood, cysteine/cystine (Cys/CySS) and glucose before and 2hrs after ingestion of 75 gms of an oral glucose solution. Subjects were 16 to 40 years of age and consisted of 15 healthy controls (Con) with normal glucose tolerance (NGT); 11 CF with NGT; 16 CF with prediabetes (CFPD, defined as fasting plasma glucose between 100 and 125 mg/dl and/or 2hr between 140 and 199 mg/dl); and 11 with CFRD (2hr glucose > 199 mg/dl with 1 subject also having fasting > 125 mg/dl). Statistical differences between the 4 groups were determined by ANOVA. At baseline, Con-NGT and CF-NGT had plasma redox status in the normal range (Eh Cys/CySS [x±SEM] = -86±2.34 mV and -80±4.17 mV respectively) whereas CFPD and CFRD showed significant redox imbalance to the oxidizing state (Eh Cys/CySS = -73±3.62 mV and -71±4.36 mV respectively, p<0.05, note that less negative = more oxidizing). This degree of imbalance is large. For example, redox status becomes more oxidizing in adults with aging at a rate of 1 mV per 5 years. Thus, these teens and young adults with CFPD and CFRD have the redox values of an octogenarian. At 2hrs after glucose challenge, CF-NGT had a marked acute redox imbalance to the oxidizing state, CFPD and CFRD had worsening redox imbalance, and Con-NGT redox status was unchanged. This resulted in CF-NGT, CFPD, and CFRD all having the same level of redox imbalance to the oxidizing state at 2hrs post glucose which was significantly different from control (2 hour Eh Cys/CySS: Con-NGT = -84±2.59, CF-NGT = -63±4.67, CFPD = -60±4.22, CFRD = -63±2.45, p<0.05). Finally, there was no correlation between the degree of hyperglycemia and the degree of redox imbalance. In summary, subjects with CF prediabetes or diabetes have severe systemic redox imbalance to the oxidizing state. Furthermore, glucose challenge in CF causes acute redox imbalance without necessarily causing hyperglycemia. Since the degree of redox imbalance seen is potentially severe enough to damage islet cells, we speculate this discovery needs further evaluation as a mechanism for the development of CFRD. Supported by: CFFSTECEN07A0 and R01 FD003527-01.

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ELEVATED AIRWAY AND SYSTEMIC GLUCOSE IMPEDES LUNG BACTERIAL CLEARANCE IN A MURINE MODEL OF CYSTIC FIBROSIS RELATED DIABETES

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Cystic fibrosis-related diabetes (CFRD) is now recognized as the most common co-morbidity associated with cystic fibrosis (CF), affecting more than 50% of patients over the age of 30. Despite profound morbidity and increased mortality associated with CFRD, little is known about the pathology of this disease, in part due to unavailability of CFRD animal models. Utilizing a murine model of CFRD, we explored our hypothesis that elevated airway glucose in CFRD is associated with reduced lung bacterial clearance.

Gut-corrected CFTR knockout mice (*Cfr^{tm1Unc}* Tg(FABPCFTR)-1Jaw/J) (CFKO) and their CFTR expressing wild-type littermates (WT) were administered low doses (50 mg/kg) of streptozotocin (STZ) for 5 days. Body weights were followed over time as were fasting serum glucose measures. An intraperitoneal glucose tolerance test (IGTT) was performed 2 weeks following STZ treatments. Fourteen days later the mice were challenged with an intratracheal inoculation of *P. aeruginosa* (PAO1) (75 µL of 1-5x10⁶ cfu/mL) for up to 24 hours. Bronchoalveolar lavage fluid (BALF) was collected for glucose concentration, cell counts, histology and bacterial culture. The right upper lung lobe was also resected, homogenized and cultured.

There was a significant increase in fasting serum glucose levels in STZ-treated CFKO mice. Consistent with diabetes, STZ-treated CFKO mice developed significant weight loss and had an exaggerated and prolonged elevation in serum glucose levels during the IGTT. More notably, the STZ-treated CFKO mice had airway glucose concentrations that were significantly higher compared to even wild type hyperglycemic mice, despite having similar levels of fasting hyperglycemia. These findings are analogous to that which is seen in humans with CFRD. There was a profound neutrophilic response to PAO1 instillation in the STZ-treated CFKO mice compared to controls and STZ-treated WT mice. Additionally, both BALF and lung bacterial culture counts were elevated in the STZ-treated CFKO mice compared to controls.

The administration of STZ created a CFKO mouse with fasting hyperglycemia and overt characteristics of diabetes similar to what is observed in human patients with CFRD. Namely, they developed fasting hyperglycemia, poor serum glycemic regulation in response to a glucose challenge, significant weight loss and excessively elevated airway glucose concentrations. Additionally, CFRD mice demonstrated an exaggerated, but less effective, inflammatory cell response to intratracheal PAO1 challenge. To our knowledge this is the first murine model of CFRD that has been utilized to study lung bacterial clearance. These results suggest that CFRD is not a mere marker of worsening CF lung disease, but may promote a more rapid decline in lung function. (Support: Center for CF Research CF Mouse Core, NIH DK056481-08 (to NAM), and NHLBI T32 training grant HL076118-08 (to WRH))

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OSTEOPOROSIS AND OSTEOPENIA IN ADULT PATIENTS ATTENDING A REGIONAL UK CENTRE: REDUCTION IN PREVALENCE AND ASSOCIATED RISK FACTORS

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Introduction: Reduced bone mineral density (BMD) is prevalent in adults with CF and results from suboptimal bone mass accrual in childhood and premature bone loss in adult life. The pathophysiology of bone disease in CF is multifactorial. Low BMD leads to bone fragility, fractures, pain,

increased disability and an accelerated decline in lung function. With improved survival early prevention strategies and interventions to reduce risk factors are important and may alter the natural course of CF related bone disease.

Aim: To compare the prevalence of osteoporosis and osteopenia (WHO criteria) and associated risk factors in a large adult CF population in 2011 to previously published data (1) from the same centre.

Methods: BMD was measured using dual energy X-ray absorptiometry (DXA). Clinical and demographic data (age, FEV1, BMI, CFRD, oral corticosteroid treatment, *Pseudomonas* status, vitamin D and testosterone level and transplant status) were collected from electronic patient records (EMIS®) in 2011. These data were compared to previously published data (1) from the same centre (2000). Multiple regression analysis was performed in each cohort to determine which of the significantly associated variables predicted BMD measurements.

Results: BMD results from 346 patients in 2011 (57% males, median age 28yrs, FEV1 65% predicted, BMI 21.8) were compared to 114 patients in an earlier study (46% males, age 24.5yrs, FEV1 47% predicted, BMI 20.2). Forty-two patients (12%) in 2011 were post transplantation vs. none in the earlier cohort. No significant difference in prevalence of CFRD or DF508/DF508 was seen between the two cohorts. In the 2011 cohort there was a significant increase in age and male sex, improved clinical status (lung function and BMI) with reduction in prevalence of patients chronically colonised with *Pseudomonas* (89% vs. 50.9%, $p<0.001$). The prevalence of patients with low BMD (osteopenia or osteoporosis) fell from 66% to 39%, $p<0.001$. The prevalence of osteoporosis fell from 18% to 6%, $p<0.001$ and osteopenia from 48% to 33%, $p<0.01$. In the earlier cohort low BMD was significantly associated with male sex, disease severity (FEV1 and BMI) and oral corticosteroid use. In 2011 additional risk factors for low BMD were low testosterone level and post transplant. There was no significant association between low BMD and age, genotype, vitamin D level, inhaled steroids or CFRD. In 2011 cohort 62 patients (18%) were prescribed bisphosphonate treatment (18% alendronate, 43% risendronate and 39% pamidronate).

Conclusions: Significant reduction was seen in the prevalence of osteoporosis and osteopenia in adult patients attending a regional CF centre. This reduced prevalence of low BMD is due to meticulous attention to nutrition, exercise and endocrine function, aggressive treatment of respiratory infections and early eradication protocols, judicious use of oral corticosteroids, annual screening and prompt diagnosis of CFRD and increased use of bisphosphonates. Risk factors predictive for low BMD are male patients with severe disease, low testosterone level, post transplant and oral steroid use.

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CYSTIC FIBROSIS BONE DISEASE: INTERACTIONS BETWEEN OSTEOBLASTS AND OSTEOCLASTS MAY BE THE KEY TO INCREASED BONE LOSS

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The incidence of cystic fibrosis (CF) bone disease continues to increase, as life expectancy improves. *Cfr*-null mice (*Cfr* S489X-/- mice, further identified as *Cfr*-/-) have reduced bone density, despite lack of lung or pancreatic disease, suggesting an inherent defect in bone metabolism. Additionally, defects in bone homeostasis of CF individuals and mouse models imply an uncoupled bone turnover, with reduced bone formation and increased bone resorption. To pursue mechanisms for CF bone metabolism, we tested for the presence and effects of CFTR, utilizing *ex vivo* osteoblast and osteoclast cultures from *Cfr*-/- and wildtype (*Cfr*+/-) mice.

CFTR expression in osteoblasts and osteoclasts was determined by mRNA analysis and immunostaining. Next, calvarial-derived osteoblasts were grown in culture for 14 days post-confluence, and RNA was collected at 7 and 14 days. Gene expression analysis was determined for osteoprotegerin (OPG) and RANKL. We then collected bone marrow aspirates from femurs and humeri of *Cfr*-/- and *Cfr*+/- mice at 8-9 weeks of age, for dynamic osteoclast cell cultures. Osteoclasts were TRAP stained at 8 days, and counted via visual microscopy.

Cfr message was found in murine primary osteoblasts by quantitative RT-PCR, and CFTR protein was also detected by immunostaining. *Cfr* gene expression was not detected in either undifferentiated or differentiated osteoclasts, nor was CFTR protein found by immunostaining. Since CFTR was not detected in osteoclasts but osteoclastic bone resorption is increased with CF, we examined the RANKL/OPG pathway to discover the mechanism for this apparent discrepancy. Activated osteoblasts stimulate osteoclasts through production of RANKL, and inhibit osteoclast activity with production of the competitive inhibitor OPG. No significant difference in *Rankl* expression was detected between *Cfr*-/- and *Cfr*+/- at either day 7 or 14. However, calvarial osteoblast cultures demonstrated reduced *Opg* expression from *Cfr*-/- compared to *Cfr*+/- cultures at day 7 (0.4 ± 0.05 vs. 1.0 ± 0.18 , $p=0.03$), but not at day 14 (0.2 ± 0.02 vs. 0.6 ± 0.17 , $p=0.07$). The decrease in OPG translated into an increased number of osteoclasts measured in *Cfr*-/- vs. *Cfr*+/- culture wells (415.3 ± 26.4 vs. 173.3 ± 50.9 cells per well, $p=0.0135$).

Expression of CFTR in osteoblasts suggests an intrinsic defect in bone metabolism. However, absence of expression in osteoclasts implies an interaction between the two cell lines, contributing to increased bone resorption. *Ex vivo* CFTR-deficient osteoblasts demonstrate similar gene expression of the osteoclast stimulating RANKL, but reduced expression of the competitive inhibitor OPG, leading to increased influences on bone resorption. Furthermore, osteoclast dynamic cultures, originating from murine bone marrow, revealed increased development and differentiation of *Cfr*-/- osteoclasts. These mechanistic interactions between CFTR-deficient osteoblasts and osteoclasts lend evidence to the pathogenesis of reduced bone density seen in CF.

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CYSTIC FIBROSIS AND OVARIAN RESERVE

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Traditionally, it has been believed that the primary barrier impacting the fertility of women with cystic fibrosis (CF) was thick cervical mucous. The role of the CFTR gene in ovarian function is unclear. However, it is known that female CF mouse models have impaired ovarian function. In addition, up to 66% of women with CF have cycle irregularity, 50% of women with CF who have regular cycles do not regularly ovulate and women with CF develop symptoms of perimenopause prematurely. These studies suggest that women with CF may not have the same ovarian function as healthy women.

The aim of the current study, therefore, is to assess the ovarian reserve of women with CF in comparison to healthy controls.

Materials and Methods: Twenty women with CF from the adult CF clinic at St. Michael's Hospital and 20 healthy women from St. Michael's Hospital Family Health Team and Taddle Creek Family Health Team between the ages of 18 and 34 were recruited. In reproductive medicine, the most accurate measure of ovarian reserve available is serum anti-müllerian hormone (AMH). Transvaginal assessment of antral follicle count (AFC), serum follicle stimulating hormone (FSH) and serum estradiol (E2) are traditional markers and, although less accurate than AMH, are also utilized for this purpose. On day 3, 4 or 5 of their cycle, participants completed a questionnaire about their reproductive history and underwent assessment of their AMH, AFC, FSH and E2. Clinical demographics and lung function were obtained from the patient charts. Results were analyzed using a Student's t-test.

Results: Data from 18 women with CF and 20 healthy women are presented. FSH and E2 data were excluded for two women; one woman was mid-cycle and the values were not available for another due to lab error. Women with CF were younger than the controls (mean age 25.3 vs 29.2 years; $p<0.001$) and had a mean FEV1% of 66% +/- 20.8% (range 33% to 96%). Overall, women with CF had significantly lower AMH values than healthy women (18.1 vs 33.2 pmol/L respectively; $p=0.01$). The mean AMH of our controls was equal to the 50th percentile of age-matched controls. In contrast, the AMH of women with CF is comparable to the 25th percentile of age-matched controls. Mean E2, FSH and AFC did not differ between the two groups. On secondary analysis, to rule out the potential confounder of polycystic ovarian syndrome (defined as AFC >30) which can cause an abnormally elevated AMH, 10 women (4 with CF and 6 healthy controls)

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were excluded. The difference in AMH remained significant (17.2 vs 30.7 pmol/L; $P=0.02$).

Conclusions: Our results confirm that the mean AMH in women with CF is lower than that of healthy women, suggesting that reduced ovarian reserve may play a significant role in the fertility of women with CF. Future research is needed to determine how the ovarian reserve of women with CF changes over time, how it affects their reproductive success and if these findings apply to female CF carriers.

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COMPARISON OF THE METABOLIC EFFECTS OF A MIXED MEAL AND STANDARD ORAL GLUCOSE TOLERANCE TEST IN CYSTIC FIBROSIS AND HEALTHY CONTROLS

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Introduction: Although the oral glucose tolerance test (OGTT) is the recommended diagnostic test for diabetes, it is not physiological and in CF it has poor reproducibility. Non-glucose dietary constituents also influence insulin secretion, and although the metabolic responses to different types of meal have been well described in normal and diabetic subjects¹, there have been no studies in CF. The purpose of this study was to compare the metabolic responses of a standardized validated mixed meal test (MMT) with the OGTT in CF subjects.

Methods: Following an overnight fast, on 2 different days 20 CF subjects and 6 healthy age and BMI matched controls underwent an OGTT (75 grams glucose) and MMT (75 grams carbohydrate), the composition being similar to a continental breakfast. Plasma glucose, insulin and C-peptide were measured and the areas under the curve (AUC) calculated. First (1-Ph) and second (2-Ph) phase insulin responses (markers of β -cell function) were determined using the Stumvoll equations².

Results: See Table. AUC_{glucose} in CF was higher after both OGTT ($p=0.002$) and MMT ($p<0.0001$), and the 1-Ph ($p=0.02$) and 2-Ph ($p=0.04$) responses were diminished, compared to the controls. Furthermore, the MMT 1-PH response was lower ($p=0.03$) in CF subjects compared to the OGTT. There was a significant correlation between the OGTT and MMT for AUC_{glucose} ($r=0.84$; $p<0.0001$) and AUC_{insulin} ($r=0.47$; $p=0.02$). Finally, in the CF subjects the 120-minute plasma glucose levels showed a strong correlation ($r=0.53$; $p=0.005$) between the MMT and OGTT.

Conclusions: This study shows that a diminished first phase insulin response, an early marker of CFRD, occurs in adult CF patients without known diabetes. Furthermore, the mixed meal test, a more physiological representation of daily carbohydrate intake, correlates well with the OGTT and can be used to assess glucose handling in this at risk patient group. The clinical application of the mixed meal as a standard test for diagnostic purposes in those with CF deserves further investigation for the early assessment of β -cell function.

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	Fasting plasma glucose (mmol/L)	AUC _{glucose} (mmol/L)	Fasting insulin (μU/mL)	AUC _{insulin} (μU/mL)	AUC _{C-peptide} (ng/mL)	1-PH (pmol/L)	2-PH (pmol/L)
Controls: OGTT	4.3 ± 0.2	573 ± 42	7.9 ± 2.2	5290 ± 607	1007 ± 82	1610.8 ± 246	394 ± 17
Controls: MMT	4.5 ± 0.1	500 ± 27	6.5 ± 1.1	4128 ± 626	705 ± 132	1509 ± 194.8	337 ± 25.4
CF: OGTT	4.7 ± 0.1	982 ± 50 [†]	7.8 ± 0.6	3900 ± 450	800 ± 92	911 ± 103 [^]	281 ± 22 [^]
CF: MMT	4.8 ± 0.1	20 ± 3 ^{††}	7.7 ± 0.8	3369 ± 399	660 ± 58	849 ± 81 [^]	264 ± 20 [^]

Results expressed as Mean ± SEM. Comparison of tests within a group: [†] $p<0.0001$; ^{††} $p=0.03$; Comparison with healthy controls: [^] $p<0.0001$; [^] $p=0.0005$; [^] $p=0.02$

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ENDOCRINE PANCREATIC FATIGUE IN CYSTIC FIBROSIS

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Background: In normal individuals, the early insulin response to an oral glucose load is greatest early in the day¹ and contributes to the rapid glucose clearance seen in the morning. However, although CF patients without known CF-related diabetes (CFRD) have poor glucose handling, their pattern of insulin release in response to a glycemic stimulus throughout the day has not previously been studied. To look at this further, we compared the pancreatic β -cell response in CF patients without CFRD with a healthy control population to identical meals at different times in the day.

Method: Following an overnight fast, 17 pancreatic insufficient CF subjects and 6 normal (age, sex matched) controls consumed within 10 minutes a standard mixed meal (the gold standard measure of endogenous insulin secretion²) at 0800, 1300 and 1800 hours on the same day. Insulin and glucose levels were measured at 0,30,60,90 and 120 minutes for each test. The area under the curve for the early (first 30-minutes, AUC₃₀), the entire (120-minutes, AUC₁₂₀) durations for glucose and insulin, and the mean time to reach a peak insulin level were calculated. In addition the first (1-Ph) and second (2-Ph) phases of insulin release (markers of β cell function³) were determined.

Results: See Table (mean ± SEM). CF subjects had a higher glucose AUC₃₀ and AUC₁₂₀ and lower insulin AUC₃₀ and AUC₁₂₀ than healthy controls. There was a more marked increase ($p=0.02$) in the time to peak insulin and decrease in insulin AUC₃₀ ($p=0.002$) in the afternoon and a decrease in the 1-Ph response through the day, compared to healthy controls. However, CF subjects showed an increased 1-Ph response in the afternoon ($p=0.02$) in keeping with a lower glucose AUC₃₀ and AUC₁₂₀. The 2-Ph response was lower than in healthy controls in the morning ($p=0.001$) and evening ($p=0.02$).

Conclusions: This study has demonstrated an increase in glucose intolerance and a longer time for insulin release to achieve glucostasis, as the day progresses. This has important clinical implications as this study shows the variability of pancreatic β -cell functioning through the day and introduces the concept of "pancreatic fatigue" in CF. This raises the question of whether dynamic testing for CFRD should be carried out later in the day, when the pancreatic endocrine function may be more vulnerable.

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	Control			CF		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening
Peak Insulin (μU/mL)	63 ± 11	60 ± 8.3	49 ± 8.5	45 ± 6.9	34 ± 4.4* [†]	37 ± 4.6
Time to peak insulin (min)	45 ± 7	40 ± 10	60 ± 11	65 ± 6*	69 ± 7	76 ± 7
AUC ₃₀ Insulin (μU/mL)	1880 ± 304	982 ± 136	835 ± 184	1877 ± 331	569 ± 89*	595 ± 78
AUC ₁₂₀ Insulin (μU/mL)	4128 ± 626	3662 ± 548	3582 ± 634	3239 ± 434	2846 ± 442 [§]	2983 ± 357
AUC ₃₀ Glucose (mmol/L)	138 ± 12	145 ± 5	149 ± 6 [†]	183 ± 6*	169 ± 5* [†]	185 ± 5*
AUC ₁₂₀ Glucose (mmol/L)	500 ± 27	560 ± 9	572 ± 18	824 ± 33 [‡]	702 ± 23* [^]	819 ± 29 [^]
Insulin (1-Ph)	1509 ± 195	1570 ± 105	1402 ± 133	797 ± 88*	965 ± 90* [†]	914 ± 95*
Insulin (2-Ph)	337 ± 25	327 ± 23	338 ± 32	242 ± 18*	276 ± 20	267 ± 21*

Comparison vs controls: * $p=0.02$; [†] $p=0.001$; [‡] $p<0.0001$; Within group: vs Morning: [†] $p=0.02$; [§] $p=0.002$; [^] $p<0.0001$; vs afternoon: [^] $p<0.0001$

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CROSS SECTIONAL STUDY OF BONE HEALTH IN CHILDREN WITH CYSTIC FIBROSIS IN QUEBEC, CANADA

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Background: Controversy exists regarding bone health status of children with cystic fibrosis (CF). Our objective was to assess bone density and fracture rates within a genetically heterogeneous population in the province of Quebec.

