Received: 19 June 2020 | Revised: 4 December 2020 | Accepted: 14 January 2021

DOI: 10.1002/hsr2.246

Health Science Reports Open Access WILEY

RESEARCH LETTER

Change in circulating proteins during treatment of pulmonary exacerbation in patients with cystic fibrosis

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Funding information
Cystic Fibrosis Foundation, Grant/Award Numbers: ZEMANI12A0, ZEMANI16A0; National Center for Research Resources, Grant/Award Number: UL1 TR001082; National Heart, Lung, and Blood Institute, Grant/Award Numbers: K23HL114883, R25 HL131486-01

1 | INTRODUCTION

Recurrent pulmonary exacerbations (Pex) contribute to significant morbidity and loss of lung function in cystic fibrosis (CF).[1,2] Biomarkers responsive to treatment during Pex may lead to improved understanding of disease mechanisms, allow treatment stratification, or facilitate earlier intervention by identifying patients at increased risk.[3] SOMAmer-based protein array technology is a novel approach allowing for the simultaneous measurement of thousands of circulating proteins, which may assist in the identification of influential disease biomarkers.[4] We sought to identify circulating proteins associated with treatment response during a Pex in CF patients utilizing a high-dimensional aptamer-based technology.

2 | METHODS

A prospective study of 25 patients aged 10-22 years with CF, admitted for intravenous (IV) antibiotic therapy for a Pex was completed. All participants were treated following standard clinical guidelines, at the discretion of their physician. Written informed consent was obtained from all patients aged 18 years or older and from parents/legal guardians for patients under 18 years of age, and assent was obtained from patients aged 10-17 years. The study was approved by the Colorado Multiple Institutional Review Board (COMIRB #07-0365). Blood samples were collected from patients at two timepoints: hospital admission for Pex (T1) and hospital discharge after medical stabilization (T2). Blood samples were then analyzed using SOMAscan (SomaLogic Inc., Boulder, Colorado), an aptamer-based proteomic technology, described elsewhere.[4] SomaLogic's Slow Off-rate Modified Aptamer (SOMAmer) method is based on the use of single-stranded DNA or RNA molecules that bind to preselected targets including proteins and peptides with high affinity and specificity. This approach resulted in the measurement of 3620 aptamers, which were recorded for each patient alongside clinically important outcomes, specifically lung function. Four patients were excluded due to missing pulmonary function data or a missing blood sample.

Change in forced expiratory volume in 1 second (FEV_1) percent predicted for each patient was calculated as the difference between T2 and T1, and change in protein level as the difference in log (base 2) transformed relative florescent units between T2 and T1. Identification of changes in protein levels that were associated with response to treatment (eg, change in FEV_1) was achieved using random forests. Forests including all aptamers were fit to identify and rank proteins, as indicated by the node purity values (mean decrease in node impurity when splitting a protein in the decision tree). Each forest contained 2000 regression trees, defaults for all other hyperparameters were used. One hundred forests were run to assess the consistency of the calculated

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node purities and account for between-forest error. Node purities were averaged for each protein across the 100 forests, and the top 17 proteins were then selected based on their clear separation from the averaged node purities of all other proteins (not shown). Node purity was used instead of accuracy as a measure of variable importance since it typically results in a less uniform distribution[5] and is therefore better at identifying separation. All analyses were performed using the R statistical software (version 4.6-12; randomForest package).

3 | RESULTS

Patients had a mean (SD) age of 16 (2.7) years, 12 (57%) had F508 homozygous genotype, and 6 cultured positive for *Pseudomonas aeruginosa*. Mean baseline FEV_1 percent predicted (SD) was 79.2 (19.6) with a median absolute increase in percent predicted FEV_1 with treatment of 6% (range −14% to 34%). The 17 selected proteins and their biological processes are presented in Table 1. The univariate associations between the change in selected proteins and change in FEV_1 are shown in Figure 1. In our analysis, decreasing levels of CRP, PARC, DR3, TAGL, GM-CSF, K1161, Coagulation Factor IX, TPSNR, SPACA1, Apo E4, GCKR, and TARC were associated with larger increases in FEV_1 after treatment. Conversely, increasing levels of KYNU, Osteocalcin, SET, GDF 11, CEA20 were associated with larger increases in FEV_1 after treatment. Mean squared error compared with original FEV1 change using the 17 selected proteins was 80.7 and explained 32.6% of the variance.