Methods: All children followed in 2 CF clinics (Quebec City and Chicoutimi) were invited to take part of the study. Lumbar and total body bone mineral densities (BMD) were examined using dual energy x ray absorptiometry (Hologic QDR 4500 and LUNAR). Determinants of bone health and disease severity were collected including, but not limited to, mutations in the CFTR gene, FEV1, corticosteroid exposure, calcium and vitamin D intakes, level of physical activity, anthropometrics and serum and urine minerals and 25-OH-D. In addition, a thoracolumbar spine anteroposterior radiograph was done for each child.

Results: To date, about 100 children have been recruited and data on 19 children from 1 center (Chicoutimi) are presented. The cohort was constituted of Caucasians, 63% of girls and 37% of boys, with ages ranging from 6 to 16 years old (mean 10 years). Mutation analysis revealed that 3 patients were homozygote $\Delta F508$, 9 patients were heterozygote $\Delta F508$ and 7 patients had different mutations. The majority of the children had a mild pulmonary disease (15/19), a normal weight and an insufficiency in vitamin D (14/19). Bone ages were concordant with chronological ages. The BMD Z scores were all normal except for one case; the mean was negative (-0.74) and values ranged from -2 to 1.2. BMD were corrected for height. Bone mineral apparent densities (BMAD) were calculated. No patients presented spine fractures on thoracolumbar spine radiographs, but 3 reported fractures of superior extremities.

Conclusions: To date, we report a normal bone health status in this subsample of children with a mild CF. No fragility fractures were described. Future analysis will be done with the entire cohort to identify the best predictors of BMD in CF children. Moreover, a link between BMD and CF mutations will be sought.

Acknowledgements: This study would not have been possible without the help of the cystic fibrosis teams from the *Centre hospitalier de l'Université Laval*, the *Centre de santé et de services sociaux de Chicoutimi* and the families.

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EFFECT OF CYSTIC FIBROSIS RELATED DIABETES (CFRD) ON SENSATION, GAIT CHARACTERISTICS AND BALANCE

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Introduction and Purpose: Cystic fibrosis related diabetes (CFRD) is a growing complication in adults with cystic fibrosis (CF). However, little is known about how CFRD affects peripheral nerves and their function related to gait parameters, sensation and balance. Deficits in gait and balance characteristics have been noted in Type 1 and 2 diabetes, and have been found to correlate with fall risk in these populations. Therefore, the purpose of this study was to determine if adults with CFRD manifested any deficits in sensation, gait characteristics or balance abilities when compared to CF adults without CFRD.

Methods: Seven adults with CFRD (6 males, 1 female, mean age 28 yrs, mean BMI 21) and 3 adults with CF only (2 males, 1 female, mean age 28, mean BMI 22) underwent testing of both feet for touch, temperature, vibration senses and reflexes. They performed gait analysis on a computer-

ized pressure mat and performed the Sensory Organization Test battery to assess components of balance reactions. Comparisons between groups on all variables were performed via t-test, with $p < 0.05$ considered significant.

Results: There were no differences between groups in sensation or balance tests. Only 1 CFRD participant showed any abnormalities in sensation. In balance testing, both groups scored lowest in comparison to population norms in their vestibular function. In gait analysis, the CFRD group had a significantly ($p < 0.05$) slower gait velocity (1.18 m/sec vs. 1.43 m/sec), shorter step length (64 cm vs. 75 cm), and shorter stride length (128 cm vs. 155 cm) than the CF only group. All but 1 in the CFRD group had gait velocities below norms for age-related healthy populations.

Conclusions: In our preliminary study, CFRD appears to decrease gait speed compared to adults with CF alone. This decrease in gait velocity is below norms for young adults without CF. Participants with CFRD altered their gait characteristics perhaps to compensate for peripheral nerve impairment. There were no detectable impairments of sensation or balance related to CFRD. CF disease or its treatment appears to impair vestibular function, regardless of CFRD status. This finding is consistent with other literature in CF and may be related to use of ototoxic antibiotic regimens.

Clinical Implications: Adults with CFRD have changes in gait patterns that may increase their risk of falling. These changes are not detectable with testing of sensation. Gait velocity has been implicated in predicting overall health and mobility status for other populations and may be an important test for adults with CF as well. Adults with CF may have impaired vestibular function, which may further restrict their mobility and functional status. These deficits are targets for physical therapy intervention to maximize the health and function of adults with CF, particularly those with CFRD.

Gait Characteristics

Parameter	velocity (m/sec)	step length left leg (cm)	step length right leg (cm)	stride length left (cm)	stride length right (cm)	base of support left foot (cm)
CFRD mean	1.18	64.13	64.48	128.40	128.84	11.36
CF only mean	1.43	75.32	75.95	151.16	151.56	8.58
p value	0.02	0.07	0.03	0.04	0.04	0.10

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RISE IN HbA1C IS ASSOCIATED WITH DETERIORATION IN LUNG FUNCTION IN YOUNG CHILDREN WITH CYSTIC FIBROSIS AND NORMAL GLUCOSE TOLERANCE

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Background: In children with cystic fibrosis (CF), impaired glucose tolerance (IGT) and CF-related diabetes (CFRD) typically develop from adolescence onwards and coincides with deteriorating lung function. We hypothesized that chronic hyperglycaemia is common even before the onset of IGT/CFRD and is associated with early reduction in lung function.

Methods: At a tertiary care CF clinic HbA1c was recorded annually in 70 (46 boys, 76% homozygous, 23% heterozygous for deltaF508 mutation) children between 2005 to 2010 along with age appropriate pulmonary function testing (n=41). OGTTs were performed annually from age 10yrs onwards according to national guidelines (n=35). Twelve children who developed impaired glucose tolerance or diabetes as defined by OGTT-based WHO criteria, or HbA1c > 6.5 %, had their data censored at the time of ascertainment of those conditions. Chronic hyperglycaemia was defined as HbA1c > 5.7 %. Within-individual changes in HbA1c, or OGTT parameters (glucose and insulin), were related to changes in lung function parameters (FEF25-75, FEV1 and FVC, each expressed as percentage expected for age, sex and height) using mixed models with age and BMI z-score as covariates.

Results: Mean age at first assessment was 6.9(1-15)yrs when mean(SD) FEV1 was 89(20)% predicted. Mean number of annual assessments was 2 (range 1-5). Prevalence of chronic hyperglycaemia increased with age from 22.4% in <5yrs, 41.2% at 5-10yrs, and 59.6% at >10yrs. Within-individual rises in HbA1c were associated with reductions in predicted FEF25-75 (a marker of small airway flow) (44% decline per 1% rise in HbA1c; $p = 0.002$)

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and predicted FEV1 (15% decline per 1% rise in HbA1c; $p=0.1$), but not with FVC. Among the 35 children aged >10yrs, changes in fasting or stimulated insulin and glucose levels were not associated with changes in lung function.

Conclusion: In young children with CF, in the absence of IGT or CFRD, the prevalence of chronic hyperglycaemia increased with age. Rises in HbA1c were associated with deterioration in small airways function. Further studies to identify the causal mechanisms may lead to early intervention strategies.

Acknowledgements: This work is presented on behalf of the Eastern Region Paediatric CF Group.

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APPLYING AVAILABLE CFF GUIDELINES TO DATA IN OUR CFF PATIENT REGISTRY TO DETERMINE WHEN TO OBTAIN DXA SCANS IN CHILDREN WITH CYSTIC FIBROSIS

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Bone disease is a known complication of cystic fibrosis (CF) and has a multifactorial etiology. In 2005, a consensus statement developed during a 2002 CF Foundation (CFF) meeting on Bone Health in CF was published which included guidelines for screening for bone disease in children with CF. There is currently little analysis of whether these recommendations identify the children who are at highest risk for bone disease or what the prevalence of abnormal DXA scans is in those who meet the suggested criteria.

The guidelines recommend using DXAs to screen all children 8 to 18 years if they have at least one of the following risk factors; <90% ideal body weight (IBW), FEV1 < 50% predicted, use of glucocorticoids of $\geq 5\text{mg/kg/day}$ for ≥ 90 days/year, delayed puberty or a history of fractures.

We used the CFF Registry data available through June 2011 to identify retrospectively patients 8-18 years of age with at least one of the five risk factors listed in the guidelines. The registry did not include data on percent ideal body weight or pubertal status. Therefore, ideal body weight was calculated manually by CDC growth charts for all patients 8-18 years of age. To assess for delayed puberty (DP) we prospectively identified patients using a self-report questionnaire. DXA scans were recommended for all patients with at least one identified risk factor.

We identified 86 persons with CF between the ages of 8-18 years. A total of 66 patients were assessed for DP. Two girls were identified as being delayed. Twenty-eight patients had at least one of the risk factors. Ten of them had DXA reports available. Screening and BMD results are listed in the table. Of the 10, 3 had a low BMD for age and sex at either the Lumbar Spine (LS) or Total Body.

The absence of pubertal data and IBW in the registry hinders the use of the current guidelines, and consideration should be given to adding these to the registry data obtained prospectively. Continued data collection is underway, however, our preliminary data indicate that current guidelines yield a rate of ascertainment of low BMD of about 30%. Further, prospective DXA data are needed to see whether the guidelines are of sufficient sensitivity to identify most patients with low BMD.

Age (yrs)	Gender*	Identified risk factor(s)	DXA Z-score
10.1	Female	History of fractures	Total body -0.3 L-spine -0.1
12.3	Male	IBW <90% FEV1 <50% predicted	Total body -1.3 L-spine -1.1
13.3	Male	IBW <90% Steroid use	Total body -1.6 L-spine -2.3
14.6	Male	IBW <90% FEV1 <50% predicted	Total body -0.7 L-spine -1.4
14.7	Female	FEV1 <50% predicted	Total body -1.0 L-spine -1.4
14.9	Female	FEV1 <50% predicted	Total body -0.7 L-spine -1.7
15.8	Female	FEV1 <50% predicted	Total body -2.2 L-spine -2.2
16.0	Male	IBW <90% FEV1 <50% predicted	Total body -1.6 L-spine -1.7
17.1	Female	Steroid use	Total body -0.5 L-spine -1.2
17.9	Female	IBW <90%	Total body -1.6 L-spine -2.4

*No delayed puberty identified in any of these patients.

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POSTPRANDIAL GLUCOSE REGULATION IN NON-CF AND CF FERRETS

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Many cystic fibrosis (CF) patients suffer postprandial glucose abnormalities including hyperglycemia and reactive hypoglycemia. The most important factors thought to contribute to these abnormalities include impaired first phase insulin secretion and maladaptive second phase insulin secretion. However, additional contributing factors may also include altered secretion of glucagon, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulintropic polypeptide (GIP). Impaired first phase insulin secretion and altered second phase insulin secretion are two features that the CF ferret models shares with human CF patients. Because there are no reports evaluating mixed meal tolerance test (MMTT) in ferret, the first goal of this study was to establish MMTT procedures in juvenile wild-type ferrets and define normal baseline postprandial glucose and hormone secretion profiles for insulin, glucagon, GIP and GLP-1. Juvenile ferrets (5-12 weeks) were fasted for 3 hrs prior to the MMTTs. The MMTT was initiated by feeding the animals a quantity of food normalized to a body surface area estimate (BSAE in cm^2) of each animal (body weight [g] x body length [cm])0.5. For unweaned kits, MMTTs utilized only liquid diet (Elecure). For weaned kits, a mixture of Elecure and canned food was used. Hormone and glucose profiles were measured at 15-30 minute intervals during the MMTT. In WT animals (N=5), postprandial glucose was well maintained within a 110-160 mg/dL range, with peaks in plasma insulin, GLP-1, and GIP by 30 minutes and a slow return to near baseline by 120 minutes. By contrast, changes in plasma glucagon levels were generally inversely correlated with changes in plasma insulin (i.e., glucagon levels decreased as insulin levels increased and vice versa). These postprandial hormone responses in the ferret are similar to those in humans. MMTT analysis of three sibling pairs of juvenile CF and non-CF ferrets (age 60-90 days of age) demonstrated a variety of abnormal glucose responses in CF animals. Although all CF animals demonstrated postprandial peak glucose >200 mg/dL, the kinetics of the rise and fall in blood glucose varied between animals. For example, two CF animals retained significantly elevated Gluc120min values (389 $\pm$ 56 mg/dL, N=6 measurements on 2 animals) as compared to non-CF controls (134 $\pm$ 9 mg/dL, N=9 measurements on 3 animals). By contrast, the third CF animal demonstrated postprandial Gluc120min values (128 $\pm$ 36 mg/dL, N=3 measurements on 1 animal) not significantly different from normal. Despite these observed differences in MMTT profiles of CF animals, the average glucose profile for CF animals was significantly different from non-CF animals at all time points in the glucose curve. Additionally, AUC0-120min was also significantly ($P<0.0001$) higher for CF (37,281 $\pm$ 4520) as compared to non-CF (15,293 $\pm$ 1005) animals. These studies lay an important foundation for understanding postprandial alterations in islet and enteroinsular axis hormones responsible for abnormal glucose regulation in CF following feeding.

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INACTIVATION OF CFTR LEADS TO IMPROPERLY REGULATED INSULIN SECRETION IN NEWBORN FERRETS AND ISOLATED ISLETS

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Cystic fibrosis related diabetes (CFRD) is a common co-morbidity in cystic fibrosis. Pancreatic endocrine destruction and impaired insulin responses are features of CFRD. It remains unclear whether alterations in insulin secretion in CF are solely secondary to exocrine pancreatic damage or if they also involve islet-intrinsic defects. To address this question, we evaluated insulin secretion in newborn CF and non-CF ferrets and isolated islets. Newborn CF kits demonstrated significant abnormalities in the kinetics of insulin secretion, including impaired first phase insulin (FPI) secretion (6-fold) and dramatically accentuated late phase insulin (~10-fold) in response to glucose and/or L-arginine challenge. Interestingly, in the non-fasted state, newborn CF kits demonstrated widely variant and elevated insulin, insulin/glucose, C-peptide, and C-peptide/glucose levels in comparison to controls. The molar ratio of plasma C-peptide/insulin was not significantly different between CF and non-CF animals, indicating that the elevated insulin levels in CF kits represents bona fide insulin hypersecretion. In non-CF nursing newborns, glucose and insulin levels were positively correlated ($P < 0.0468$), but this was not the case in CF kits ($P = 0.7743$), supporting the hypothesis that insulin secretion by the CF pancreas is not properly regulated. To evaluate whether components of this insulin dysregulation are intrinsic to the CF islet, we performed studies in isolated cultured neonatal CF and non-CF ferret islets. Interestingly, CF islets had a significantly higher (5.3-fold) percent insulin secretion at 2 mM glucose in comparison to non-CF controls. Hypersecretion of insulin by CF islets at low glucose was blocked by activation of K-ATP channels with 100 μ M diazoxide, suggesting that CFTR defects in the β -cell inhibit K-ATP channels to potentiate insulin secretion in a glucose-independent manner. Similar to in vivo findings in nursing CF kits, the variance in values for percent insulin secretion by CF islets at low glucose was significantly greater than their non-CF controls ($P < 0.0001$), suggesting dysregulated insulin secretion by the CF islet. Following stimulation with 30 mM glucose, non-CF islets demonstrated a significant induction in percent insulin secretion (5.1-fold), while the percent insulin secretion for CF islets was not significantly different at low and high glucose. These alterations were reflected in a 4-fold reduced insulin secretory index in response to glucose challenge for CF islets as compared to non-CF islets. These findings provide strong supports that islet-intrinsic CFTR defects alter glucose-responsive insulin secretion by the β -cell in CF.

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GLUCOSE UTILIZATION IS IMPAIRED IN SKELETAL MUSCLE OF CF MICE

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Full blown cystic fibrosis-related diabetes (CFRD) occurs in about 40-50% of adults with CF, however, as many as 75% of CF patients exhibit some form of glucose intolerance. It is not known why only certain patients develop CFRD, even though the majority of patients are exocrine pancreatic insufficient, indicating that atrophy/fibrosis of the pancreas is not the only culprit. The CF mouse model does not exhibit pancreatic insufficiency; therefore, the CF mouse represents a good model to study insulin regulation by tissues in the CF context without pancreas atrophy.

We measured glucose fasting levels in 7-8-week-old Δ F508 (mutation that is pancreatic insufficient in humans) and R117H (pancreatic sufficient in humans) male and female mice and their WT littermates, C57BL/6J background, after a 6-hour fast. Glucose values (mg/dL) for males: WT 205.4 ± 8.7 , $n = 10$; Δ F508 171.6 ± 17.8 , $n = 5$; R117H 170.8 ± 12.7 , $n = 8$, $p = 0.034$. For females: WT 182 ± 7.8 , $n = 12$; Δ F508 127.7 ± 14.6 , $n = 6$, $p = 0.002$; R117H 170.8 ± 12.7 , $n = 8$, $p = 0.04$. In all cases, fasting glucose level was statistically lower in CF mice, except for Δ F508 males where significance was not reached, but the same trend was observed. Lower glucose fasting values in CF mice could reflect a different size of glycogen pools since CF mice are smaller, and have less fat and muscle mass than WT. To

further explore the role of muscle, if any, we extracted RNA from calf muscles of pristine 8-week-old Δ F508 female mice and their WT littermates at rest (4 mice per group). cRNA was amplified and hybridized to Illumina's MouseRef-8 v2 Expression BeadChip. Out of the 10,890 genes considered present in skeletal muscle at rest, 678 were differentially expressed in CF vs. WT, 400 up-regulated and 278 down-regulated. Pathway Studio was used to find correlations between the differentially expressed genes and known diseases/perturbed states. Among the most prevalent ones were diabetes mellitus, fibrosis, inflammation, and ubiquitin-dependent protein degradation. NF- κ B has been shown to be involved in these processes leading to muscle-wasting and skeletal muscle insulin resistance, with the latter occurring even before there is β -cell failure. Nutrient supply through insulin/PI3K and muscle contraction through AMPK are the main signaling pathways regulating GLUT4 translocation in muscle. Several genes in both pathways (Insr, p110, Akt, AMPK γ) have higher mRNA expression levels in CF skeletal muscle, which supposedly will lead to higher glucose utilization by muscle. Both pathways converge at the level of AS160, whose phosphorylation removes the "brake" of Rab proteins and allows the mobilization of GLUT4. An isoform of AS160, Tbc1d1, has decreased expression in CF skeletal muscle. At the moment, we do not know if this decrease in message, reflects a decrease in phosphorylation of AS160 and how this affects GLUT4 mobilization. Since the majority of the proteins involved in these pathways exert their action by changing their phosphorylated states, we are in the process of looking at global phosphorylation in the skeletal muscle of our CF mice at rest and during exercise. Our preliminary data indicates impairment of glucose utilization in skeletal muscle of CF mice in the absence of pancreatic insufficiency. Supported by NIH grant GM088823.

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IMPROVING SCREENING FOR ADULT CFRD: A NURSE-INITIATED CLINIC PROTOCOL

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Introduction: Screening for CFRD with an oral glucose tolerance test (OGTT) is time consuming and often requires a separate laboratory appointment to complete. Given the busy schedules of adults, many patients are not able to complete the CFF recommendation for annual CFRD screening, which is reflected by the consistently low national rates. As a quality improvement project at our center, we examined the feasibility of a nurse-initiated OGGT protocol within the context of a routine clinic appointment.

Methods: We developed an algorithm to incorporate non-fasting screening similar to those used in gestational diabetes into routine clinic appointments. We also validated the use of point-of-care (POC) glucose testing by comparing this with venous sampling drawn simultaneously and sent for standard testing. All adult patients without a diagnosis of CFRD were eligible for the In-Clinic OGTT protocol (IC-OGTT). Patients underwent POC glucose testing at presentation to clinic and ingested a 75g glucose solution under nursing supervision if the POC glucose level was below a pre-determined threshold. Following the completion of the clinic appointment, a timed venous blood draw was collected at 2-hours in conjunction with a repeat POC glucose to assess for variance in these two testing techniques. Patients were considered to be screen negative if 2-hour venous glucose was below 140, as per CFRD guideline values. Serum values of >140 were referred for formal laboratory testing.

Results: We screened 5 patients for the IC-OGTT protocol, with all eligible patients meeting criteria to proceed with IC-OGTT. Baseline POC testing values were low (median 98 ± 14 mg/dL) and significantly below the pre-established cutoff suggesting glucose administration to be safe. Normal 2-hour serum glucose values were obtained in 4 of 5 patients using this technique. The mean POC testing values was significantly higher than simultaneously collected venous samples (median 113 ± 45 vs 71 ± 45 mg/mL for POC and venous respectively, $p < 0.05$) and no POC value underestimated the venous sample value. Our data suggest that POC testing may overestimate the glucose values. The results suggest that In-Clinic OGTT testing is a safe and feasible screening method for CFRD in adults with CF. Imple-

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mentation of nurse administered testing may help to increase the rates of CFRD screening by improving the excessive time burden on patients.

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THE USE OF THE FRAX CALCULATOR IN THE EVALUATION OF BONE HEALTH IN PATIENTS WITH CYSTIC FIBROSIS

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Background: Patients with cystic fibrosis (CF) have a high prevalence of bone disease with recent meta-analysis estimates of 23.5% and 38% for osteoporosis and osteopenia respectively. Fracture rates are elevated as well (14% for vertebral fractures and 19.7% for nonvertebral fractures) and are comparable to untreated, postmenopausal osteoporotic women. While bisphosphonate therapy based on bone mineral density as determined by DXA (Dual-energy X-ray absorptiometry) for T/Z score of ≤ -2 should be strongly considered in this population, the approach to patients with less severe abnormalities on DXA is unclear. The FRAX calculator is a tool developed to estimate a 10 year fracture risk for major osteoporotic and hip fracture which can be used to identify patients who are candidates for osteoporosis treatment. Its use in the CF patient population has not previously been explored.

Objective: To determine whether the FRAX calculator is a useful tool for identifying CF patients for pharmacotherapy for low bone density.

Methods: Chart review of CF patients aged 18 or older to identify clinical risk factors related to low bone density, DXA results, and fracture history. FRAX calculator was used to determine a 10 year fracture risk for major osteoporotic fracture or hip fracture both with and without DXA bone mineral density information.

Results: Of the 95 charts reviewed, 46 patients had DXA scores readily available. Eleven patients suffered a previous fracture, two of which occurred in patients over 40 years old. Mean DXA T-scores were statistically lower for the femoral neck (-1.762 vs. -1.305 ($p = 0.001$)) and lumbar spine (-1.503 vs. -1.178 ($p = 0.034$)) for those with history of fracture when compared to those without fractures. FRAX risk estimates were not significantly different in the group with fracture history. Interestingly, the FRAX risk estimate for major osteoporotic fractures for 4 of 11 patients with fractures actually decreased when the DXA T-scores were added to the calculation. In all patients, FRAX risk estimation for hip fracture was significantly greater when calculated with DXA T-scores vs. without the DXA T-scores (1.322 vs 0.4432 $p = 0.0058$). However, the FRAX risk estimate with the DXA did not approach a level where one would consider pharmacotherapy. There was only one patient out of 95 who had a FRAX score which would have prompted consideration for pharmacotherapy for low bone mass, whereas there were 16 patients who would be considered for treatment based on DXA scores. The patient with elevated FRAX estimation did not have a fracture history.

Conclusion: In our small study sample, we have found that the FRAX calculator did not improve estimation of fracture risk in the adult CF population. DXA scores of the femoral neck and lumbar spine did positively correlate with fracture history. Further study is needed to evaluate the effectiveness of the FRAX calculator in the adult CF population.

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PROSPECTIVE EVALUATION OF THE DETERIORATION OF INSULIN SECRETORY PARAMETERS MEASURED DURING ORAL GLUCOSE TOLERANCE TESTS IN CYSTIC FIBROSIS PATIENTS

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Cystic fibrosis related diabetes is a complication of cystic fibrosis (CF) associated with pulmonary and nutritional deterioration years before evident hyperglycemia, possibly because of progressive insulin deficiency and resistance. We have previously shown in a cross-sectional study that model-

derived beta cell function measured during OGTT is negatively associated with age in CF patients.

The aim of this study was to quantify prospectively the deterioration of insulin secretion and insulin sensitivity associated with glucose tolerance in a cohort of CF patients undergoing repeated standard 3-h oral glucose tolerance tests (1.75 g/kg, max 75 g), with sampling at basal and at 30-min intervals of plasma glucose, serum insulin, and C-peptide concentrations.

We selected 435 OGTTs conducted in 100 CF patients (age 16.6 $\pm$ 4.6 yrs, 48 females) who received at least 2 studies spaced more than 3.5 years apart (median 4 studies/patient in 6 years of median follow-up duration). None of the subjects received insulin or oral hypoglycemic agents for diabetes for the duration of the follow-up.

During this time FEV1 declined within subjects by -2.2% (CI -2.8 to -1.7) each year, roughly corresponding to 2-3% of initial values. OGTT peak and mean glucose concentrations increased by 1.7 mg/dL (CI 0.04 to 3.35) and 1.6 mg/dL (CI 0.7 to 2.4) each year, respectively. In contrast, mean insulin concentrations decreased by -1.0 μ U/mL (CI -2.0 to -0.2) and peak insulin and C-peptide responses delayed by 1.9 min (CI 0.4 to 3.3) and 2.3 min (CI 0.3 to 4.3), respectively, each year. Insulin sensitivity measured by HOMA index did not change significantly, whereas the early phase insulin secretion (the insulinogenic index calculated as [(30 min insulin - fasting insulin) / 30 min glucose]) decreased by -0.16 μ U/mL per mmol/L (CI -0.27 to -0.07) each year. Overall, OGTT glucose responses increased by 1-1.5% yearly with respect to basal values, whereas insulin responses decreased by 3% and delayed by 2.5% with respect to the basal time to peak. Consequently, the early phase insulin response was reduced by 4.9% of basal value each year. In conclusion the rate of progression of insulin secretory defects seems to be comparable to that of pulmonary function in this cohort of CF patients.

PSYCHOSOCIAL/BEHAVIORAL

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IMPACT OF LIFE EVENTS IN ADULTS WITH CF

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Stressful life events are known to be related to higher levels of depressive symptoms, poorer quality of life, and overall psychosocial adjustment in adults with other chronic illness. Little is known about the role of life events on psychosocial outcomes in adults with CF.

Objective: The purpose of this study was to examine the association between life events and social support, depressive symptoms, and quality of life in adult patients with cystic fibrosis.

Methods: We recruited and completed a baseline survey with 124 adults (mean age =29.1 $\pm$ 10.9; range 16-63 years; 52% male; 93% Caucasian) treated at the Johns Hopkins CF care centers. Participants completed a baseline assessment that included measures of demographics, Centers for Epidemiological Studies Depression (CES-D), Life Experiences Survey (LES), Medical Outcome Study Social Support Survey (MOS) and Cystic Fibrosis Questionnaire-Revised (CFQ-R). The LES is a 29-item survey that asks whether a particular event occurred (frequency) and if it occurred was it positively or negatively perceived (valence) using a 5-point Likert scale ranging from 1 = very good to 5 = extremely bad. Baseline health outcome data included FEV1 % predicted and BMI.

Results: The ten most common life events are reported and associated perceived valence are shown in the table. Preliminary results indicated that total frequency of life events and average valence were both positively correlated with depressive symptoms (spearman $\rho=0.35$, $p<0.01$ and spearman $\rho=0.40$, $p<0.01$ respectively). Only valence was negatively correlated with social support (spearman $\rho=-0.21$, $p=0.02$). Frequency and valence of life events was associated with poorer health-related quality of life on 5 (Vitality, Emotion, Body, Respiratory, and Digestion) and 9 (Vitality, Emotion, Body, Respiratory, Digestion, Health Perceptions, Social, Physical, and Role) of the 11 CFQ-R subscales, respectively. There were no significant correlations between life events and health outcomes (FEV1 % and BMI) by either frequency or valence.

Conclusions: This study demonstrated that many adult patients with CF are experiencing a wide range of significant life events that may impact their adjustment and health outcomes. Furthermore, increased frequency of events and perceived negative valence of the life events is associated with higher levels of depressive symptoms and poorer health-related quality of

life. Also, individuals with higher levels of social support report positive valence of events. Supported by NHLBI-HL087997.

Most frequent life events In the last year did you or were you...	N (%)	Median perceived valence (IQR)
Achieve something important	69 (56)	1 (1,2)
Close friend/family member have serious illness	43 (35)	4 (4, 4)
Have a serious illness/injury	40 (32)	4 (4, 4)
Close friend/family member die	37 (30)	4 (4, 5)
Move to a new place	36 (29)	1 (1, 2)
Have trouble with superiors at work	28 (23)	4 (3, 4)
Begin a new job	27 (22)	1 (1, 2)
Go deeply into debt	24 (19)	4 (3, 5)
Break up with partner	24 (19)	4 (3, 4)
Household income decrease a lot	23 (18)	4 (3, 4)

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WORK EXPERIENCES AND CAREER DEVELOPMENT IN INDIVIDUALS WITH CYSTIC FIBROSIS: A GROUNDED THEORY STUDY

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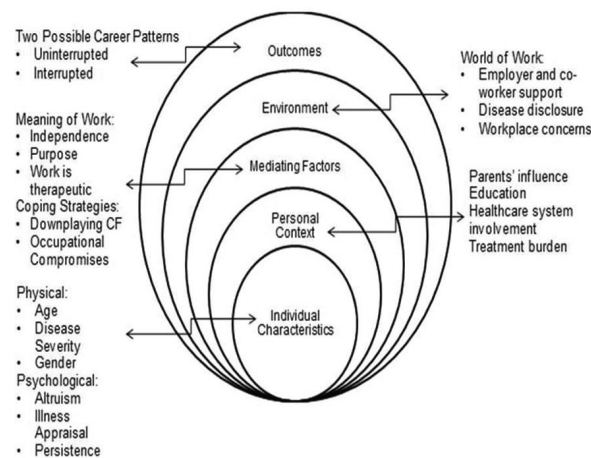
Background: Many qualitative studies have examined the lived experience of individuals with cystic fibrosis (CF) but the role of work and career development have previously received only cursory investigation. Individuals with CF are living into adulthood with a regularity that was unknown a generation ago and are now entering the workforce. The primary goal of the study was to achieve an understanding of the employment experiences and career development of people with CF.

Methods: The study employed the grounded theory method of qualitative research. Ten semi-structured qualitative interviews were conducted. Analysis of transcripts followed the constant comparative approach to coding, which identified core themes and sub-themes and culminated in a conceptual framework of variables influencing employment and career development.

Results: Four major themes and several sub-themes (1. individual characteristics [age, altruism, gender, disease severity, illness appraisal, and persistence], 2. personal contextual factors, 3. mediating factors [downplaying CF, occupational compromise] and 4. workplace characteristics) that influence employment and career development were identified. These themes were present for all participants, but specific experiences and outcomes varied by disease severity and gender.

Two patterns of career development outcomes emerged - the uninterrupted and the interrupted. The uninterrupted pattern, closely resembling the path taken by healthy peers, is a seamless transition from school to post-secondary education or work. The interrupted pattern of career development is marked by interruptions and disruptions in school and work and an uneven transition path from school to post-secondary education or work.

Conclusion: The grounded theory that emerged proposes that work experiences and career development are influenced by the interaction of certain individual characteristics, contextual factors, mediating factors, and environmental variables. By understanding the interplay of these ecological variables, CF centers can assist with career decisions and the transition from school to work.



Ecological Model of Career Development in Individuals with Cystic Fibrosis

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ADULTS WITH CF FACE COMPLEX LEGAL PROBLEMS

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As new treatments increase the life expectancy of people with CF more children are living into adulthood. Adults with CF face many issues as they try to balance treatment time with the pursuit of higher education, employment and family life. In addition, the burden of maintaining insurance coverage and paying for care and medication can be overwhelming.

Adults with CF need accurate guidance at various stages of their life regarding these important issues. Young adults who do not have family support continue to be the most vulnerable section of the population. Their issues are compounded by a lack of financial assistance, access to health insurance and help with daily care.

In October 1998, the CF Legal Information Hotline ("Hotline") began to provide free and confidential information to people with CF, family members and healthcare providers in the U.S. The Hotline is funded by a grant from the CF Foundation and is staffed by 4 attorneys. Information regarding the content of each call is recorded on an intake form and includes the age of the person with CF and their question.

Between October 1998 and April 2012, the CF Hotline has received a total of 26,842 calls.

From January 2012 to April 2012 the CF Legal Information Hotline has received a total of 1,792 calls from or about children and adults with CF. During this time period the Hotline received 530 calls regarding children under the age of 18 and 1,262 calls regarding adults 18 years old and over. The callers requested information relating to health insurance coverage, government benefits, the pursuit of education or problems pursuing or retaining employment. Callers are people with CF, parents of children or adults with CF, spouses of adults with CF and CF care center team members.

Based on calls to the Hotline, in 2012, adults with CF seem to face more obstacles to care than children. Children are more likely to be able to obtain insurance coverage from their state Medicaid program than adults with CF or have insurance coverage provided to them by their parents. The Hotline provides important information to both patients and healthcare providers.

Understanding the legal rights of people with CF continues to reduce obstacles to obtaining care, optimizing education and employment, and increases access to retirement benefits.

The CF Hotline continues to provide valuable information to members of the CF community. Increasingly the Hotline is responding to more calls from or about adults with CF who are facing obstacles to education or employment or who need health insurance coverage or government benefits. Those adults without family support are in need of information that will help them plan for the future and understand eligibility requirements for government benefits that may help fill the gap left by not having family support.

Adults with CF who have information about their legal rights are more likely to face less issues when trying to access education or employment opportunities. In addition, information on health coverage options or Social

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Security benefits can result in adults with CF obtaining coverage and access to care and medication needed to treat CF.

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WOMEN'S HEALTH AND SEXUALITY: IMPORTANT CONSIDERATIONS FOR WOMEN WITH CF

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Persons with cystic fibrosis (CF) have improved life expectancy compared to the 2000 cohort. While the focus of CF research has been primarily aimed at disease and symptom management, reproductive health and sexuality are underreported. These issues are becoming increasingly important as our female patients are reaching child-bearing age. The objective of this study was to better elucidate women's health issues in CF. Women were recruited at their routine scheduled clinic visit at the Adult CF Center at the Keck Hospital of USC under an IRB-approved protocol. An anonymous survey was distributed to all women with CF. Data collected included patient demographics, age of menarche, menstruation cycles, regular women's health follow up with pap smears, performance of breast self-examinations, presence or absence of urinary incontinence and how CF affects quality of life. The survey was given to patients as they came to their quarterly visit. Thirteen of 76 (17%) female CF patients were given and 13/13 responded to the survey. Median age was 32 years (range 25 – 40 years). Median age of menarche was 13 years (range 9-16 years). Eight of 13 (61%) reported normal menstruation. Four of 13 (31%) reported hemoptysis at the time of the menstrual cycle. The median age of sexual activity was 19.5 years, while only 10/13 (77%) reported having a pap smear in the last 2 years. Two of 13 (15%) reported performing monthly breast self-examinations. Seven of 13 (54%) reported occasionally performing breast self-examination. Nine of 13 (69%) reported urinary incontinence ranging from occasional to up to 5 or more years. With respect to sexuality, 5/13 (38%) viewed themselves as looking different from other women, with 2/13 (15%) not liking their physical appearance. Three of 13 (23%) stated that CF affected their desire of getting into a long-term relationship. Four of 13 (31%) perceived that men would not want to be involved in a relationship with them and similar numbers believed that CF made them less desirable. Women who did not have these perceptions tended to have a higher number of sexual partners. With better insight into reproductive health issues and sexuality, women with CF may receive better comprehensive care.

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DEMYSTIFYING SEX: A GYNECOLOGICAL VIEW OF WOMEN WITH CF

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Persons with cystic fibrosis (CF) have improved life expectancy compared to 2000 cohort data. With these improvements, women with CF are at risk for sexual and psychosocial health-related issues. While the focus of CF research has been primarily aimed at disease and symptom management, there are limited data on how women with CF understand and deal with their sexual and psychosocial health. Dealing with issues related to sexuality is becoming increasingly important as patients are reaching child-bearing age. The objective of the study is to better elucidate gynecological health issues in women with CF. Women were recruited at their routine scheduled clinic visit at the Adult CF Center at the Keck Hospital of USC under an IRB-approved protocol. An anonymous survey was distributed to all women with CF. Data collected included demographics, age of menarche, sexual activity, types of contraception, pregnancy, and sexual relationships. Survey was given to 13 of the 76 female CF patients as they presented for their quarterly visit. Thirteen of 13 responded to the survey. Median age was 32 years (range 25 – 40 years). The median age of menarche was 13 years (range 9-

16 years). The median time for initiation of sexual activity was 18 years. Although 13 respondents were in monogamous relationships at the time of the survey, the median number of sexual partners was 5 (1-24). Eleven of 13 (85%) women used some method of birth control method, with the majority 5/13 (38%) of women utilizing the withdrawal method as their primary mode of birth control. Unprotected sexual intercourse was reported in 8/12 women (66%). None of the women reported having had an abortion. Five of 13 (38%) had contracted a sexually transmitted disease (STD). STDs documented were: herpes simplex virus (2), Chlamydia (2), human papilloma virus (5), and gonorrhea (1). Three of 13 or 23% admitted to experiencing sexual abuse and 3/13 (23%) physical abuse. Six of 13 (46%) admitted to experiencing emotional abuse in their lifetime. Three of 13 (23%) wanted to become pregnant in the future. Three of 13 (23%) did not want to have a child in the future. In response to whether their health care provider answered questions about sexual health, 9/13 (69%) agreed that the provider did address their sex questions. Lastly, 9/13 (69%) of the women were comfortable discussing sex with the CF provider. With better insight into sexual and psychosocial health related issues, women with CF may receive better comprehensive care.

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A NOVEL PARTNERSHIP: PALLIATIVE CARE AND CYSTIC FIBROSIS

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Novel therapies in the treatment of cystic fibrosis (CF) have resulted in a significant improvement in life expectancy. Though the majority of CF patients now transition to adult care, CF remains a fatal disease. It is well documented that care for CF patients is challenging, as they suffer from complex medical and psychosocial issues. By virtue of their limited life expectancy, CF patients are often forced to consider end of life decisions at an age when most of their peers are planning for marriage, children and new careers. As a result, CF teams are challenged with not only providing medical care to prolong life, but also simultaneously addressing psychosocial care, complex symptom management and end of life planning. Our CF providers shared with us their challenges in trying to accomplish these goals, and felt that earlier involvement of palliative care (PC) would enhance their ability to care for their patients.

PC aims to ease suffering and maximize quality of life for patients and families dealing with serious illness such as cancer. In addition, PC teams assist in the development of realistic goals of care and facilitate advance care planning. Over the last several years, PC teams have increasingly become involved in the care of patients with chronic diseases such as heart failure and chronic lung disease, but integration of PC into the CF population remains minimal.

Our CF providers initially consulted PC on inpatients whose life expectancy was days. Discussions with CF providers revealed that these consultations were "too late to provide significant action." To address this challenge, we developed a model that incorporates the PC provider into the outpatient CF team. In addition, PC is now consulted for most all CF admissions, not just those with limited life expectancy.

We will review our experience with this new clinical model that integrates PC upstream into the care of CF patients. We will discuss the frequency and severity of symptoms and common psychosocial barriers to optimal care. We will report the prevalence of advance care directive documentation before and after implementation of this new model and our experience with patients' willingness to discuss death and dying. Finally, we will present our innovative "Cystic Fibrosis Goals of Care Form" and how this dovetails into ongoing advance care directive and symptom management assessments.

Care for CF patients will continue to become more complex as life expectancy increases. Previous studies on early integration of PC into chronic disease care has demonstrated improved symptom management, enhanced advance care planning and in some cases increased life expectancy. We hope to see similar clinical benefits as a result of this new model that integrates palliative care into the chronic care model for CF.

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PAIN IN ADOLESCENTS AND YOUNG ADULTS WITH CF: LOCATION, SEVERITY, AND PREDICTORS

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Background: Cystic fibrosis (CF) is a progressive disease affecting multiple organ systems, including the lungs, pancreas and gastrointestinal system. Pain is a significant, but under-studied complication of CF and may be related to disease progression, which can include chronic pulmonary infections and decreased lung function, and increased treatments, such as airway clearance and intravenous antibiotics. The relationship between pain and other outcome variables, such as emotional functioning and health-related quality of life (HRQOL), is largely unknown in this population. The purpose of this study is to systematically assess pain in adolescents and young adults with CF and evaluate associations between pain, internalizing psychological symptoms, and HRQOL.

Methods: The current study is part of an ongoing, NIH SBIR Phase II randomized, controlled trial being conducted at 6 CF centers. Participants complete a battery of measures during four consecutive clinic visits assessing pain, symptoms of anxiety and depression, and HRQOL. Participants also completed supplementary online pain diaries for the 6 days following each clinic visit. Diaries assessed pain intensity, location, duration, affective rating, and coping response.

Results: Preliminary results were analyzed from the baseline visit (N=94). Mean age was 15.69 years (SD=2.91), 62% were female; 57% Caucasian, 16% Hispanic, and 9% African American. Mean FEV1% predicted of 79.89% (SD = 25.90) was in the mild range of severity. An average of 4.4 (out of 6 or 73.33%) of pain diaries were completed, with approximately 50% of participants completing all 6 diaries. At least one pain episode was reported by 34% of participants across the 6 days. Of those who experienced pain, mean intensity was 3.81 (out of 10 points). The most frequent location of pain was the stomach (66%), head/neck (50%), and chest (50%). Most frequently used coping mechanisms were: resting (72%) and taking medication (66%). Structural Equation Modeling evaluated the unique contributions of internalizing symptoms on pain and HRQOL. Pain intensity was regressed on age and disease severity (FEV1% predicted) to control for demographic characteristics. Next, internalizing symptoms (anxiety and depression) were included as independent, exogenous variables in the model. The final step included a latent, endogenous HRQOL variable, with indicators from the Cystic Fibrosis Questionnaire-Revised (CFQ-R), including Respiratory Symptoms, Treatment Burden, and Social Functioning. The final model fit the data, $\chi^2(32) = 16.14$, $p = .097$ (RMSEA = .079, SRMR = .07). Results indicated that symptoms of depression and/or anxiety significantly predicted both reports of pain and HRQOL, after controlling for age and disease severity.