TABLE 1 List of top 17 proteins associated with FEV_1

Gene name	Name	Uniprot #	GO biological process
Negatively-associated with treatment response			
CRP	C-reactive protein	P02741	Acute phase; defense response to Gram-positive bacterium; inflammatory response
TAGL	Transgelin	Q01995	Epithelial cell differentiation; muscle organ development
GM-CSF	Granulocyte-macrophage colony-stimulating factor	P04141	Macrophage activation
K1161	Myogenesis-regulating glycosidase	Q6NSJ0	Carbohydrate metabolic process; skeletal muscle fiber development; positive regulation of insulin-like growth factor receptor signaling pathway
PARC	C-C motif chemokine 18 (CCL18)	P55774	Chemokine-mediated signaling pathway; chemotaxis; inflammatory response
Coagulation Factor IX	Coagulation Factor IX	P00740	Blood coagulation; hemostasis
TPSNR	Tapasin-related protein	Q9BX59	Immune system response
TARC	C-C motif chemokine 17 (CCL17)	Q92583	Chemokine-mediated signaling pathway; chemotaxis; inflammatory response
DR3	Tumor necrosis factor receptor superfamily member 25	Q93038	Apoptosis; immune response; inflammatory response
SPACA1	Sperm acrosome membrane-associated protein 1	Q9HBV2	Acrosome assembly; cell wall macromolecule catabolic process; defense response to bacterium (both Gram-negative and Gram-positive)
Apo E4	Apolipoprotein E	P02649	Cholesterol metabolism; lipid metabolism; lipid transport; steroid metabolism; transport; negative regulation of inflammatory response
GCKR	Glucokinase regulatory protein	Q14397	Carbohydrate metabolism; response to fructose
Increasing levels associated with treatment response			
SET	Protein SET	Q01105	Host-virus interaction
GDF.11	Growth differentiation factor 11	O95390	Nervous system development; pancreas development; regulation of apoptotic process
Osteocalcin	Osteocalcin	P02818	Biomineralization; bone development; cellular response to growth factor stimulus; cellular response to vitamins D and K
CEA20	Carcinoembryonic antigen-related cell adhesion molecule 20	Q6UY09	Immune system response
KYNU	Kynureninase	Q16719	Pyridine nucleotide biosynthesis