Conclusions: These results indicate that pain is experienced quite commonly by adolescents and young adults with CF and illustrate the importance of measuring other factors that may affect pain perceptions, such as psychological distress. Analyses indicated that patients with more anxious and depressive symptoms reported more pain and worse HRQOL. Regular assessment of pain, psychological distress, and HRQOL is recommended.

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CFFONE: A SOCIAL NETWORKING SITE FOR ADOLESCENTS AND YOUNG ADULTS WITH CF

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Background: Adolescence and emerging adulthood are two of the most challenging phases of development, and include a shift toward greater independence and responsibility, and in those with cystic fibrosis (CF), new challenges related to the progression of the disease and decreases in treatment adherence. Infection control guidelines prohibit face-to-face contact and thus, most peer-to-peer support has been drastically reduced. The major

aim of Cffone was to evaluate the benefits of an online peer support program for adolescents and young adults with CF on outcomes, such as knowledge of disease management, perceived social support, psychological well-being, and HRQOL. The aim of the current study was to examine the social networking (SN) behaviors of these participants prior to engaging in the Cffone intervention.

Methods: This SBIR Phase II randomized, controlled trial is being conducted at 6 CF centers across the U.S. Before being randomized to either Cffone.com or a CF educational website, participants completed the following measures: 1) Knowledge of Disease Management (General Health, Lung Health, Nutrition, and Treatment), 2) Hospital Anxiety and Depression Scale (HADS; anxiety and depression), 3) Cystic Fibrosis Questionnaire-Revised (CFQ-R; Treatment Burden, Social Functioning, and Respiratory Symptoms), 4) Daily Pain Assessment Questionnaire for CF (DPAQ-CF), and 5) Social Networking Survey.

Results: Baseline data on 100 patients with CF were collected; mean age of participants was 15.69 years (SD=2.91), 62% were female, 57% Caucasian, 16% Hispanic, and 9% African American. Mean FEV1% predicted was 79.89% (SD = 25.90) indicating mild disease severity. At baseline, 60% of participants used SN sites to communicate with friends; only 20% communicated online with others with CF. Surprisingly, only 20% of participants searched for CF-related resources on the internet - predominantly on cff.org. When viewing CF-related sites, 20% were looking for information about CF, 8% were looking for information about new treatments, 7% were interested in support, 6% were providing support, and 9% were trying to meet new people. Adolescents' knowledge scores on the KDM were highest on Lung Health (M=66.8; SD=20.1) and lowest on Nutrition (M=50.2%; SD=19.3), indicating significant gaps in knowledge. Preliminary results indicated that those who felt more supported by their CF friends had lower depressive ($r = -.26$, $p < .01$) and anxious symptoms ($r = -.19$, $p = .04$), and higher Social Functioning scores ($r = .22$, $p = .02$). Importantly, those who had a personal cell phone at Baseline also reported higher Social Functioning scores on the CFQ-R ($T = 2.3$, $p = .02$).

Conclusions: Social networking sites for adolescents and young adults with CF may be useful for increasing knowledge of disease management, increasing perceptions of social support and improving mental health and quality of life. Our next step will be a prospective evaluation of the effects of Cffone vs. an educational control condition on the outcomes described above.

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USE OF A DECISION AID FOR MECHANICAL VENTILATION IN PATIENTS WITH ADVANCED CYSTIC FIBROSIS LUNG DISEASE: A PILOT STUDY

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Introduction: Many patients with advanced CF lung disease are treated with mechanical ventilation despite uncertain benefits. Adults with CF want more information about treatment options, and bereaved family members of CF patients report inadequate communication with physicians about treatment options for respiratory failure. Decision aids provide objective information about medical treatments and are intended to facilitate patient participation in important treatment decisions. We developed and pilot tested a decision aid for mechanical ventilation with adults with advanced CF.

Methods: We used existing literature on outcomes of mechanical ventilation, recommendations from interviews with bereaved caregivers and interviews with CF physicians to design a decision aid explaining causes of respiratory failure, treatments for respiratory failure including mechanical ventilation, risks and benefits of mechanical ventilation, and available data on treatment outcomes. We assessed knowledge of treatment options before and after exposure to the decision aid. Participants reported their treatment choice before and after reviewing the decision aid, and we used the Decisional Conflict Scale (DCS) to measure decisional conflict about mechanical ventilation as a treatment for respiratory failure. We solicited feedback on utility, perceived bias, and applications of the decision aid.

Results: Ten adults with advanced lung disease (mean age 26.5 years, mean FEV1 31% predicted) and no prior use of mechanical ventilation for respiratory failure completed the study. Half reported having discussed treatment options for respiratory failure with a physician. At baseline, they

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exhibited low knowledge of purpose and outcomes of mechanical ventilation; number of correct responses to multiple choice questions about mechanical ventilation improved after review of the decision aid. Before reviewing the decision aid, one reported a preference for mechanical ventilation for respiratory failure under any circumstances, none preferred a time-limited trial, five stated they would accept mechanical ventilation for "reversible causes" of respiratory failure and four would "never" accept this treatment. After reviewing the decision aid, these preferences were unchanged but decisional conflict was significantly reduced (mean DCS score 20.4 v. 10.9; $P=0.02$). Bias of the decision aid toward mechanical ventilation was perceived by two participants; eight thought it was balanced and unbiased. All participants found the decision aid useful and all but one felt it would facilitate communication with family members and medical providers. All suggested development of decision aids for other CF treatments.

Conclusions: Use of a decision aid for mechanical ventilation is feasible for adults with advanced CF lung disease and may reduce decisional conflict about this complicated treatment decision. Revision of and further testing of this decision aid may lead to a useful tool to facilitate communication. Patients are likely to support the development and distribution of decision tools for other CF treatments.

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OBSTACLES TO PALLIATIVE OR END-OF-LIFE CARE IN CYSTIC FIBROSIS

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Background: Advancements in CF treatment have resulted in significant improvements in median life expectancy. Patients living with advanced disease have an increased symptom burden resulting from increased disease acuity, CF complications, and co-morbidities and may greatly benefit from palliative care. Despite advancements in treatment, CF remains life limiting with a median age of survival of 38.3 years and 407 total deaths in 2010. A multi-center survey of CF clinicians in Canada showed that most people with CF die in the hospital with palliative care provided only in the last few days of life. Why, in a disease known to be life limiting is palliative care provided in mostly the final days? It has been suggested that additional research and training is necessary for CF clinicians, specifically in palliative and end of life care. It is essential that education address both basic palliative principles and potential obstacles to palliative care in CF.

Purpose: 1) Assess CF team member perception of effect of known obstacles to palliative or end-of-life care. 2) Identify obstacles to the provision of palliative or end-of-life care specific to CF.

Methods: A questionnaire was distributed through established CF list serves to assess team member perceived obstacles to end of life care. The tool was developed based on barriers to palliative care listed in the International Association of Hospice and Palliative Care Manual of Palliative Care 2nd Edition. Also included were potential obstacles from published articles on end of life or palliative care in CF and potential obstacles identified through experience of the author. 26 obstacles were included and consisted of those related primarily to the CF team, patient and family, and specific to CF.

Results: A total of 317 clinicians responded to the survey consisting of 21% dietitians, 19% social workers, 18% registered nurses, 15% physicians, 11% nurse practitioners/physician assistants, 9% respiratory therapists, 5% physical therapists and 5% others. 35% of respondents reported caring primarily for pediatric patients, 33% for both adult and pediatrics and 32% for primarily adults.

The ten obstacles reported to have the largest effect in CF were:

1. Lack of advanced care planning
2. Patient unrealistic expectation of disease process
3. Referral for lung transplant
4. Lack of clear delineation between aggressive and palliative care in cystic fibrosis
5. CF team members lack of communication skill to discuss end-of-life issues
6. Patient-family disagreement about treatment options
7. Patient belief that prognosis is better than what they are told
8. Close relationships developed between teams and family members

9. CF team member lack of education in CF specific palliative and end-of-life care

10. Lack of comfort of CF teams to conduct difficult discussions or deliver bad news

Conclusion: As new therapies emerge and excitement builds in the quest for a cure, it is essential to remember those who may not live to realize that dream. CF teams must endeavor to provide the highest quality care across the continuum, including end of life. Educational opportunities must be provided to CF teams that address general principles of palliative care as well as the most common obstacles to provision of palliative or end-of-life care in CF.

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LIGHTING UP DEPRESSION IN CYSTIC FIBROSIS

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With the increasing lifespan of cystic fibrosis (CF) patients, much attention has been dedicated to improving quality of life and monitoring for symptoms of mood disturbances that can be associated with a chronic, progressive disease. Depression rates in CF are variable, but the most recent CF registry data indicates more than 21% of adults have a diagnosis of depression. Moreover, depression is known to impact health in CF as it correlates inversely with lung function and is associated with worse health related quality of life and longer hospital stays. These factors highlight an area for improving inpatient care in CF by decreasing depressive symptoms in order to shorten hospital length of stay. In addition to natural sunlight exposure, intense phototherapy is the only therapy shown to acutely improve depressed symptoms in hospitalized patients. Light therapy has not been studied in CF, but offers a potentially inexpensive way to improve symptoms for depressed CF patients who are having prolonged hospitalizations complicated by the co-morbid condition.

In order to determine the potential impact of light therapy in hospitalized CF patients we used an actiwatch to measure light absorbance during hospital stays for adult CF patients. Light was measured continuously as Lux over 1 week duration. Depressive symptoms were screened at admission and discharge with the Center for Epidemiologic Studies Depression Scale (CES-D >16 positive for depression) and the CFQ-R was used for quality of life scoring at admission onset and discharge. Twenty patients hospitalized in the winter months November-March (mean age 26.9 years) were enrolled, with 59% of the patients screening positive for depression (mean CES-D 31). There was a non-significant difference in length of stay between depressed CF patients (13.6 days) and non-depressed patients (12.3 days). All CFQ-R scores positively increased at discharge except role (-5.7) and treatment burden (-7.0). The largest increase in CFQ-R scoring was seen in the respiratory domain at 30.9.

Only 25% (5/20) of patients had more than 60 minutes of cumulative bright light exposure (>1000 lux) over one week. No patients had more than 60 cumulative minutes of light exposure greater than 10000 lux, which would be the equivalent time frame of a single therapeutic bright light session. There were also no patients with more than 60 minutes of cumulative light exposure greater than 2500 lux which would equal therapeutic bright light exposure if applied 4 hours continuously. The average awake light exposure for all patients was 64.2 lux, far below the bright light threshold. A cohort of spring and summer admitted patients are currently being followed for comparison.

These results underscore the abysmal bright light exposure hospitalized CF patients are achieving, and highlight an area of further research in order to impact psychosocial health of hospitalized CF patients and potentially decrease length of stay through acute treatment of co-morbid depression with intense phototherapy.

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THE IMPACT OF CYSTIC FIBROSIS ON THE HUTTERITE POPULATION

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There exists research related to the genetic mutations of cystic fibrosis that occur in the Hutterite population, but no research currently exists to explain the impact of quality of life that a diagnosis of cystic fibrosis has on this group of genetic isolates. Seven of the nine Hutterite families, representing 6 of the 8 affected colonies in Montana were interviewed and asked a

series of questions to determine the impact of cystic fibrosis on their day to day life and, conversely, the perceived impact that being Hutterite has on their disease management. Eight out of eight medical professionals specializing in cystic fibrosis who respond to the needs of the Hutterite population in Montana were interviewed. Interview questions were designed to determine how medical professionals perceive Hutterite patients, the impact that being Hutterite has on cystic fibrosis care and the responsiveness of the medical community to this population's needs. The results indicated that both the Hutterites and the medical professionals perceive that medical care received is as good as the general population. Both groups acknowledged the negative impact that farm work has on cystic fibrosis management. The Hutterites perceive colony life as an overall benefit and their beliefs seem to limit stigmas commonly attached to cystic fibrosis. The medical professionals perceive colony life to more negatively impact care. This study was limited to Montanans who all share the same genetic mutation and no comparative quality of life study was performed on English Montanans diagnosed with cystic fibrosis. Comparative interviews need to take place in other areas of the United States and Canada to more fully understand the risks and benefits to disease management and quality of life that come with being Hutterite diagnosed with cystic fibrosis.

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DOES ADHERENCE LITERATURE HELP US TO ADHERE?: A PARENT'S PERSPECTIVE

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Objective: Daily care regimens for cystic fibrosis (CF) are demanding, and maximizing adherence to treatment is a challenge for patients, families, and healthcare providers alike. However, the bulk of adherence is completed in non-medical settings by patients and their families. The objective of this patient-centered literature review is to discuss adherence from the perspective of these "adherence practitioners," and to examine how adherence research can inform and be informed by them.

Methods: For the literature review, PubMed was queried using the search phrase ("Cystic Fibrosis"[Mesh]) AND ("Health Behavior"[Major]) and PsychInfo was queried using the search phrase (DE "Cystic Fibrosis") AND (MM "Treatment Compliance"). The searches were limited to journal articles dated 1/1/2001 – 12/31/2010 and in English.

Results: Adherence literature for CF was fragmented in terms of reporting adherence outcomes; where outcomes were reported, they were often process-based (e.g., % treatments completed) and non-comparable across studies. "Adherence practitioners" expressed the importance of seeing adherence linked to tangible results; consistently reporting health-based outcomes (i.e., FEV1 and BMI) would align adherence research with patient goals as well as increasing comparability across studies. Major factors identified as impacting adherence could help "adherence practitioners" set priorities and identify opportunities to improve adherence; in particular, the drop-off in adherence in the mid-teen years demonstrates the need for parents to develop improved strategies for preparing for adolescence. Techniques such as motivational interviewing were researched for use by healthcare providers, but might also be relevant for parent-to-child or peer-to-peer interactions among "adherence practitioners." Finally, research documented the occurrence of overadherence (e.g., ineffective precautions due to fear of *P. aeruginosa* exposure); this problem is of potential importance to "adherence practitioners" because overadherence consumes resources that might be better used in other ways.

Conclusions: Research literature on adherence in CF offers some insights that can be operationalized to increase adherence in the home. However, adherence research runs the risk of treating adherence as something that is done to, rather than done by, patients and their families. It is essential to involve "adherence practitioners" in the formative stages of adherence research, not only to ensure that such research is asking the right questions, but also to test the possibility that empowerment is, in and of itself, an effective adherence intervention.

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BEADS FOR BREATH: A PILOT STUDY OF AN ARTS IN HEALTH INTERVENTION

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The diagnosis of a chronic illness has significant emotional impacts for both the patient and family. Many studies have focused on maladaptive coping techniques of people living with chronic illness. One of the primary roles of psychosocial professionals (social workers, child life therapists, psychologists) is to help patients and families identify and use a variety of coping strategies. Due to infection control issues in CF, children are not allowed to participate in activities like camps or support groups involving interpersonal contact. A literature review was conducted by the authors for alternative coping strategies that could be implemented. Bead programs are being widely used in other areas of chronic illness for pediatric patients, such as oncology and congenital heart defects (Beads of Courage®, Heart Beads, Bravery Beads). The bead programs have served as a coping strategy and narrative to the child's journey with their disease. Research has also described how parents' use of emotional support assisted in their adaptation to their child's CF.

Methods: Patients 6 and over will be asked during routine clinic or hospital visits about program enrollment. Patients will be assessed prior to program enrollment by using a validated questionnaire (CFQ-R) to assess their quality of life. Parents of CF patients will also complete the CFQ-R for those children under the age of 12, to rate their perception of child's quality of life. For adolescent patients, the PASS-CF will also be administered to assess adolescents' perceived social support and coping methods. Enrolled patients will receive a strand for their beads, and begin collecting beads according to the Beads for Breath guide, assigning meaning to various events, accomplishments or milestones. Patients and their parents will also be given a journal to record their experiences with the program journey. After 3 months of enrollment, patients and their parents will be reevaluated with the CFQ-R, PASS-CF, and a program specific survey.

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MANAGING CYSTIC FIBROSIS IN SCHOOL: EVALUATING A PSYCHOSOCIAL WORKSHOP FOR SECONDARY SCHOOL STAFF

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Background: Schools play an important role in supporting families who have children with cystic fibrosis (CF). It is common for nurses and physiotherapists to provide training to schools around medical management. However less is known about the use of training for school staff around the psychosocial impact of living with CF.

Aim: To evaluate school staff's experience and understanding of CF before and after training in psychosocial issues in CF during adolescence.

Methods: One half-day workshop will be delivered on 16 May 2012 by two paediatric clinical psychologists to 14 staff from secondary schools in East Anglia, UK where it is known a child with CF attends. Roles include first aiders, pastoral support and special education need coordinators (SEN-COS). The workshop will include two teaching slots followed by group work and discussion. Topics to be covered are chronic illness and development, including adherence and transitions, adolescents' experiences of CF, and the potential impact on schooling. The group work will focus on managing: i) difficulties experienced at school when working with adolescents with CF; and ii) challenges faced by professionals.

Results: All workshop participants will complete an evaluation form. Descriptive statistics and qualitative analysis will be used to analyse the data. It is hoped the workshop will enable professionals to share ideas and encourage greater collaboration between the school and medical settings to support CF families.

Supported by: CF medical team.

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TREATMENT ADHERENCE AMONG ADOLESCENTS WITH CYSTIC FIBROSIS

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Adherence to CF treatments is a significant public health problem, especially during adolescence when patients begin assuming responsibility for their health and this responsibility is shifted away from the parents. Poor adherence can have serious consequences, including increased morbidity and mortality, reduced quality of life, and greater health care costs. The purpose of this study is to identify baseline levels of treatment adherence in the University of Florida pediatric cystic fibrosis (CF) population and to understand the factors that influence treatment adherence.

The study is being conducted through distribution of a two-part survey to patients between the ages of 10 and 19 at the University of Florida Pediatric CF Clinic. It is anticipated that between 40 and 50 surveys will be completed by participants. The two-part survey measure includes the following components: part (1) is a fifteen-item self-report questionnaire used previously by Myers and other CF researchers that measures adherence amongst CF patients in particular on a Likert-scale; part (2) of the survey measure is a thirty-two-item self-report questionnaire also on a Likert-scale created by a Dutch researcher (being used with permission) under the supervision of field experts following previous research by Modi, a specialist in the field. This second part measures factors influencing adherence as identified from the literature. The study is currently in progress and anticipated to be completed in July 2012.

Quantitative data will be analyzed using Statistical package for the Social Sciences (SPSS). Analysis for part one (treatment adherence) will consist of analyzing how many participants require each of the sixteen treatment components and of those requiring each treatment component, how many participants report being adherent, as a percentage.

Part two (influencing factors) analysis will consist of identifying how many participants agree, how many disagree and how many do not disagree or agree with each of the 32 statements regarding factors that impact adherence.

Results will be expressed as mean and range for the quantitative variables. Means will then be compared using a t-test in which a p-value of <0.05 will be statistically significant.

Conclusion and discussion will elaborate on findings related to which beliefs affect adherence in the CF teen population.

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DEPRESSION AND HEALTH-RELATED OUTCOMES IN ADOLESCENTS WITH CYSTIC FIBROSIS

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Background: Depression and anxiety in the cystic fibrosis (CF) population has been associated with lower quality of life, worse respiratory function and reduced treatment adherence. Our study aims to describe the impact of depressive disorders on medical outcomes in adolescents with CF.

Methods: This is a retrospective study of adolescents with CF aged 12-21 years who were diagnosed at the time of an "index" clinical encounter with a depressive disorder by Children's Hospital Boston Psychiatry Consultation Service between 2009-2011. This group was matched to a group of nondepressed adolescents with CF on the basis of age, gender, and severity of lung disease at the time of an index clinical encounter. Data was collected for both groups for the 12 months prior to and 12 months following the respective index clinical encounter. The following variables were examined across both groups: FEV1, BMI, frequency of pulmonary exacerbations, Vitamin D and HgbA1c values, respiratory colonization and CF-related complications. Analyses between health care variables and mental health outcomes included descriptive statistics and t-tests.

Results: Among the group of 20 adolescents diagnosed with a depressive disorder, 12 (60%) were male and the mean age was 16.6 years (SD=2.5). Both the depressed and nondepressed group had moderate illness severity in the 12 months prior to and after their index encounter, with neither group showing a significant change in lung function over these two

periods of time. Both groups had borderline underweight-healthy BMI. There was a significant decrease in BMI for the depressed group after diagnosis of depression ($p<.01$) that was not found in the nondepressed group. The depressed group also showed trends of increased frequency of exacerbations and elevated HgbA1c values post-index time that were not found in the non-depressed group. Both groups were described in terms of health care outcomes including: Vitamin D values, respiratory colonization and CF-associated complications, which did not demonstrate statistical significance.

Conclusions: Adolescent CF patients diagnosed with a depressive disorder appear to have moderate illness severity, and demonstrate a statistically significant decrease in BMI following diagnosis, along with trends of increasing frequency of exacerbations and elevated HgbA1c values. As BMI is often used as a primary predictor of future decline in lung function, and exacerbation frequency is an important marker of disease severity, these findings highlight the negative impact depressive disorders have on critical health outcomes for this population and suggest depressive disorders as a potential target for clinical intervention. Future directions of study include larger sample groups, longer time frames, earlier identification of depressive disorders, and investigation of impact of other disorders such as anxiety on medical outcomes. Lastly, further study holds the promise of improving morbidity and mortality in the adolescent CF population through timely recognition and treatment of depressive disorders.

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EFFECTS OF CLINICAL SUPERVISION ON TREATMENT FIDELITY IN ICARE

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Background: Research indicates that behavioral interventions are effective in improving adherence; however, training and support are needed to transport these interventions to medical settings. The degree to which interventions are implemented as intended, or "treatment fidelity," can significantly enhance their effectiveness. Fidelity is unlikely to improve as a result of experience alone, but has been shown to increase with ongoing clinical supervision, in which a supervisor reviews each session and provides feedback. In the context of a problem-solving (PS) intervention for teens with CF (iCARE), the current study examined predictors of treatment fidelity over time, including number of PS sessions conducted, number of supervision sessions received, prior mental health training, and initial fidelity.

Methods: iCARE is a multi-site, randomized study of the effectiveness of a Comprehensive Adherence Program (CAP). Trained behavioral interventionists (BIs), including nurses, social workers, physicians, and other multidisciplinary team members, conduct PS sessions with adolescents and parents to identify adherence barriers and brainstorm solutions. The first session with each participant is video-taped and reviewed by a clinical supervisor, who provides ongoing supervision and feedback by phone. Taped sessions are rated for treatment fidelity by 3 trained raters on a scale of 0 to 35.

Results: Preliminary results are reported on 235 sessions by 34 BIs (52% mental health (MH) professionals) at 13 CF centers. Across BIs, mean number of completed sessions was 30.52 (range: 2-94) and mean number of supervisions was 4.12 (range: 1-11). No statistically significant differences in initial fidelity scores (prior to any supervision) were found between MH ($M=21.53$, $SD=4.49$) and non-MH professionals ($M=19.12$, $SD=4.75$, $t(26)=-1.37$, $p>.05$). After supervision sessions were implemented, the strongest predictor of most recent fidelity score was number of supervision sessions received ($\beta=.63$, $p<.01$), followed by MH professional status ($\beta=.43$, $p<.01$). In contrast, number of PS sessions conducted was not related to fidelity ($\beta=-.14$, $p>.05$). Finally, initial fidelity score (prior to supervision) was not predictive of subsequent fidelity ($\beta=.25$, $p>.05$). The overall model explained 54% of variance in treatment fidelity ($R=.73$, $R\text{ square}=.54$).

Conclusions: Results indicated that phone supervision significantly improved interventionists' fidelity to the PS intervention; experience alone did not. Although MH professionals did not initially score higher on fidelity than those with less formal MH training (e.g., physicians), they were more responsive to supervision over time. In sum, clinical supervision was the strongest predictor of treatment fidelity, highlighting its effectiveness at supporting HCPs in implementing behavioral interventions in CF clinics.

Future analyses will examine treatment fidelity in conjunction with supervision with a larger sample of BIs and PS sessions.

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ADAPTING A BEHAVIORAL ADHERENCE INTERVENTION FOR PATIENTS WITH AUTISTIC FEATURES: A CASE STUDY

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Background: Autism Spectrum Disorders (ASDs) are characterized by impaired social interactions, delayed communication, and restricted or repetitive behaviors. The CDC estimates that 1 in 88 children meet the diagnostic threshold for an ASD (1). Due to limited organizational and self-care skills, adolescents with ASDs may face adherence challenges beyond those faced by their typically-developing peers. iCARE is a multisite, randomized control trial testing the efficacy of a behavioral adherence intervention for adolescents with CF. We describe adaptations made to the Problem-Solving (PS) component of iCARE for an adolescent with high-functioning ASD. Additionally, we present initial short-term outcomes.

Methods: A 13 year old male presented to a CF clinic with impaired social skills (e.g. eye contact, perspective-taking), limited speech intonation, restricted interests (e.g., plays video games exclusively), and difficulties with planning. The healthcare provider (HCP) facilitated a PS session with the teen and parent. Short phrases and multiple examples were used to aid comprehension of PS steps. Other modifications included frequent praise for on-task behavior, guided questions, and modeling of PS techniques. Due to the adolescent's limited writing skills, the HCP documented the solutions generated during PS.

Results: In Session 1 of PS, the adolescent problem-solved vitamin adherence (barrier: forgetting). Possible solutions included using a pill box for day of the week and computerized reminders. Unique barriers emerged, such as the adolescent's self-reported difficulty remembering the day of the week. He opted to reduce memory demands by taking two vitamins once a day, instead of one vitamin twice daily. Future sessions will: 1) utilize a colorful visual flow-chart depicting each PS step, and 2) allow the adolescent to draw pictures of each solution to maintain his engagement. Results of two upcoming PS sessions and the adolescent's success at implementing solutions are forthcoming.

Conclusions: The high rates of ASDs indicate that PS should be adapted appropriately, with concomitant training of HCPs to implement modifications. Some features of ASDs may predict greater success of behavioral adherence interventions, including preference for routines and an absence of "social desirability" responding (e.g., exaggerating adherence behaviors). Further, teens with ASDs benefit from many of the same behavioral strategies as most adolescents, including labeled praise, immediate feedback, cell phone reminders, and modeling. This case study demonstrated that iCARE can be adapted for an adolescent with both CF and an ASD.

Funding provided by Novartis Pharmaceuticals Corporation, Genentech, Inc., and Cystic Fibrosis Foundation.

Reference: 1. Center for Disease Control. Prevalence of Autism Spectrum Disorders – Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. Surveillance Summaries, Morbidity and Mortality Weekly Report. 2012;61(SS03):1-19.

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UNDERSTANDING THE LIVES: HOW TEENS WITH CF VIEW THEIR WORLDS

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The project to be presented is based on the qualitative analysis of transcripts of videotapes made by teens with CF about their lives. Common themes about teens' interests, needs and concerns will be provided, illustrated and discussed.

Health care providers are often puzzled or frustrated by the fact that youth with cystic fibrosis and their families do not follow the treatment plan as completely as the healthcare team would like. Teens and their parents, on the other hand, may be frustrated when healthcare providers do not understand their "whole lives" – the additional demands placed on them, competing needs and priorities, beliefs about health and how they balance healthcare with other life events. These differing views can lead to a lack of trust on both sides and a stalemate in the treatment process, resulting in a major barrier to successful healthcare. The goal of this project was to reverse this negative cycle: to empower teens with cystic fibrosis to show healthcare providers about their lives and to help healthcare providers understand and appreciate the lives of participants. As providers better understand families and families feel more understood, trust and partnership will develop, enhancing the treatment process.

The goals of this project were: 1) to learn from teens about their lives with cystic fibrosis and 2) to empower these teens in their own care. These goals were accomplished by loaning camcorders to teens with cystic fibrosis and asking them to "teach your provider about your condition." Teens were encouraged to film family members, interests and activities, school and work, friends and social life, medications and treatments, views of healthcare and any other aspect of life they consider important in understanding them. Teens were also asked to use the camcorder to create a "video diary," talking about their feelings and observations about events and relationships in their lives.

Videotapes were then transcribed and are currently being analyzed qualitatively for themes. VIA is based in grounded theory which examines and structures data to build theoretical models rather than use data to test a pre-determined hypothesis. We plan to identify primary themes related to adherence, relationship with the healthcare provider, outside interests and health beliefs.

Video Intervention/Prevention Assessment, or VIA, is a well established technique, developed at Boston Children's Hospital (BCH). Patients are given camcorders and are asked to videotape their day-to-day lives, with an emphasis on depicting how their illness affects their lives. From the analyzed data, health care providers and health professions students learn about the needs, interests, values and concerns of teens with cystic fibrosis as a group. More on the BCH VIA program can be found at <http://www.viaproject.org/>.

This project was funded in part by the Herbert Bearman Foundation.

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PARENTAL REPORT OF CHILDREN'S VIEWS OF THE SHORTENED LIFE EXPECTANCY ASSOCIATED WITH CYSTIC FIBROSIS

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Introduction: The purpose of this study is to assess how aware children with cystic fibrosis (CF) ages 7 -20 are of the shortened life expectancy (SLE) associated with CF, as reported by their parents/caregivers. The study also explored the ways the children learned about the SLE in CF and the impact this awareness had on them. Finally, the study solicited recommendations and questions from parents/caregivers about how this topic should be handled. IRB restrictions precluded asking the children directly.

Background: Currently, only limited and outdated literature—written when CF patients died at a very young age—exists on the subject of pediatric patients' awareness of the SLE associated with CF. Existing studies on SLE in children focus on illnesses such as cancer, when death is imminent. These studies note that attempting to shield children from the truth can be harmful, preventing them from communicating with and confiding in those doing the shielding and leading to anxiety, isolation and a sense of loss of control. Understanding the topic of how best to discuss SLE and the impact this has on children can inform and improve the care we provide.

Methods: Surveys containing 31 multiple choice and open-ended questions were given to interested parents/caregivers of children with CF between the ages of 7-21 at the University of Florida Pediatric Pulmonary Clinic. Due to the sensitive nature of this topic letters informing parents/caregivers that they would be approached about this study were sent to their homes, in advance of the study. In clinic, the social worker asked whether or not the parents would be interested in participating and, if so, asked them to sign a consent form. Parents/caregivers were then instructed to complete the survey and leave it in a designated box near the check-out

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desk. The box was emptied only every 3-4 weeks, so that it would not be possible to link families attending clinic on a given day with the surveys in the box.

Interim Analyses: Interim (pilot) analyses were conducted with 19 surveys. The small sample size thus far allowed only descriptive analyses of the data. Twelve of the 19 respondents reported that their child was aware, two weren't sure and five said their child was unaware, of the SLE in CF. Average age when children became aware was 10.4. In six cases parents had informed the child about SLE. In four instances, physicians (n=3) or other members of the CF healthcare team (n=1) had discussed SLE with the child. When asked their preferences, all respondents felt that a parent should be involved in the process. Nine respondents preferred that a parent and team members be involved. Attitudes of children in response to learning about the SLE varied between neutral (n=6) and scared/sad (n=6). Current attitudes of these children varied between motivated to fight CF (n=8), neutral (n=2) and discouraged (n=1). Parents' beliefs and attitudes were often conveyed in the surveys.

Future Plans: Final analyses will be conducted in September 2012 and will include updated descriptive analyses, as well as correlations between sociodemographic variables and patients' attitudes related to SLE.

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AIRWAY CLEARANCE ADHERENCE: INFLUENCE OF PARENTAL SPIRITUALITY AND DEPRESSION

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Introduction: Religious/spiritual (R/S) beliefs are important to many Americans. However, little is known about the role of R/S beliefs in CF treatment adherence, nor about their potential interaction with parental depression. R/S coping, defined as, "the search for significance, in ways related to the sacred" has mixed health associations, although negative R/S coping (attributing CF to divine punishment, the work of the devil, or feeling abandoned by the divine or one's congregation) is usually related to poorer health outcomes. Using the Theory of Reasoned Action (TRA) as a conceptual model, we hypothesize that R/S beliefs relate to adherence determinants (perceived treatment effectiveness, perceived norms, and self-efficacy) and that depression is related to both negative R/S coping and adherence determinants.

Methods: N=130 parents of children with CF aged 3 months to 13 years are being recruited from 2 centers (Cincinnati, OH and Birmingham, AL) to complete questionnaires based on TRA constructs and R/S constructs. Daily phone diaries are being used to obtain treatment adherence behavior.

Results: Preliminary analysis is based on n=96 parents (67% female; n=130 anticipated by 7/1/12). Mean child age is 6.4 years (SD 4.2); 49% female. Mean adherence to airway clearance was 63±24%. Body sanctification (the imbuing of one's body (or one's child's body) with sacred significance) was associated with adherence intentions for mothers and fathers (rho=0.192; p=0.065; n=93). Fathers who imbued their child's body with sacred significance had higher levels of perceived treatment effectiveness (rho=0.360, p=0.043, n=32). Almost half (47%) of parents reported depressive symptoms above cutoff for clinical significance; depression correlated inversely with motivation for treatment completion (rho=-0.28; p=0.008); body sanctification (rho=-0.0304, p=0.004) and positively with negative R/S coping (rho=0.520; p<0.01; n=87). Self-efficacy for completing airway clearance correlated with treatment intentions (rho=0.403, p<0.001), as did perceived behavioral norms (rho=0.309, p=0.003).

Discussion: R/S beliefs relate to adherence determinants and adherence behaviors. The prevalence of depression in this sample is 6x the national average and depression is related to adherence determinants, body sanctification, negative R/S coping and adherence behavior. These findings extend the literature by incorporating important psychosocial factors into the understanding of adherence and have implications for clinical interventions. To the extent that depression relates to negative R/S coping, addressing either issue may function to decrease both. Future studies will explore integration of R/S issues into disease self-management and test potential R/S interventions to improve adherence.

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A FINANCIAL SURVEY OF CYSTIC FIBROSIS FAMILIES IN ONE CF CENTER

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Background: The financial stressors involved in cystic fibrosis (CF) medical care are widely recognized. However, current assessment is limited to anecdotal social worker assessments with limited data captured by the CFF registry. To better understand the economic difficulties that our families face, the Cystic Fibrosis Center of Chicago developed a financial survey. Our intent was to identify the major economic challenges so that priority could be given to the most pressing needs.

Methods: We developed a nine question financial survey that was introduced to families upon check-in for routine office visits. While families were encouraged to complete the survey, it was not mandatory. Questions were selected to address insurance status and stability, to identify difficulties paying for healthcare, to determine gaps in healthcare coverage (i.e., services and medications), to assess awareness of assistance programs, and to evaluate desire for additional financial counseling. All surveys collected over the past 4.5 years were reviewed. While families completed multiple surveys over this time period, 106 (73% of a possible 146) represented the most recent survey and were included for analysis.

Results: Results indicated that the majority of individuals had PPO insurance coverage followed by Medicaid. The majority did not anticipate a change in insurance over the upcoming 12 months. The ability to make all co-pays and pay for all CF services was surprisingly high (88%, 94% respectively). The majority of patients were aware of dornase alfa, tobramycin inhalation solution and Cystic Fibrosis Institute financial assistance programs. A minimal percentage of patients felt they required additional financial counseling. Of the families who identified a not-covered service, most cited vitamins, followed by g-tube feedings, nutritional supplements and physical therapy.

Discussion: The low rate of uninsured patients is consistent with the private practice patient population. The lack of difficulty with co-pays and payment of CF services may also be indicative of this population; however, it most likely reflects the high baseline level of attention given to our families' financial needs. The high percentage of patients who were aware of assistance programs coupled with the low rate of families who sought additional financial counseling is consistent with this theory. On several occasions utilization of this survey identified potential problems that were able to be proactively addressed. One limitation of this analysis was that data was not collected on 27% of our center population. This may be reflective of low utilization of the survey at off-site locations, patients whose health status interfered with routine office visits, and patients who were uninterested in completing the voluntary survey. Given the ease of administration, immediate benefit of information provided, and more global utility of this data analysis, regular utilization of the survey will be encouraged.

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ADOLESCENT DEPRESSION SCREENING IN CYSTIC FIBROSIS

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Issue: Depression co-exists with chronic illness and decreases capacity for self-management and adherence to medical treatment recommendations. Guidelines recommend depression screening and advocacy for follow up treatment as a strategy to support self-management in chronic conditions. Our cystic fibrosis (CF) clinic did not have a formal process for screening adolescents with CF for depression.

Objective: A fellowship was undertaken to develop a process for depression screening in adolescents with CF and a formal referral system for those who have signs of depression.

Methods: The Registered Nurses' Association of Ontario Best Practice Guideline Implementation Model and Toolkit was used to guide the fellow-

ship. A literature review was conducted on adolescent depression and the use of screening tools. Health care professionals were surveyed to assess current practice in U.S. and Canadian CF clinics for adolescent depression screening. Structured interviews were held with key stakeholders to obtain input on the development of a depression screening and referral process.

Results: Fifty-four responses to the survey were received. Survey results suggest that very few CF clinics have a formalized system for adolescent depression screening at this time. Almost 60% of survey respondents reported a lack of confidence with screening for depression. Eight CF and mental health professionals participated in structured interviews. The Beck Youth Inventory (BYI-II) was chosen as the adolescent mental health screening tool. A formal collaboration was established between our CF program and the Mental Health Program Mood and Anxiety Team.

Conclusion: We anticipate implementation of this screening and intervention program will improve the quality of patient care through our adoption of evidence-based mental health screening practices and a collaboration between the CF and Mental Health teams.

This fellowship work was supported by the Ontario Ministry of Health and Long Term Care through the Registered Nurses Association of Ontario, and Hotel Dieu Hospital, Kingston, Ontario, Canada.

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THE USE OF SELF-ADMINISTERED MEDICINAL CANNABIS FOR CYSTIC FIBROSIS SYMPTOM MANAGEMENT: PATIENT-REPORTED EXPERIENCES

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Background and Purpose of Study: Cystic fibrosis (CF) patients commonly report complaints such as persistent pain and anorexia which negatively impact on their quality of life (QOL). A number of patients have volunteered their use of cannabis for symptom control. Although there have been many studies regarding the use of medicinal cannabis for symptom management in HIV/AIDS and cancer, to our knowledge there have been no studies done to explore its efficacy in the CF population.

Objective: This survey explores the reasons why our patients are self-medicating with cannabis and how successful it has been from the patient's perspective. The results could help clinicians identify patients who may benefit from the Canadian Health Canada Marihuana Medical Access Division Program.

Methods: We anonymously surveyed all the patients registered with the Adult CF Clinic at St. Paul's Hospital via a self-administered web-based survey. A letter inviting all 215 patients to participate in the survey was mailed. The survey was open for 9 weeks. We used the approach described by Dillman et al. to assess: how many patients are self-medicating with cannabis for medicinal reasons related to CF; what CF symptoms they are specifically trying to manage; and how medicinal cannabis use has affected their QOL. Questions focused on the use and success of cannabis for the management of poor appetite, pain, insomnia, bronchoconstriction, anxiety, and other symptoms identified by the patients. Four-Six category Likert scales were employed. The primary outcome is the rate of cannabis use for CF symptom control. Secondary outcomes include self-reported efficacy scores for overall symptom control, as well as for the individual symptoms listed above.

Results: Preliminary results are as follows: 85 patients responded to the survey and 76 completed the survey; 57% of the respondents were male and 41% were female and one identified as transgendered.

19 patients identified with using marijuana for medicinal reasons: 21% used it less than once per week, 16% used it 1-3 times per week, 5% used it 4-6 times per week and 58% used it daily. Before these patients started taking marijuana, 16% indicated CF affected their QOL mildly, 68% indicated CF affected their QOL moderately and 16% indicated CF affected their QOL severely. After they began taking marijuana, 94% indicated CF affected their QOL mildly, 6% indicated CF affected their QOL moderately and 0% indicated CF affected their QOL severely. 83% of these 19 patients stated it significantly improved appetite, 58% stated it significantly improved pain, 72% stated it significantly improved sleep, 53% stated it significantly improved bronchoconstriction, and 53% stated it significantly improved anxiety. 6% indicated there was no effect on sleep, 18% indicated there was no effect on bronchoconstriction and 5% indicated no effect on anxiety. 6%

of these patients indicated a worsening of bronchoconstriction and 5% worsening anxiety.

Conclusions and Recommendations: Cannabis is commonly used by adult CF patients who ascribe benefits in QOL. This data provides a rationale to study its impact in a prospective study.

Pending analysis of data.

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RELIGIOUS COPING AND PSYCHOSOCIAL DISTRESS AMONG ADULTS WITH CYSTIC FIBROSIS

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Religious coping has drawn considerable attention from health investigators in recent years, but surprisingly little work has focused on adults with CF. Many patients draw on religious/spiritual resources to assist them in managing the demands of chronic illness. This study examined relationships between distinct types of positive and negative religious coping and psychosocial adjustment, among adults followed in a CF Center in the southern US.

Average age was 27.2, and median education was 12 years. Psychosocial distress was assessed using the Hospital Anxiety and Depression Scale (HADS). Discrete facets of religious coping were evaluated using 4 scales from the RCOPE. It was hypothesized that religious struggle in response to CF (i.e., punishing God reappraisals; spiritual discontent) would be tied to increased distress. No hypotheses were offered regarding use of positive religious coping strategies (i.e., benevolent religious reappraisal; spiritual connection), since findings in other illnesses have been mixed.

In bivariate analyses, coping strategies reflecting religious struggle were related to increased concurrent distress, as predicted (punishing God: $r = .26$, $p = .03$; spiritual discontent: $r = .49$, $p = .00002$). Benevolent religious reappraisal was related to less distress ($r = -.24$, $p = .045$); effects for spiritual connection were not significant. In multiple regression analyses that examined significant religious coping strategies simultaneously, controlling for medical and demographic covariates, spiritual discontent remained an independent predictor of concurrent distress ($\beta = .44$, $p = .0007$).

Findings suggest that religious/spiritual coping efforts warrant closer attention by CF investigators and clinicians. In particular, responses that reflect religious struggle or alienation are related to heightened psychosocial distress.

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RELATIONSHIPS BETWEEN ILLNESS BELIEFS AND QUALITY-OF-LIFE OUTCOMES AMONG ADULT CYSTIC FIBROSIS PATIENTS

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The manner in which patients interpret and understand their condition appears to have a salient effect on adjustment to serious illness. CF patients are confronted with daunting burdens. Illness beliefs may have a notable impact on health outcomes, as posited by Leventhal's self-regulation model; however there have been few efforts to examine these beliefs among adults with CF.