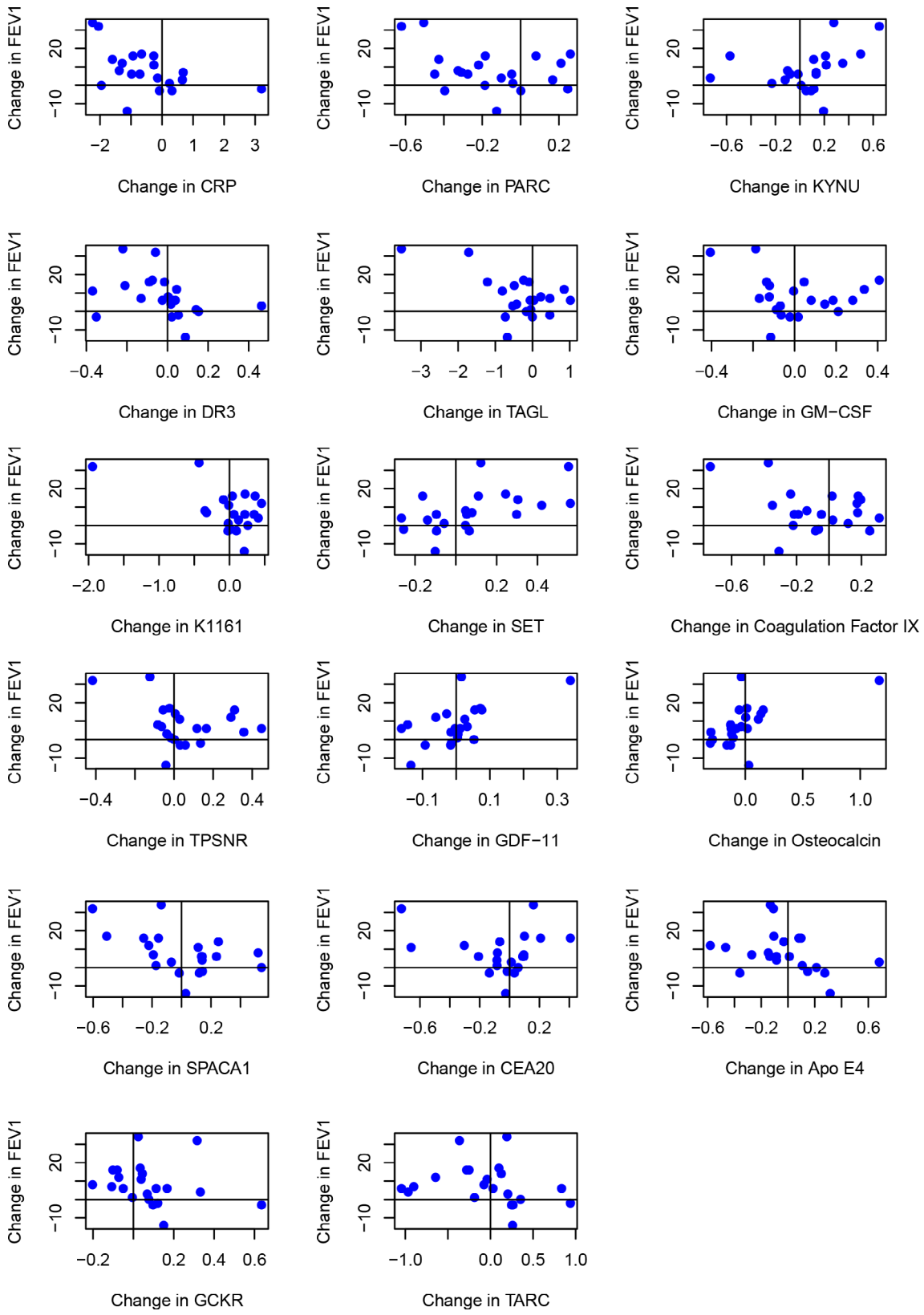

FIGURE 1 Scatterplots for the 17 selected proteins showing the association between the change in FEV1 % predicted (y-axis) and the change in protein measurements with treatment for a pulmonary exacerbation (x-axis). Reference lines at 0 values are plotted

4 | DISCUSSION

Using an aptamer-based approach to examine changes in circulating proteins with IV antibiotic treatment of CF Pex, 17 proteins associated with change in FEV_1 were identified. Proteins with known associations with Pex include increased KYNU and decreased PARC, DR3, CRP, and GM-CSF. CRP has been well studied in CF.[2,3] Several of the proteins we identified are related to macrophage activation and phenotype differentiation (GM-CSF, PARC, TARC, DR3), which has been investigated in CF.[3,6] Both KYNU and PARC have been identified in the literature as potential biomarkers for *Pseudomonas aeruginosa* infection in CF. [7,8] Proteins not commonly associated with CF Pex were also identified. Coagulation factor IX has been linked with Vitamin K deficiency in CF patients.[9] Osteocalcin has been previously noted in the literature with potential importance in relation to FEV_1, though not yet investigated in its relation to Pex.[10]

Strengths of the study include paired sampling allowing the changes within a subject to be determined and the ability to investigate a large number of protein targets simultaneously. Limitations include a small sample size (n = 21), which hindered our ability to adjust for other relevant clinical variables and could have affected our interpretation of lung function change. Change in FEV_1 was used as a surrogate for treatment response, which may be affected by age and disease severity. This concern is mitigated by the low correlation between FEV_1 change with age and baseline FEV_1 in this study. However, even if a strong correlation with change in FEV_1 was observed, this would not necessarily negate the potential utility of protein markers in elucidating mechanisms involved in response to treatment. Lung function change with treatment also may not fully reflect clinical response to treatment (eg, clinical signs and symptoms may have improved even in those with worsening FEV_1). However, poor lung function response to pulmonary exacerbation treatment is associated with persistent decline in lung function and earlier time to next admission.[1] The timing of the two sample collections were based on clinically meaningful states (beginning and end of Pex) and the time between collections varied across participants, though pulmonary function tests and blood samples were collected on the same day so the timing is consistent within participants. Also, the use of circulating proteins may not reflect activity in the airways. However, children with CF are often not able to expectorate sputum, thus identifying circulating markers may preclude the need to collect lower airway samples. Examination of differential interactions between the proteome and treatment method, for example, specific antibiotic regimens, as well as the culture results is a next logical step for future study.

Several well-established proteins were identified in this analysis corroborating the results. In addition, some potentially novel proteins were selected that may provide insight into the mechanism of response to treatment of a Pex. Further investigation into the effect of the selected proteins is needed to validate any association between changes in levels of these proteins and FEV_1 with treatment for a Pex. This investigation serves to augment the current CF literature and introduce potentially novel proteins related to CF and treatment of Pex.

ACKNOWLEDGMENTS

Summer Institute for Biostatistics Participants: Asem Berkalieva, Monica Borges, Grant Fleming, Noah Graham, Emily Peterson. SIBS Instructors: Brandie D. Wagner, Edith T. Zemanick, and John Kittelson.

FUNDING

This work was supported by the NIH/NHLBI Summer Institute in Biostatistics grant R25 HL131486-01 (John Kittelson, Principal Investigator [PI]). The study population consisted of subjects enrolled in a prospective study funded by the NIH (K23HL114883) and the Cystic Fibrosis Foundation (ZEMANI12A0, ZEMANI16A0) and was also supported by Colorado Clinical and Translational Sciences Institute (NIH/NCRR Colorado CTSI Grant Number UL1 TR001082). The funders were not involved in analysis, interpretation of data; writing of the report; or the decision to submit the report for publication.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

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All authors have read and approved the final version of the manuscript.

Brandie D. Wagner had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

Data are available on request due to privacy/ethical restrictions.

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How to cite this article: Wagner BD, Berkalieva A, Borges M, et al. Change in circulating proteins during treatment of pulmonary exacerbation in patients with cystic fibrosis. *Health Sci Rep*. 2021;4:e246. https://doi.org/10.1002/hsr2.246