This study evaluated adults treated in a regional CF center. Mean age was 27.2 (9.6) years, and mean FEV1% was 67.8 (25.4). Illness beliefs were

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assessed with selected scales from the Illness Perception Questionnaire-Revised. Health-related quality-of-life was evaluated with the SF-12. It was hypothesized that greater perceptions of (1) illness coherence, (2) personal control over the illness, and (3) treatment control, would each be related to improved daily functioning in mental health and physical domains. No predictions were offered regarding perceptions of the illness as chronic (as opposed to brief), given the protracted course of CF.

In bivariate analyses, better mental health functioning was significantly related to greater illness coherence ($p = .003$) and treatment control ($p = .019$). Better physical functioning was related to greater illness coherence ($p = .045$), treatment control ($p = .001$) and personal control ($p = .015$). Timeline beliefs did not predict either outcome. In multiple regression models that examined these predictors simultaneously, adjusting for significant medical and demographic covariates, better mental health functioning was significantly associated with illness coherence ($\beta = .27, p = .02$), and marginally associated with treatment control ($\beta = .18, p = .098$). None of the illness beliefs was independently related to physical functioning in the multivariate model.

Results suggest that a clearer understanding of one's condition (i.e. illness coherence) is tied to better emotional well-being among adult CF patients. Longitudinal research is needed to further examine these findings; ultimately, illness coherence may represent an important, modifiable target for intervention. Beliefs about control over the illness (personal control or treatment control) merit further study as well.

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ASSOCIATIONS BETWEEN PERSONAL RESOURCES AND HEALTH-RELATED QUALITY-OF-LIFE AMONG ADULTS WITH CYSTIC FIBROSIS

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In recent years, growing research attention has focused on positive personal strengths or resources (e.g., optimism, spirituality, gratitude, forgiveness), and their potential salutary effects on health. Relationships between personal resources and quality-of-life (QOL) outcomes rarely have been examined among adult CF patients.

The current study examined associations between several important personal resources and health-related QOL in an adult CF center serving a rural southern region. Of participants, 40.9% were women, most were white, and average household income was modest. It was anticipated that higher levels of gratitude (Gratitude Questionnaire), forgiveness (Trait Forgiveness Scale), and religious commitment would be related to better QOL outcomes in several CF-specific domains of functioning. QOL was evaluated with selected scales from the Cystic Fibrosis Questionnaire-Revised (i.e., vitality, emotional, social, eating, and respiratory domains).

Gratitude was associated with significantly better functioning on all outcomes, in bivariate analyses (all p 's $< .05$). Forgiveness was associated with better functioning on all outcomes (all p 's $\leq .003$) except the respiratory domain. Religious commitment was tied to better vitality ($p = .009$), emotional functioning ($p = .006$), and eating ($p = .005$), and marginally better respiratory functioning ($p = .09$). Multiple regression analyses examined the simultaneous effects of all 3 personal resources, controlling for medical and demographic covariates: gratitude remained an independent predictor of all outcomes (all p 's $\leq .01$) except respiratory functioning.

Personal resources, especially gratitude, may be tied to improved functioning in a number of CF-specific QOL domains. Longitudinal studies will be important to further examine the temporal and causal nature of these relationships.

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HOW IS LIVING WITH CYSTIC FIBROSIS FOR ADOLESCENTS?

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Objectives: To understand how adolescents deal with cystic fibrosis (CF) and how they feel about their actual life and future.

Methods: A clinical-qualitative method was used with semi-structured interviews with open questions: (1) How is CF for you? (2) Do you have any special needs due to CF? (3) How do you see your future?

Forty-two adolescents (10-19 years old; 23 females) were interviewed. A qualitative analysis of the answers showed: (1) Subjective views about dealing with CF included fears of death, of being abandoned by a partner and of the opinions of others. Shame, anguish, anger and tiredness were also found. Losses suffered in the patients' views included: loss of school life, routine, friends and freedom. (2) Real needs included: needing to adjust to a tight routine for treatment, taking drugs daily and time required to prepare special diets. Psychological needs included: equability and social acceptance. Understanding needs included: about the disease and its knowledge by society. (3) Expectations regarding CF: uncertainty and insecurity about the future, changed plans after diagnosis and hope of cure. Personal plans: Having a family, studying and working.

Conclusion: Understanding the issues presented here of how to deal with CF contributes to understanding the psychosocial processes, making possible future investigations and therapies that may ease the disease impact, leading to a better treatment compliance and also a better quality of life for adolescents with CF.

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SURVEY OF CONTRACEPTIVE PRACTICES IN WOMEN ATTENDING A LARGE REGIONAL UK CENTRE

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Introduction: Women with CF have similar sexual lifestyles to their peers and unlike males most are fertile. With improved survival it is vital that effective contraception and sexual health choices are made, with the aim that these do not negatively impact on their CF or co-existing conditions. In recent years several new contraceptive methods have become available and patients seek sexual health information from a number of healthcare professionals.

Aim: The aim of this study was to determine the number of women using any form of contraception, the different methods used and the source of their sexual health advice.

Methods: A survey was performed in women attending the unit between July and November 2011. Patients were questioned during their routine clinic visit and data recorded on their electronic patient record (EMIS®). Clinical and demographic data were retrieved from computer records (age, BMI, FEV1, presence of CFRD and CFLD, *Pseudomonas* status, pancreatic insufficiency, DEXA and transplant status).

Results: Data were available in 150 (92%) women. Median (range) age 28(17-62) yrs, FEV1 62(14-113)% predicted and BMI 21.3(15.3-41.2). Of these, 132(88%) were pancreatic insufficient, 55(36%) had CFRD, 46% were colonised with *Pseudomonas* and 22(15%) post-lung transplantation. Ninety women (60%) were taking contraception, 28 (19%) did not require and 32 (21%) were not using any contraception (lower than the figure of 30-50% previously published). Reasons for not requiring contraception: 2 currently pregnant, 8 trying to conceive, 4 known conception problems, 7 no partner or were not heterosexual, and 7 post-menopausal. The most common method of contraception used in 90 women was the OCP (34%) followed by condoms (20%), depot (17%), implant (13%), coil (10%), tubal ligation (4%), hysterectomy (1%) and male vasectomy (1%). Thirteen different brands of COCP were used (12% on 20µg, 46% on 30µg and 42% on 35µg strength of ethinylestradiol). Women using the implant or depot were significantly younger (median age 21yrs and 23yrs) than those using OCP (26 yrs), coil (27yrs) and condoms (33 yrs). Fifty-three percent of women received contraceptive advice from their GP with only 19% from the CF team.

Conclusions: We report the largest review to date of contraceptive practices in women with CF. The majority of women are making active contra-

ceptive choices, however 1 in 5 are not using any contraception. Accurate documentation of contraceptive choice is thus an essential part of reproductive health counseling to highlight those at risk of an unplanned pregnancy. CF teams need training in the full range of modern contraceptive methods available.

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MOTHER-CHILD ATTACHMENT STYLE IN CYSTIC FIBROSIS - PRELIMINARY RESULTS

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Background: Cystic fibrosis (CF) is diagnosed at about two months of age at our center and causes difficulty in the early mother-child relationship and more generally in the family as a whole. Cystic fibrosis is a chronic progressive disease, still fatal to this day, and it creates an important feeling of loss in the parent's life. This could affect the style of attachment in the mother-child relationship, and it is hypothesized that it could affect the child's emotional and psychological growth characteristics.

Attachment style refers to a relational strategy, a configuration of information processing and strategy for identifying danger and responding. It is expressed in various styles according to the experiences that the child has with the reference figure: anxious-avoidant, low and high index from A1 to A3; safe from B1 to B3; anxious-resistant low and high index from C1 to C6; and mixed strategies A/C.

Aims: To estimate the attachment style distribution in situations of early important child experience.

Methods: The School-age Assessment of Attachment (SAA) was administered before the scheduled office visit to 12 children (7 males, 5 females), aged between 6 and 11 years (average age 9 years), followed at the Regional CF Center Meyer Hospital in Florence. The assessment was first explained to families by a letter that described the purpose of the study and asked for the participant's agreement.

Results: Results to date show the following distribution of different styles of attachment in the sample:

Attachment A: high index, n=3 children; low index, n=0 children

Attachment B: n=2 children

Attachment C: high index, n=5 children; low index, n=1 child

Attachment A/C: high index, n=1 child; low index, n=1 child

Conclusions: The results show that half of the sample is distributed in attachment style with high index where, as is described in the literature, there is a high risk of psychopathological disorder and an inappropriate mother-child relationship. In the sample there is a prevalence of unsure attachment with the reference figure, and deficient strategies in affective information processing (A); cognitive information processing (C); or in cognitive or affective information processing (A/C) in a different primary relationship. This preliminary study suggests there may be correlation between CF and a pathological attachment style. A future study with a larger sample and the introduction of medical variables is needed to test this hypothesis.

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I WOULD LIKE THEM TO KNOW MORE ABOUT CYSTIC FIBROSIS

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We spend a lot of time informing families and patients with cystic fibrosis about the disease, medication, treatments, complications and social aspects. In general, therefore, affected patients and families are very well informed.

We have, however, over the years often heard of the problems of informing e.g. day care centers, schools, relatives, friends and neighbours. There are articles and pamphlets, short informational DVDs available or even special visits to schools etc. Still, families are unsure of how and what to tell, and the outer circle around the family too unsure and insecure to offer help and support. How often have we not heard "I wish they knew more about CF."

We as a CF team, decided to issue invitations to an "I wish they knew more about CF" meeting. Our agenda was to give facts, to show medications

and treatments and to answer questions. We sent invitations out to all families and patients, asking them to forward the invitations on to everyone they wanted to know more about CF. As affected patients or families, they were not themselves invited to come, but anyone else was, regardless of possible relationships. We thereby thought the questions would be more open and direct as no one had to worry about how the affected patients would react.

Along with the invitation, was also a questionnaire about how often they had been thinking like this and how often they had heard, "I would be glad to help if I only knew more." They could add special wishes to be part of the agenda. We also wanted to know how many people they forwarded the invitation to, and in order to provide coffee and snacks, the number of people accepting to come.

The response and interest was overwhelming, the hall was filled and a very intensive evening started, involving the whole team.

A month later, another questionnaire was sent out to evaluate the meeting. Patients and families were all very positive and felt they had much more support and help than earlier and asked, "When will the next meeting be?"

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DIFFICULT DECISIONS: PATTERNS OF (NON) DISCLOSURE OF THE CYSTIC FIBROSIS DIAGNOSIS, MALE INFERTILITY AND USE OF ARTIFICIAL INSEMINATION BY DONOR IN MEN WITH CLASSIC CYSTIC FIBROSIS AND CF DIAGNOSED IN ADULTHOOD

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We report on a case series illustrating patterns of non-disclosure in five men with cystic fibrosis (CF) who have conceived their children using insemination by donor sperm (IDS). All five of the men are active in the parenting process. Three men were diagnosed with CF via positive sweat test in childhood, and two were diagnosed with CF in adulthood by genotype and sweat chloride levels many years after their children were born.

All three classically diagnosed patients have disclosed their diagnosis of cystic fibrosis to their children, but none has disclosed his infertility status or the decision to conceive children using IDS. While each of the three classically diagnosed patients has children who are either late adolescents or adults, none of these men are grandfathers. None of the three has reported having been asked by their children about the need to be tested for CF carrier status. One classically diagnosed male has expressed that he and his wife have begun to talk about how and when to raise the issue of IDS with their twins, who do not look like their father.

For the two men diagnosed in adulthood, the issue of disclosure of the CF diagnosis is intimately tied to their decisions not to disclose that their children were conceived via IDS. One of the adult-diagnosed patients has adolescent children, and one became a grandfather prior to his CF diagnosis. With collaboration from their spouses, both men hide not only their diagnosis but also their medications and therapies for fear that their diagnosis will be revealed to the children.

Our multidisciplinary team has employed numerous strategies to help families move toward acceptance of living with cystic fibrosis as well as to help them cope with the difficulties in disclosing information pertaining to diagnosis and infertility. The CF team has reached out to our ethics consultation service and held individual sessions and multidisciplinary meetings with the patients and spouses to explore some key questions: how will the knowledge of a new diagnosis that includes male infertility impact the parent/child relationships? What is the appropriate age to discuss the issue of non-biological parents with children and how does this impact on the child's sense of identity? What impact will the "secret-keeping" have on the family dynamic? Providing appropriate, tailored support and guidance to the patients surrounding issues of disclosure of the CF diagnosis and infertility is an evolving and ongoing challenge.

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POSTTRAUMATIC GROWTH AMONG ADULT CYSTIC FIBROSIS PATIENTS

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There has been growing recognition that some patients perceive positive life changes ("posttraumatic growth") as well as more debilitating ones in response to severe illness. Evaluation of these sequelae allows for a more complex understanding of adaptation to life-threatening disease. However, to our knowledge, posttraumatic growth and its determinants have yet to be examined among adults with CF.

Participants were adults receiving care at a regional CF center. Median age at diagnosis was 1.1 years, median age at study enrollment was 25 years, and average socioeconomic status was modest. Positive and negative life changes were evaluated with the Posttraumatic Growth and Depreciation Inventory. Consistent with Tedeschi and Calhoun's conceptual model, we anticipated that posttraumatic growth would be related to (1) more stressful appraisals of the illness, (2) increased coping through positive reframing, and (3) reevaluation of core beliefs.

On average, participants reported a "moderate" level of growth or positive changes stemming from their illness (item M = 3.8 on 1-6 Likert scale). In bivariate analyses, increased posttraumatic growth was related to increased positive reframing coping ($p = .002$) and reevaluation of core beliefs ($p = .002$). The perceived stressfulness of the illness was not significant. These variables remained significant independent predictors of posttraumatic growth in multiple regression analyses, which controlled for demographic and medical covariates (positive reframing: $\beta = .26$, $p = .03$; core beliefs: $\beta = .33$, $p = .004$).

As far as we are aware, this investigation is among the first explorations of posttraumatic growth in the adult CF population. Results suggest that some CF patients perceive positive life changes as well as negative ones in the context of their illness. Support was obtained for some though not all of the factors thought to contribute to perceived growth, providing an important foundation for longitudinal investigations.

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AN AUDIT COMPARING INPATIENT PRESCRIBED AND SIGNED DRUG CHARTS TO DOWNLOADED DATA FROM PATIENTS' NEBULISERS

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An audit was undertaken to establish what nebulised therapy is taken by children with cystic fibrosis (CF) during inpatient admissions at a paediatric regional CF centre.

Poor adherence to medication is well documented within the CF population; both the CF specialist physiotherapist and nurses anecdotally were suspicious that adherence to treatment with inpatient nebulised therapy was poor. Modern nebulisers make it possible to accurately measure when nebulisers are taken, and the duration and timings of treatment through downloadable data. An audit was therefore undertaken to compare the number of nebulisers taken by CF patients to the number of "signed" drugs on their prescription charts during an inpatient episode. Twelve patients' (7-16yrs) data and prescription charts were audited for each admission over a one year period. This resulted in 26 admissions totaling 325 inpatient days.

Overall the results highlighted poor adherence (36%) to daily drug regimes and an individual drug dose adherence of 51%. Further analysis of the data revealed only 3 of the admissions reaching adherence rates of 75% and above. The audit findings were presented to the ward managers.

The audit clearly exposed poor adherence but does not tell us why. It quite clearly demonstrated a variety of issues.

1. Ward staff are not ensuring that patients take their prescribed treatments.

2. Staff are unaware of the level of non-adherence.

3. There is an assumption that non-adherence is not an issue with inpatient care

There is need for further work to be undertaken to understand these issues. Education for both ward staff and the multidisciplinary team is being undertaken to address these issues and formulate ways in which the patients can be better supported. A follow up audit is planned.

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PREPARATION AND SUPPORT; USING CHILD LIFE SPECIALISTS DURING PICC PLACEMENT TO AVOID SEDATION

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Background: Sedation is often used for child and adolescent patients requiring PICC (peripherally inserted central catheter) placements for long term antibiotics. Sedation increases the risks, costs and time commitment associated with PICC placement. Members of the Lurie Children's Cystic Fibrosis Center's multidisciplinary team have been working to provide positive experiences for patients during PICC placement by offering Child Life (CL) support in lieu of sedation. In addition to avoiding the pitfalls of sedation, CL prepares the family, reduces anxiety and creates positive patient experiences, while promoting the use of effective coping mechanisms during future medical procedures.

Objective: To assess the effectiveness and viability of Child Life support as a substitute for sedation for patients with cystic fibrosis (CF) during PICC placements.

Design: The CF team developed a process to assess if CL support is an effective substitute for sedation for patients who would previously have been sedated. Doctors identified patients who need PICCs for long term antibiotics. The team and patient caregivers worked together to determine if a patient would benefit from PICC placement without sedation and with CL education and procedural support. These patients have generally demonstrated an ability to focus on deep breathing exercises or distraction items with support from a Child Life Specialist (CLS). Once the patient and family choose to undergo a PICC without sedation, a CLS provided pre-procedure education for the patient and family by reviewing a PICC preparation book, which includes photos and descriptions of what the child will be exposed to during the procedure. A CLS determined the appropriate timing for preparation and how to provide developmentally appropriate information for the individual patient. A CLS accompanied patients to Interventional Radiology and provided emotional support and distraction throughout the procedure.

Results: Out of 45 patients who required PICCs since January 2011, 29 most likely would have needed sedation had it not been for CL support. CL has been substituted for sedation for 20 patients since January 2011. The patient ages have ranged from 4 to 21 years old. None of the patients who have received CL support during PICC placement required sedation. Requests by patients and parents for CL support for subsequent medical procedures have increased. Patients have shown less anxiety and distress during medical procedures. In addition, the medical staff felt able to focus entirely on the procedure and completed the procedure more quickly. The patient recovery period also shortened.

Conclusion: Parents and patients at our cystic fibrosis center showed a decrease in sedation use during PICC placements when CL support was available. Having CL education and support before and during PICC placements allowed patients to have positive experiences and promoted effective coping during medical procedures, including future medical procedures. Decreasing the need for sedation is beneficial for the patient, family and medical staff. CL support will continue to be offered to patients requiring PICC placement when appropriate.

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DELAY, OMISSION AND REFUSAL SCALE (DORS); A VALID MEASURE OF ADHERENCE FOR CHILDREN WITH CYSTIC FIBROSIS

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Background: Adherence is crucial to maintain health in the paediatric cystic fibrosis population. A valid measure that records adherence to medications and airway clearance in a young population would be a useful tool for both research and clinical practice. We developed, in collaboration with CF specialists from several centres, the Delay, Omission and Refusal Scale (DORS). We refined it in focus groups with caregivers and tested inter-rater reliability (0.91). The measure uses a parent completed checklist followed by a telephone interview to improve recall and data quality. DORS focuses on adherence behaviours rather than total amount of medication taken in order to obtain a more relevant account of adherence.

Aims: To test if a high DORS score is associated with a history of adverse behaviours in children with CF.

Methods: At baseline caregivers of children aged between 3 to 13 years with a diagnosis of CF were asked about their child's mental health history and if they had ever been referred for clinical support with "CF related adverse behaviours." At a second time point parents completed the DORS. They recorded episodes of delay, omission and refusal of airway clearance, oral and inhaled medications on a checklist for two weeks. A telephone call at the end of the two weeks collated and expanded on each event to improve data quality. The raw data were converted into a 7 point scale, where 0 = total adherence, 2 = delays occurred due to child refusal, 3 = at least one dose was missed and delays from child refusal, 6 = total refusal to a prescribed medication, and 7 = total refusal to all prescribed medication.

Analysis: Children were grouped according to the presence or absence of a history of CF related adverse behaviours and Mann Whitney U-tests were conducted to compare median DORS scores, significance at 0.05 level. Odds ratios are calculated to determine the effect size of the relationship.

Results: In all, 113 caregivers reported their child's mental health status at baseline, of those 43 were followed up at the second time point and completed DORS. Fifteen of 43 children were identified as having CF related adverse behaviours. These children had significantly higher median DORS score (4.0) than children with no history (0) ($z = -3.53$, $df = 42$, $p < 0.001$). Odds ratios (OR) show that children were 6.8 (95% CI: 1.7-28.1) times more likely to have a history of CF related adverse behaviours when they scored 3 or more on the DORS scale. The OR increased to 16.3 (95% CI: 2.97, 88.9) when they scored 2 or more on the DORS scale.

Conclusions: Children with a DORS score greater than 2 are significantly more likely to have previous CF related adverse behaviours reported by their parents/caregivers. These results further strengthen the validity of this tool as a measure of adherence in children with CF.

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PREGNANCY INTENTION AMONG WOMEN WITH CF: A QUALITATIVE INVESTIGATION OF INFLUENCING FACTORS

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As the population of adults with CF continues to grow, new "adult" issues are becoming more prevalent. Among women with CF, the issue of family planning and sexual reproduction is becoming a more salient issue. According to the CFF (2011) the rate of pregnancies among women with CF continues to rise annually. A better understanding of the salient factors can inform and enhance interventions and approaches that clinical care teams provide for women with CF. This and future work can influence CF centers' policies and procedures in regards to pregnancy among women with CF.

Objective: Based upon the Theory of Planned Behavior (TPB), this qualitative study will examine a variety of behavioral influences to identify what factors influence pregnancy intention among women with CF.

Methods: Nonprobability, purposive sampling of women with CF who attend an accredited CF center in the Midwest region of the United States will occur. Semi-structured elicitation interviews will be conducted with individual females with CF who are 18 and older using an interview guide based upon the TPB constructs. A purposive sample size will be determined by data saturation. Six in-depth semi-structured interviews will be initially recorded, transcribed, and themes will be coded. Thereafter, further interviews will be collected, with transcription and coding after each interview until saturation occurs or 12 interviews are collected, whichever occurs first. Transcripts will be content analyzed to identify the constructs that contribute to the pregnancy intention of women with CF.

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RESTLESS LEGS SYNDROME, DAYTIME SLEEPINESS, AND EXECUTIVE FUNCTION IN CYSTIC FIBROSIS

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Objective: Restless legs syndrome (RLS) occurs in approximately 10% of the general population; patients with cystic fibrosis (CF) may be more at risk for RLS due to low iron and other aspects of the disease. Furthermore, disruptions in sleep may lead to increased daytime sleepiness and worse executive functioning (e.g. memory, attention). This cross-sectional, observational study examines these variables and their relationships in adults with CF.

Methods: During a regular visit, adult patients in our CF clinic completed 2 questionnaires regarding RLS: a diagnostic tool for RLS (the Cambridge-Hopkins questionnaire for ascertainment of RLS) and a 10-question rating scale used to assess severity (the International Restless Legs Syndrome Study Group Rating Scale for RLS). In addition, patients completed the Epworth Sleepiness Scale (ESS) and the Behavior Rating Inventory of Executive Function (BRIEF). Medical record review was conducted to obtain demographic characteristics and lung function.

Results: Participants included 38 adults (52.6% male) who ranged in age from 19 to 69 years ($M = 33.90$, $SD = 12.69$). Disease severity, as measured by forced expiratory volume in one second (FEV_1) percent predicted, was moderate on average ($M = 49.67$, $SD = 20.01$) but ranged from severe ($FEV_1 = 22$) to normal ($FEV_1 = 97$).

RLS criteria were met for 4 out of 38 participants (11%). ESS scores were elevated (ESS score > 10) in 4 out of 33 participants (12%), which is consistent with a prevalence of 10-20% found in the general population. More daytime sleepiness on the ESS was related to more deficits in Working Memory on the BRIEF ($r = .36$, $p < .05$). RLS symptoms were not associated with sleepiness on the ESS or executive function on the BRIEF; however, RLS symptoms in our CF population were only mild.

Conclusions: We found similar rates of RLS and daytime sleepiness in our adult CF sample, compared to the general population. As expected, increased sleepiness was associated with worse working memory. Further research is needed to determine the relationship between RLS and CF in the general CF population.

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DIRECT OBSERVATION OF CHILD AND PARENT BEHAVIOR DURING CF RESPIRATORY TREATMENTS: WHAT LEADS TO IMPROVED ADHERENCE?

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Background: Chronic lung infections are the leading cause of morbidity and mortality in cystic fibrosis (CF). Although effective respiratory treatments exist to prevent and treat lung infections, adherence to these treatments is suboptimal with estimates at 49%. Examination of barriers to adherence has identified child oppositional behavior as playing a key role. However, to date, no studies directly observing parent-child interactions while children are performing respiratory treatments have been conducted. The objective of this study was to examine parent and child behavior during individual respiratory treatments in their home environment to determine what factors lead to improved adherence. It was expected that parents who provide specific directions and a positive atmosphere during respiratory

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treatments would have children who were more compliant during those treatments and were more adherent to their respiratory medical regimen over three months.

Methods: Participants included 15, 6-12-year-old children diagnosed with CF observed performing their nebulized medication and oscillating vest treatment on three different occasions in their home environment. These interactions were videotaped and coded using a standardized coding method. Percent adherence was measured through oscillating vest time use and six 24-hour recalls of respiratory adherence collected over three months. An overall respiratory adherence percentage was calculated by averaging these two scores. Pearson product-moment correlations and a t-test were used to analyze data.

Results: Preliminary data analysis was conducted on 10 participants (6 boys, mean age=8.50, SD=1.96). As hypothesized, parent praise and direct instructions were positively correlated with child compliance during individual treatments ($r=0.69$, $p<.05$; $r=0.79$, $p<.01$, respectively), and parent criticism and indirect instructions were positively correlated with child non-compliance ($r=0.93$, $p<.01$; $r=0.93$, $p<.01$, respectively). The average three-month adherence rate for respiratory treatments was 72.25% (SD=24.86, Min=26.38, Max=96.80). Child compliance during observed respiratory treatments was significantly correlated with three-month adherence rates ($r=0.72$, $p<.05$). Parent praise was not correlated with three-month adherence rates, but there was a trend for parent direct instructions to be positively correlated with adherence rates ($r=0.63$, $p=.07$). Parent criticism and indirect instructions were not correlated with three-month adherence rates. Parents who were present in the room with their children during an individual respiratory treatment were found to have children with higher three-month adherence rates (mean=89.70%) than parents who were only intermittently present (mean=50.45%; $t(7)=-3.97$, $p<.01$).

Conclusion: Results suggest that parents who are present with their children and provide a positive atmosphere during respiratory treatments have children who are more cooperative during individual treatments and have higher respiratory adherence rates over three months. Information gathered has the potential to directly identify parent and child behaviors to target in future behavioral interventions designed to improve child adherence to respiratory treatments.

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EFFECT OF SNOEZLEN ON PAIN PERCEPTION AND ANXIETY IN CF INFANTS DURING SPUTUM SUCTION

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Background: The CF clinic visit is of paramount importance in ongoing CF care and the CF team aims to make it a safe haven for CF families. However, patients often require painful procedures e.g. oropharyngeal suctioning for sputum for culture. In young children, pain and anxiety are closely interrelated and CF infants subsequently develop a generalized anxiety response related to clinic visits, thus lowering their pain threshold and increasing suffering.

The Snoezlen approach provides a controlled, multi-sensory environment combining gentle lighting and special visual effects, calming music and vibrations. The method has been proven to improve quality of life in subjects suffering from anxiety, pain and restlessness.

Aim: To evaluate the effect of a controlled, Snoezlen environment within the pulmonary and CF clinic on the response of infants and their parents to an oropharyngeal suction procedure for obtaining induced sputum.

Subjects: Non-expectorating infants ages 0-3 years with CF or other chronic pulmonary disease requiring repeated oropharyngeal sputum suction for bacterial culture.

Methods: Snoezlen equipment was introduced into the pulmonary and CF clinic. This included oil and bubble column with changing colors, a vibro-acoustic armchair, and a projected rotating sea images wheel for the ceiling. Room lighting was dimmed. CF team members underwent specialized training for using the Snoezlen system.

Patients had sputum suction repeated twice, when clinically required, at two separate visits, and were randomized to have either the first or second visit within the Snoezlen environment, and the other visit under usual hospital conditions (the system was not switched on).

Patients were monitored by measuring pulse, oxygen saturation, FLACC score for pain and anxiety, questionnaires evaluating response of parents.

Results: Preliminary results to date are available for 6 patients (2 females, 4 males). Paired comparison of parental response indicated a significantly more pleasant experience with the Snoezlen environment compared to without ($p=0.04$). Parents had a lower mean score out of 5 for perception of a traumatic experience (2.67) compared to untreated (4.67) ($p=0.04$). In both groups, FLACC pain scores decreased similarly from a high at the beginning of the procedure (1.17 untreated and 1.33 among treated) to 0.67 and 0.5 after 5 minutes, 0.17 and 0.5 after 10 to 15 minutes, to 0.2 and 0 after 35 minutes.

Conclusions: Preliminary results suggest that the Snoezlen system may have an important role in ameliorating the unpleasant procedure of sputum suctioning for infants and their parents in the CF clinic, enabling infants and parents to have a more positive clinic experience. This research is on-going.

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DEPRESSION, ANXIETY, AND SUICIDE SCREENING IN CYSTIC FIBROSIS: PATIENT ACCESSIBILITY TO MENTAL HEALTH TREATMENT

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Objective: Mental health is a crucial component of comprehensive care for patients with cystic fibrosis (CF). Elevated levels of depression and anxiety have previously been associated with declining respiratory function, lower quality of life, and reduced therapeutic regimen adherence in patients with CF. Our objective is to report on a pilot study assessing the feasibility of a mental health screening program initiated within the CF center at Children's Hospital Boston. Two critical components of this study include: (a) suicide screening, and (b) providing mental health referrals and assessing participant ability to engage in outpatient (OP) referrals to mental health treatment.

Methods: Participants ($n = 36$) were consecutively admitted patients (8 years and older) receiving inpatient medical care at Children's Hospital Boston from November 2011 - April 2012. Patients in the age group of 8 - 17 years ($n = 18$) were administered the Children's Depression Inventory (CDI) and Multidimensional Anxiety Scale for Children (MASC). Adult participants ($n = 18$) completed the Patient Health Questionnaire-9 (PHQ-9; a depression diagnostic measure), and the Beck Anxiety Inventory (BAI). Patients reporting suicidal ideation or intent on either the CDI or PHQ-9 underwent immediate risk assessment by the study principal investigator. Participants were also assessed at a post-discharge appointment or via telephone to determine if mental health treatment (i.e. outpatient psychotherapy/pharmacology, or other advanced level of care) was accessible and initiated after screening.

Results: Participants were 52.8% female (19/36), had a mean age of 19.3 years (SD = 7.6; range of 8 - 47) and were almost all Caucasian. Patients who underwent screening represent 52.2% (36/69) of the total patients approached for participation: 12 declined, 15 were missed during their admissions, 3 were of acute status, and 3 could not provide parental consent. In addition, 3 were excluded due to a documented developmental delay. Child participants reported a mean CDI score of 8.0 (SD = 6.2) out of a possible 27, with 4 patients reporting suicidal ideation ("I think about killing myself but I would not do it"). This constitutes 11% of the total screened population. Children reported a mean MASC score of 42.7 (SD = 16.1), and six participants reported clinically significant anxiety symptoms, having a t-score of 65 or above on the MASC total score or one of the subscales. The mean PHQ-9 summed score for adults was 3.7 (SD = 2.6) out of a possible 27. The mean anxiety score reported by adults on the BAI was 8.4 (SD = 7.7) out of a possible 61, with a clinically significant score of 36. Of those screened, 4 patients were given referrals for OP providers based on their mental health screening scores. In addition, 11 patients had previously received referrals as part of their care. Further analysis will compare mental health screening results with patients' BMI z-scores, FEV1 results, and psychiatric history to assess feasibility of obtaining follow-up mental health care within the context of patients' health-related outcomes.

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AN ONLINE SOCIAL NETWORKING COMMUNITY FOR ADOLESCENTS WITH CF: QUALITATIVE ANALYSIS OF ONLINE ACTIVITIES

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Objective: Social support from peers with the same chronic illness has been shown to have significant positive psychological and health benefits for the adolescent patients. However, teens with cystic fibrosis (CF) have limited opportunities for face-to-face support because of infection control guidelines. The current study evaluated peer support obtained via CFfone, an online social networking community, in terms of activities and supportive exchanges among participants over a 2 week period.

Methods: Participants were website users ranging in age from 11 to 18 years (n=50, 64.7% females). One hundred threads posted on the website in August 2011 were coded with ATLAS.ti. The following themes were examined: 1) types of social support provision (emotional support, informational support, instrumental support, companionship support), 2) types of support requested (same as above), and 3) topic being discussed (CF-related vs. general, non-CF related) during supportive exchange. Use of other website features (private messaging, reminders, educational articles, and Ask Kate) were also examined.

Results: Preliminary results indicated that companionship support (e.g. sense of belonging) was the most frequent type of support both provided and requested on the website, followed by emotional support. Companionship support was provided and received most frequently in relation to non-CF topics, whereas emotional support was provided and received primarily in relation to CF topics. The three most frequently discussed topics were: 1) greetings (e.g. hello, goodnight), 2) health (e.g. coughing), and 3) leisure activities (e.g. drawing, shopping). Results indicated that more website users engaged in posting comments on their own and other users' walls (about 54% of users; mean number of comments posted per user = 14.74, SD=18.29) than utilizing the other website features; private messaging (26% of users; M=8, SD=7.43), reminders (14% of users; M=17.57, SD=10.78), and reading educational articles (6% of users; M=1, SD=0). The "Ask Kate" feature, which allows users to see frequently asked questions and its responses, was the least used feature of CFfone (0% of users).

Conclusions: Findings suggested that CFfone may be a promising intervention for exchanging peer-to-peer support. Adolescents appeared to like and use the posting features more than the educational ones. Future analyses will document the changing patterns of communication on CFfone, over an academic year and as new users join the online community.

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PARTICIPATION IN ADULT CF SUPPORT GROUP IMPROVES PATIENT PERCEPTION OF PATIENT-PHYSICIAN RAPPORT

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Introduction: Patient support groups provide an open forum that offers adult patients the opportunity to voice their opinions and concerns in discussion with other adults with cystic fibrosis. At our center, representatives from the CF care team are present to assist in facilitating the discussion as well as to clarify information. In the support group, patients discuss how CF affects their lives and share their personal experiences. For some patients, this may be their first opportunity to have a face-to-face discussion with another adult with CF. We postulated that this opportunity to talk with other patients may improve individual confidence and level of comfort in communicating with the CF care team. Thus, we sought to evaluate whether participation in adult CF support group improves patient perception of patient-care provider communication and rapport.

Methods: We surveyed current participants in the support group for adult CF patients at our center. For comparison, we also surveyed patients from our adult CF program who did not participate in the support group. In the survey, we presented 10 statements related to various aspects of patient comfort regarding communication with others regarding their disease. All

patients were asked to respond on an ordinal scale from strongly disagree to strongly agree. The statements queried were the following:

- 1) I feel comfortable discussing medical options for my care with my CF team.
- 2) I feel comfortable asking questions at clinic about my general health.
- 3) I feel comfortable asking my CF team about reproductive health-related questions.
- 4) I feel comfortable expressing disagreement with my physician or other health care providers.
- 5) My CF team listens to me and addresses my concerns.
- 6) I feel comfortable reaching out to other patients in the CF community.
- 7) I know about resources available for CF patients.
- 8) I am knowledgeable about my disease and management.
- 9) I feel empowered in taking control of issues related to my CF and health.
- 10) I have a good relationship with my CF team.

Conclusions: Patient perception of their relationship with their CF team is likely altered by participation in the support group. Analysis of data collected from the survey administered to participating and non-participating patients in the support group will provide insights into patients' perceptions regarding their care and patient-physician rapport. Participation in a dedicated adult CF support group likely fosters patient confidence and perceived knowledge as well as improves overall interaction with the CF program. Support groups are feasible venues for CF patients to come together and discuss issues with their peers.

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INFECTION CONTROL KNOWLEDGE: IMPACT OF KNOWLEDGE ON ADHERENCE

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Health-related information helps individuals cope with chronic illnesses by increasing knowledge, reducing uncertainty, and improving emotional and social adjustment (Carpenter et al., 2010). However, according to Ullrich (2007) a significant number of parents of children with CF were dissatisfied with infection control (IC)-related education provided by health professionals. Gaining a better understanding of patient/family knowledge of IC Guidelines and the relationship between knowledge and adherence is important as research has noted suboptimal understanding of IC guidelines among health care professionals and patients/families (Miroballi et al., 2011). To date, no studies have been conducted that explore the relationship between parent and child knowledge of IC guidelines and adherence behaviors. Researchers have demonstrated a link between parent nutritional knowledge and child adherence in CF (McDonald et al., 2009), but the relationship between parent knowledge and youth adherence is somewhat contradictory in other chronic illness populations (Wysocki et al., 2011). This study attempts to examine the relationship between parent and parental IC knowledge scores to determine if the extent of agreement (as a proxy for parent/child communication) has an impact on IC adherence.

Method: Participants were recruited from a Midwestern, urban regional CF center. Written and verbal assent was obtained from all children and written consent was obtained from primary caregivers. Approval was obtained from the Institutional Review Boards and data was collected in compliance with APA ethical standards. Questionnaires assessed demographic data, treatment adherence, and IC knowledge. Participants were 36 children/adolescents with CF between the ages of 8 and 18 years (range: 8-18, M = 13.33) and a primary caregiver (range: 29 - 51 years, M = 41.19); 45.5% were female. Most parents had completed high school, with 51.6% having completed at least some college.

Results: Mean IC knowledge scores (range 0-12) for parents and youth were 9.44 (SD = 1.74) and 6.69 (SD = 3.26), respectively; mean scores on the IC knowledge questionnaire were significantly discrepant between parents and children ($t(36) = 4.736, p < .001$), with parents attaining higher scores than children. Next, adherence was inversely associated with youth IC knowledge such that youth with CF who were nonadherent had lower IC knowledge scores ($r(36) = -.339, p = .023$). Age related differences in knowledge and adherence were also found in the sample. Finally, knowl-

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edge scores were examined between parents and children; total IC knowledge scores that differed by more than 30% were categorized as “disagreement” and the remaining dyads were categorized as “agreement.” Agreement was significantly associated with IC adherence such that greater parent/child agreement was associated with pro-adherent behavior ($r(36) = -.362, p = .03$).

Conclusions: Results suggests that particularly for children/teens, knowledge of IC guidelines is inadequate. Moreover, data are suggestive of a relationship between IC knowledge and adherence and potentially transmission of IC knowledge between parent and child as a positive contribution to IC adherence.

NURSING ISSUES

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A CF CENTER NURSING INTERVENTION TO IMPROVE INFLUENZA VACCINATION RATE IN CYSTIC FIBROSIS PATIENTS

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Objective: To improve the influenza vaccination rates of the CF center's CF patient population.

Background: The CF Foundation and the CDC recommends everyone 6 months of age and older receive a seasonal influenza vaccine. Vaccination is especially important for children with CF who are at higher risk of morbidity from influenza, including those younger than 5 years of age. Despite these recommendations, many CF children may not receive an influenza vaccination in a timely manner. Nurses play a key role in identifying CF patients and ensuring access to vaccination during CF ambulatory clinic visits.

Methods: A retrospective review of CF patients receiving influenza vaccinations from April 2008 to April 2010 was completed. The CF center developed a nurse-led quality improvement initiative aimed at increasing rates of influenza vaccination in our CF population. A care map of clinic flow and process was reviewed. Letters as well as emails were sent to CF patients and their families informing them of the benefits of influenza vaccination. The influenza vaccine was made available to CF patients in clinic. A clinic tool was created to improve documentation of influenza vaccination.

Results: We reviewed the influenza vaccination rates at the CF center. We found that 25.2% (138/547) of patients were vaccinated from 4/1/2008 to 3/31/2009, and 19.1% (111/580) were vaccinated from 4/1/2009 to 3/31/2010. The first year we implemented our initiatives (4/1/2010 to 3/31/2011), the percentage of CF patients vaccinated increased to 73.9% (411/556). The second year of the initiative (4/1/2011-3/31/2012), the results remained high at 78.2% (457/584).

Conclusion: Our nursing led initiative enabled a more timely and efficient administration and documentation of influenza vaccinations to our CF patients. Nurses can improve the influenza vaccination rate in CF patient population thus decreasing severe complications related to the influenza in CF patients.

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INTEGRATING BEST PRACTICES FOR SMOKING CESSATION INTO THE UNIT NURSE'S ROLE

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The respirology unit at St. Michael's Hospital has 15 beds for adults with cystic fibrosis and other lung diseases. For CF patients, smoke exposure impairs lung function and development and may disqualify listing for lung transplant. Smoking rates by CF patients may be as high as 8-21% (Flume, 2009). Most smokers want to give up smoking. The acute care environment is smoke-free and can boost motivation to quit. While nurses can provide information, advice and emotional support to enable quitting the staff nurses at our centre were not practicing at their optimal level.

Objectives: The purpose of the project was to improve patient access to support for quitting smoking. Unit RNs would be prepared to assess and counsel smokers and have standardized mechanisms to inform the multidisciplinary team about patients' smoking status and readiness to quit.

Method: An implementation team was recruited from staff nurses in order to identify gaps in knowledge and resources. Following a baseline survey of nursing practice, knowledge and self-efficacy, nurses received brief education sessions about the optimal process for inpatients. We established a partnership with the Smokers' Helpline to ensure continued support after discharge. Two clinic nurses were trained in intensive tobacco cessation counseling and several inpatient nurses attended one day workshops in smoking cessation. The admission chart was organized to promote completion of the intake form. We used pocket cards to remind nurses of the process and distributed bedside signs to prompt smokers to initiate discussions with their nurse. When initial rates of nursing participation dropped, nurses were reminded to complete the assessment tool and asked to complete a booster session on the minimal intervention process.

Results: An initial survey showed that only 26.3% of nurses usually discussed smoking with patients due to concerns about distressing patients and perceived limitations of the nurses' role. Post-education this number increased to 55% while the number of nurses reporting sufficient training increased from 8.6% to 62.5%. Documentation in the existing electronic system did not initially show evidence of interventions. The charting process was improved to include a specialized smoking assessment tool to guide intervention and documentation. Recent audits on the written tool revealed nurses now advise, assess and assist smokers at least 50% of the time and are referring for followup at least 25% of the time.

Discussion: The current project gave us an opportunity to optimize the role of nurses and fill a gap in the provision of care. The documentation tool boosted evidence of nursing participation while bedside reminders promoted nurse-client interaction. The small patient population, short hospitalization stays and reluctance to report smoking presented challenges to data collection and analysis. Future plans to improve patient care include communicating about smoking between the inpatient and outpatient areas, optimizing the use of our trained nurses and increasing the number of referrals we make to the Smokers' Helpline and clinics.

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ANNUAL HEALTHCARE COSTS FOR PATIENTS WITH CYSTIC FIBROSIS FROM A MEDICAID PERSPECTIVE

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Objective: To assess the annual healthcare costs for patients with cystic fibrosis (CF) in a Medicaid population.

Methods: Patients (≥ 6 years) with a CF diagnosis and ≥ 18 months of continuous eligibility were identified from the MarketScan® Medicaid database (1/2005 to 12/2010). Annual healthcare costs incurred by CF patients were measured during a 12 month study period and expressed in 2010 USD. Total healthcare costs were compared across subgroups of CF patients, defined by age, gender and history of hospitalization in the 6 months preceding the study period, using Wilcoxon rank-sum tests.

Results: Among 1,731 patients with CF, the average age was 17.9 years, 50.7% were female and 27.2% were hospitalized within 6 months prior to the study period. Mean total healthcare costs (medical service and pharmacy) to Medicaid were \$37,328 $\pm$ 58,182 per patient per year, with medical service costs accounting for 55% of total healthcare costs (Table 1). Mean total healthcare costs varied significantly across age, gender and hospitalization history. As compared to patients aged 6-10 years (\$29,076 $\pm$ 43,179), costs incurred by patients aged 18-34 (\$45,045 $\pm$ 69,998, $p < .001^*$) and 11-17 (\$43,610 $\pm$ 62,982, $p < .001^*$) years were significantly higher whereas costs incurred by those aged 35-64 (\$21,382 $\pm$ 32,541, $p = .104$), and ≥ 65 years (\$6,365 $\pm$ 7,427, $p = .020^*$) were lower. Mean costs were higher in males relative to females (\$38,765 $\pm$ 56,127 vs. \$35,932 $\pm$ 60,111, $p = 0.011^*$) and in patients with at least one hospitalization during the baseline period relative to those without hospitalizations (\$72,160 $\pm$ 83,898 vs. \$24,307 $\pm$ 37,421, $p < 0.001^*$).

Conclusions: The annual healthcare expenditure for a person with CF in Medicaid averaged \$37,328 in 2010 USD. Medical service costs made up more than half of the total expenditures for people with CF.

Supported by Novartis Pharmaceuticals Corporation.

Table 1: Annual All-Cause Healthcare Costs Incurred by CF Patients

Costs	Mean $\pm$ SD
Total medication costs	16,627 $\pm$ 21,473
Medical service costs	
Inpatient hospitalization costs	13,631 $\pm$ 41,549
Outpatient costs	3,133 $\pm$ 7,645
ER costs	227 $\pm$ 818
Home health costs	3,710 $\pm$ 19,082
Total medical service costs	20,700 $\pm$ 48,943
Total healthcare costs	37,328 $\pm$ 58,182

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INCREASING CF PATIENT VISITS WITH ENDOCRINOLOGY BY ESTABLISHING COMBINED CLINICS

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A multi-specialty team of practitioners from the local pediatric CF Centers participated in the CFF sponsored CFRD Collaborative that was held from fall of 2010 to fall of 2011. During the collaborative the team mapped out an ideal process for testing, diagnosing and management of CF related diabetes. The team identified areas needing improvement. One of these was the limited number of patients who had been diagnosed with CFRD who were routinely seen by an endocrinologist. Another was lack of follow up information given to families regarding results of annual oral glucose tolerance tests. As a result of the team's recognition of problems with care and follow up, a combined Endocrine and CF clinic was developed. This combined clinic began in November of 2011 following the completion of the CFRD collaborative. Twice a month, a pediatric endocrinologist attends CF clinic. Her presence in CF clinic has led to several improvements. In the seven months that she has been embedded in CF clinic, she has had 56 patient encounters. Prior to this, 67 patient encounters occurred outside of CF clinic in a one year period. With this rate of visits, she will see 70% more patients in CF clinic than seen outside CF clinic at the completion of the first year. Her presence in CF clinic has also broadened the scope of the endocrine visits. The visits have included meeting with patients with CFRD, meeting to discuss results of the annual Oral Glucose Tolerance Test (OGTT), meeting regarding bone density issues and meeting to make recommendations related to growth and/or puberty. Prior to her presence in CF clinic, patients had primarily been seen only following a diagnosis with CFRD. Very few patients were seen following a diagnosis of osteoporosis or growth related issues. No patients were seen to review results of the OGTT if the test was normal or indeterminate. The CFRD consensus statement recommends that patients with CFRD have four visits annually with an endocrinologist. Prior to the collaborative, none of our patients met this recommendation. We believe that having an endocrinologist in CF clinic will lead to a dramatic improvement in these visits and we will follow this closely as we near the end of the first year. We hope to expand the presence of the endocrinologist in the CF clinic. Currently, she is present on the days when four of the CF pulmonologists see patients, but not on the days that two other CF pulmonologists see patients. We also hope to have the ability to perform point of care testing of HgbA1C in the CF clinic. This will have a direct impact on care management. Our team is currently collecting satisfaction data related to patient and parent perception of the value of seeing an endocrinologist during CF clinic.

Our team's involvement in the CFRD collaborative led to changes in the way we provide care for patients with multiple endocrine issues. With an endocrinologist in CF clinic, we have been able to increase the number of patients seen, increase the frequency of visits for patients with CFRD, broaden the number of issues addressed and provide a proactive, preventive medicine approach to care. We believe that satisfaction with this approach is high, but are currently gathering satisfaction data.

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IMPROVING NURSING STAFF EDUCATION REGARDING CYSTIC FIBROSIS

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Background: Cystic fibrosis (CF) education is important for inpatient nursing staff because pulmonary treatments and care of multisystem manifestations of CF (i.e. pancreatic insufficiency or CFRD) are of utmost importance on the inpatient unit. Prior to this project, education sessions had been prepared for the nurses by the multidisciplinary CF staff while the nursing staff provided care on the inpatient unit. However, these sessions have been rescheduled several times due to patient care demands. Due to this dilemma, we included the inpatient nursing staff in our CF Family Education Day to provide additional instruction.

Methods: In 2009, inpatient nursing staff from our regional adult and pediatric CF centers attended CF Family Education Day. Eight nurses were present. Their nurse manager provided them with education hours for this 5 hour session. The program was free to both parents and nursing staff. In addition to attendance at this event, select nursing staff members also attended the 2010 NACFC. After attending the meeting, the nurses and the multidisciplinary CF care team presented the information learned from the conference to the hospital CF Family Advisory Council and their nursing colleagues. Given the positive response in the past, a 4-item pre-conference survey on CF issues was administered to assess CF knowledge prior to attendance at the next CF Family Education Day. Added incentive was provided with nursing contact hours to encourage attendance.

Results: Inpatient nursing staff attended the 2012 CF Family Education Day, which included topics such as an overview of CF care, Port CF Registry center data, transitioning to adult care and a parenting skills workshop. Survey responses showed that ongoing CF specific education is beneficial. For example, the nursing staff underestimated the average life expectancy of patients with CF. However, following inclusion at CF Family Education Day and the 2010 NACFC, the pediatric inpatient nursing staff has identified ongoing opportunities for education and improvement on the inpatient unit regarding CF care. Quality improvement initiatives such as evaluation of the time it takes to initiate inpatient treatments and accuracy of care are being studied. Together, these endeavors have fostered better collaboration with the inpatient nursing staff in addition to development of new multidisciplinary projects.

Conclusion: Including the inpatient nursing staff in the CF Family Education Day was instrumental to increasing their knowledge regarding CF care and integrating them as contributing members of the CF team. The inclusion of the inpatient nursing staff has also fostered quality improvement projects specific to the care of patients with CF.

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TELEMEDICINE TO IMPROVE ACCESS TO SPECIALIZED CARE FOR PATIENTS WITH CYSTIC FIBROSIS

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The Toronto Adult Cystic Fibrosis (CF) Program at St Michael's Hospital was established in 1992 and is North America's largest adult CF program. Two satellite CF clinics were established for patients in northern Ontario communities hundreds of miles away. Frequent follow up in multidisciplinary CF clinics is known to improve patient outcomes. However, satellite clinics do not allow access to the entire multidisciplinary team, as reimbursement is limited for expenses related to physician visits. For appointments at the host hospital scheduling limitations such as accommodating patients' work or school responsibilities, availability of space, the CF physician's schedule, and inclement weather frequently led to last minute clinic cancellations.

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Objectives: The main goals of this initiative were to improve patient care and education by utilizing videoconferencing. Patients would come to Toronto once a year, attend satellite clinics twice a year and have a telemedicine appointment annually.

Methods: In July 2010, monthly telemedicine multidisciplinary CF clinics were established to address the barriers that impede access to care. Patients were interviewed by the local nurse and spirometry and bloodwork were done. Results were sent by FAX to the Toronto clinic and the patients were able to see team members by video link.

Results: The telemedicine conference visit was used in two major ways - for routine clinic visits and for episodic illness. To date, there have been 71 patients seen by telemedicine conference with 22 monthly clinics held and 10 patients seen outside of the monthly clinics. As time passed clinicians became more experienced with the format and used the telemedicine link in increasingly diverse ways. Several telemedicine clients in remote locations were cared for on home intravenous therapy. One ventilated client was being considered for transplant while hospitalized 800 miles away and was monitored and coached via telemedicine by both the CF and transplant physicians. For a newly diagnosed client the CF doctor used telehealth to explain how to use enzymes. The nurse practitioner was able to present diagrams of the pathophysiology of cystic fibrosis by video for the patient to see. The physiotherapist used telemedicine to demonstrate correct technique for airway clearance. The mother of a CF child commented that the drive to the telehealth studio took 15 minutes compared with a 10 hour trip to the Toronto clinic. One patient explained that the trip to Toronto meant interrupting her life-sustaining therapies including masks, physio and exercise.

Discussion: Telemedicine improved patient care, reduced patient costs and improved inter-professional collaboration. We were able to strengthen relationships with community partners by incorporating visits with local care teams. The telemedicine visits express our client-centred approach to care by maintaining interprofessional collaboration with the CF team while saving the client time and travel costs. Future plans include continuing to promote attendance at telehealth and possibly considering specialized clinics such as CFRD clinics that could be held via telehealth.

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ESTABLISHING THERAPEUTIC RELATIONSHIP BETWEEN NURSES AND PATIENTS TO REDUCE ANXIETY AND FEAR DURING HOSPITALIZATION

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The respiratory unit at St. Michael's Hospital has 15 beds for adults with cystic fibrosis and other lung disease. However, majority of the admissions are patients with cystic fibrosis at any given time. Most of them are admitted due to pulmonary exacerbation for treatment of IV antibiotics. However, other medical interventions including desensitization to antibiotic, steroid therapy, insulin regimen, oxygen or Bipap may also be initiated. Recent study from Germany involving 670 patients range from 12 to 64 demonstrated that anxiety score were found in 20.6% of patients with CF and recent medical events i.e. hemoptysis or diagnosis of diabetes were associated with anxiety. We too have observed a degree of anxiety and fear in our hospitalized patient as measured by Picker Scores. As part of our corporate initiatives for quality improvement, our ward nurses took on the task to reduce anxiety and fear in our hospitalized patients by utilizing Best Practice Guideline of Establishing Therapeutic Relationships to ease anxiety and fear.

Method: Pre and post-education surveys to assess nurses' knowledge and comfort level in dealing with patients' anxiety were administered to >80% of nurses on the ward. A one-hour, interactive education session about how to establish therapeutic relationship and professionalism in nursing was provided. Posters were put in patient rooms to remind patients of the nurses' interest to listen, counsel, address or communicate issues of anxiety and fear. Fifteen patients completed a survey to identify the factors that cause the most anxiety and fear during their hospitalization and these factors were printed on a pocket cards and distributed to all nurses to remind them to address the issues identified. Tape-reporting as a mean to communicate between nursing shifts were replaced by the transfer of accountability (TOA) method, bedside reporting between nurses with patient participation.

Picker Scores, chart audits and a unit-specific patient satisfaction survey were used as outcome measures of improvement.

Results: Patient identified 10 major factors causing anxiety and fear with separation from home and learning new information about their health being the most anxiety provoking. Chart audit revealed that more nurses addressed anxiety and fear after implementation of the project an increase from 75% to 95% of charts. Patient expressed that TOA provided good communication and opportunities to discuss their fear and anxiety during hospitalization. The ward Picker Score increased from 35% in 2010 to 86% in 2011 with higher scores indicating patients' felt their anxiety and fear is being addressed.

Conclusion: Patients experience fear and anxiety during the hospital stay and establishing therapeutic relationship between nurses and patients helps ease these concerns.

EDUCATION

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PARENTS OF HISPANIC CYSTIC FIBROSIS PATIENTS HAVE REDUCED HEALTH LITERACY: A SINGLE CENTER PILOT STUDY

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Background: Reduced parental health literacy has been associated with negative health behaviors and outcomes across several pediatric conditions, yet has not been previously studied in CF. CF patients of Hispanic ethnicity have worse pulmonary function and survival. The objective of this pilot study was to assess health literacy in parents of Hispanic and non-Hispanic children with CF. We hypothesized that parents of Hispanic children with CF would have lower health literacy than parents of non-Hispanic CF children.

Methods: This cross-sectional study enrolled 25 parents of Hispanic children with CF and 63 parents of non-Hispanic CF children in a convenience sample; this represented 84.6% of our eligible pediatric population. Health literacy was assessed using the Short Test of Functional Health Literacy in Adults (STOFHLA), a standardized instrument available in both English and Spanish versions. Health literacy scores and categories were compared by child's Hispanic origin as well as additional socioeconomic and demographic parameters.

Results: Eighty-four of 88 parents enrolled in the study (95.4%) demonstrated adequate health literacy. While there was a trend for parents of Hispanic CF patients to attain lower health literacy scores than parents of non-Hispanic CF patients, the difference in the mean score of each group was not statistically significant ($P=0.17$). Nonetheless, parents of Hispanic children were significantly more likely to have inadequate or marginal health literacy (16.0%) than were parents of non-Hispanic children (0.0%), $P=0.005$. Further investigation revealed that the parent's primary spoken language may be an important factor underlying this disparity, as Spanish-speaking parents attained significantly lower health literacy scores than English-speaking parents ($P=0.02$) and were far more likely to have inadequate or marginal health literacy (26.7% versus 0.0%), $P=0.001$.

Conclusions: While health literacy was largely adequate among parents of children with CF at our center, parents of Hispanic children were significantly more likely to have inadequate or marginal health literacy skills than were parents of non-Hispanic children. The proportion of individuals with inadequate or marginal health literacy was particularly high for parents who speak Spanish as their primary language, despite the opportunity for participants to take the assessment in their language of choice. Although multicenter studies are required to confirm our findings, further investigations should examine whether differences in parental health literacy contribute to outcome disparities between Hispanic and non-Hispanic CF patients. As predictors of limited health literacy, Hispanic origin and Spanish primary language could also be incorporated into future efforts to screen for this potential barrier to management of CF and to develop interventions for this high-risk population. This study was supported by the 2011 Northwestern University Feinberg School of Medicine Medical School Summer Research Program.

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DIABETES IN CYSTIC FIBROSIS – EDUCATION (DICE) – THE DEVELOPMENT OF A STRUCTURED EDUCATION PROGRAMME FOR THE MANAGEMENT OF CFRD

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Background: Diabetes is the most common secondary complication (co morbidity) in CF. In addition to the microvascular complications associated with diabetes itself, cystic fibrosis related diabetes (CFRD) is associated with an accelerated decline in lung function, weight loss and reduced survival by up to 10 years. Routine screening followed by early diagnosis and treatment has improved survival. DAFNE® (dose adjustment for normal eating) is a structured training programme of flexible intensive insulin management enabling dietary freedom which leads to improvements in glycosylated haemoglobin (HbA1c) and quality of life in type 1 diabetics. In the UK there are currently no structured education programmes for adults with CFRD and formal carbohydrate counting does not form part of routine CFRD management. To address this we have developed the Diabetes in Cystic Fibrosis – Education (DICE) programme. With permission from the DAFNE® collaborative the DAFNE® programme and resources have been adapted specifically for the management of CFRD. Patients with CFRD have been involved in the development of the materials to ensure acceptability and understanding. The course was changed from a 5 day group course to a 1:1 course which involves 4 sessions over a 6 week period. The aim of this pilot study is to determine whether the introduction of an education course teaching patients flexible intensive insulin treatment to allow dietary freedom can improve diabetic control and quality of life for patients with CFRD.

Method: Adult patients with CFRD and an HbA1c > 53 mmol/mol (7%) are eligible to participate. The course is delivered by 2 educators with support from 2 CF consultants and a DAFNE® approved diabetologist. All members of the DICE collaborative have received formal DAFNE® training. Patients attend 4 - 3 hour education sessions over a 6 week period and are taught the principles of carbohydrate counting and insulin dose adjustment as part of the programme. At recruitment, 6 and 12 months the following data is recorded – lung function, weight, height, BMI, random glucose, HbA1c, CGMS (6 days) and completion of CFQ-R, ADDQoL, DTSQ and diabetes self-efficacy questionnaires.

Results: Twelve patients have been recruited to date. Four have completed the study, 8 are in progress and recruitment continues across both sites with the aim of enrolling 30 patients. Preliminary data have identified some interesting observations; improved self efficacy in the overall management of CF and diabetes, improvements in QOL scores, better understanding of CFRD and a commitment to continue with DICE CFRD management (carbohydrate counting and insulin dose adjustment).

Conclusion: Preliminary observations on the impact of this education course have been encouraging with patients reporting improved understanding and confidence in self-management of CFRD. Recruitment is ongoing. Available data for those patients having completed the initial 6 month study period will be presented.

assess the impact of intense education related to nutrition, airway clearance, infection control, and compliance with therapies. A multisystem questionnaire was developed and implemented to study the change in nutrition and pulmonary endpoints after intense education.

Methods: A pilot study among a convenience sample of 25 pediatric patients was conducted over a one year period of time. Patients/families completed a pre-test at the first clinic visit of the year. Upon completing the pre-test, education on prescribed medication administration was provided. During each subsequent clinic visit patients were educated on different facets of the multi-system disease (visit two: airway clearance, visit three: nutrition and visit four: infection control). A post-test was completed after the final education session at the last visit of the year. Nutrition and pulmonary endpoints were collected. Height, weight and body mass index (BMI) percentile were averaged over the four visits; levels of vitamin A, D, E were documented from the study year and year prior. Average percent FEV1, FVC and FEF25-75 was recorded. Means and standard deviations were calculated, nonparametric Wilcoxon signed ranks test was utilized to determine change in nutrition and pulmonary endpoints. A p-value of 0.05 was utilized to determine significance.

Results: Among the 25 subjects no changes were observed among anthropometrics and lung function. Among patients at increased nutrition risk a clinically relevant, but non-significant increase in nutrition and pulmonary endpoints was observed. Results are summarized in the table.

Conclusions: In this one-year pilot study, only vitamin A and E status improved after intense patient education. Results indicate pediatric patients/families have moderate knowledge regarding CF disease process and associated therapies (pre-test score 78%). Post-test scores only improved by 2%, indicating more education may be required or that education sessions during the clinic visit may be ineffective. However, a sub-set of patients at increased nutrition risk did experience improved vitamin levels, anthropometrics and lung function. More research is need to determine the effect of education on nutrition and pulmonary endpoints; a larger study population over more than a one year time period may be indicated. A potential for focus could be on patients/families with poor growth.

	Education Score % (±SD)	Vitamin A (±SD)	Vitamin E (±SD)	Vitamin D (±SD)			
Study Subjects (N=25)	Pre 77.9 (13.2)	Pre 33.8 (14.1)	Pre 8.2 (3.1)	Pre 25.2 (7.5)			
	Post 79.9 (10.4)	Post 38.1 (16.6)	Post 10.6 (5.1)	Post 27.1 (7.7)			
	p=NS	p<0.05	p<0.05	P=0.15			
	Weight% (±SD)	Height % (±SD)	Vitamin A (±SD)	Vitamin D (±SD)	FEV1 % (±SD)	FVC% (±SD)	FEF 25-75 (±SD)
Patients at increased nutritional risk (BMI<50th %) (N=7)	Pre 12.9 (11.3)	Pre 13.09 (12.8)	Pre 30.7 (12.0)	Pre 23.3 (8.0)	Pre 71.3 (26.0)	Pre 79.5 (12.3)	Pre 71.1 (47.5)
	Post 13.3 (8.5)	Post 14.7 (11.2)	Post 34.7 (12.8)	Post 27.0 (10.2)	Post 74.1 (27.3)	Post 82.4 (10.8)	Post 73.3 (51.3)
	p=NS	p=NS	p=NS	p=NS	p=NS	p=NS	p=NS

NS=not significant

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IMPACT OF MULTI-SYSTEM QUESTIONNAIRE AND INTENSE EDUCATION ON NUTRITION AND PULMONARY ENDPOINTS AMONG PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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Background/Rationale: Cystic fibrosis (CF) is a complex and multi-system disease requiring a complicated and time consuming regimen of treatments. Due to the considerable intricacies of care, patients may not understand the rationale behind prescribed therapies and develop the misconception that non-adherence is acceptable. Treatment burden may also lead to non-adherence with nutrition recommendations, medication administration and airway clearance. The primary objective of the study was to