OR22-05

Background: Obesity is a heterogenous disease resulting from environmental and genetic factors and is characterized by disordered energy balance, regulated in part by the hypothalamic melanocortin-4 receptor (MC4R), including neuronal ciliary assembly and trafficking pathways.[1] Rare loss-of-function variants in genes encoding components of this pathway are associated with severe obesity and hyperphagia, with or without additional features.[2] However, such rare genetic disorders may be underestimated due to a lack of genetic screening in individuals with severe obesity.[3] Our objective was to identify and characterize rare genetic variants in a Spanish population from Madrid with childhood obesity. **Methods:** This analysis was conducted from a prospectively-collected cohort of children with obesity, generally with a BMI>+3DS. Participants were sequenced for 35 obesity-related genes, including 23 genes related to Bardet-Biedl (BBS) and Alström syndromes, plus an additional 12 genes associated with non-syndromic, monogenic causes of obesity, to identify individuals with rare (<1% frequency in gnomAD) potentially biallelic (homozygous and compound heterozygous) non-synonymous variants in protein-coding regions. **Results:** Of the 1019 Spanish patients with obesity, 493 (48.4%) were female and the mean age and BMI were 10.41 ± 3.38 years and 4.38 ± 1.76 SDS (79.8% above +3 SDS), respectively. We identified 26 rare potentially biallelic variants in 25 unique individuals, including 2 individuals with homozygous variants in *POMC*, 3 individuals with two variants in *SRC1*, one individual with two variants in *ADCY3*, and one individual with a homozygous mutation in *LEP*. In addition, we identified 18 individuals with biallelic mutations in one of 23 *BBS* or *ALMS1* genes, including two individuals with known pathogenic variants and clinically confirmed BBS. **Conclusions:** Rare and potentially biallelic sequence variants were identified in 25 individuals with childhood obesity. These results support the use of genetic testing for individuals with severe obesity who may be candidates for specific clinical interventions or additional targeted therapies.

Bone and Mineral Metabolism

PARATHYROID HORMONE TRANSLATIONAL AND CLINICAL ASPECTS

Investigating Analogues of Parathyroid Hormone (PTH) and PTH-Related Peptide (PTHrP) to Improve Anabolic: Catabolic Response Ratios Using UMR106-01 Osteocytic Cells.

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SAT-402

Osteoporosis, the most common bone disease in humans, is characterised by decreased bone mass and increased fracture risk[1]. Osteoporosis development is associated with an imbalance of bone resorption and deposition mediated by all three bone cell types; osteoblasts, osteocytes and osteoclasts[2]. Current treatments for osteoporosis either prevent further bone resorption (Bisphosphonates and anti-RANKL) or increase bone deposition through anabolism (Teriparatide and Abaloparatide)[3]. The two anabolic treatments are truncated analogues of endogenous peptides; parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP), respectively. Both are administered intermittently, act on PTH1R receptors on osteoblasts and osteocytes leading to an increase in bone mineral density and bone strength. However, both treatments also have side-effects of hypercalcemia and cortical bone porosity caused by osteoclast stimulation. We therefore compared an array of PTH and PTHrP analogues for potential stimulation of catabolic side effects and desired anabolic effects. Screening assays used are UMR106-01 osteocytic cells that have high levels of PTH1R to measure mRNAs involved in anabolic responses (suppression of WNT inhibitors SOST and Dkk1) and catabolic responses (stimulation of RANKL/OPG, IL6). Peptides were also analysed by real-time cellular impedance assays (xCELLigence) to measure unbiased receptor stimulation. xCELLigence assays showed PTH1-34, PTHrP1-34 and Abaloparatide had the highest potencies (3.7 nM,1.4 nM,1.7nM respectively) while Tyr^{1}PTH1-34 and PTH2-34 had significantly decreased potencies (64nM and 130nM), the β-arrestin biased agonist, D-$Trp^{12}Tyr^{34}$PTH7–34, had no effect. PTH1-34 potently inhibited SOST (IC_{50} 0.29nM), and catabolic genes (RANK/OPG EC_{50} 0.5nM, IL6 EC_{50} 2.5nM). PTHrP1-34 provided higher potencies for anabolic (inhibited SOST IC_{50} 0.08nM) and catabolic (RANKL/OPG EC_{50} 0.3nM, IL6 EC_{50} 1nM) genes. Altering the first amino acid to Tyrosine; Tyr^{1}PTH1-34 caused potent anabolic responses (SOST IC_{50} 0.97nM) yet showed decreased potency for catabolic responses (RANKL/OPG EC_{50} 20nM, IL6 EC_{50} > 100nM). Removing the first amino acid of PTH to PTH2-34 drastically decreases the effectiveness of the peptide (OPG, SOST and RANKL IC_{50} – no effect, IL6 EC_{50} > 100nM). These results indicate the importance of the N-terminal amino acid for PTH affinity and efficacy and suggest that Tyr^{1}PTH1-34 may offer the best combination of bone stimulation without causing hypercalcemia.

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Bone and Mineral Metabolism

BONE DISEASE FROM BENCH TO BEDSIDE

A Natural Antibody Against Oxidized Phospholipids Attenuates Age-Related Bone Loss and Prevents Age-Related Glucose Intolerance in Mice

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SUN-346

Lipid peroxidation produces oxidized phospholipids (OxPL) such as oxidized phosphatidylcholine. These compounds react with amino groups of proteins and lipids to form

adducts called oxidation specific epitopes (OSEs), which are proinflammatory moieties present on oxidized low density lipoproteins and on apoptotic cells and, unless removed, cause extensive cell damage. Natural antibodies (NAb) produced by B-1 lymphocytes, bind OxPL and prevent their inflammatory activity. E06 is a NAb that recognizes the phosphocholine moiety of OxPL. We previously showed that transgenic expression of a single chain (scFv) form of the antigen-binding domain of E06 IgM (E06-scFv) increases cancellous and cortical bone mass in both male and female mice by increasing bone formation. Age-related bone loss is characterized by a decline in osteoblast number and bone formation, associated with increased oxidative stress and lipid peroxidation. These findings, together with the evidence that serum anti-OxPL IgM titers decrease with age, suggest that increased OxPL formation and decreased anti-OxPL antibodies may contribute to age-related bone loss. Like humans, mice exhibit an age-dependent worsening in glucose tolerance, mainly due to alteration in body composition and increased fat tissue. Chronic low grade inflammation and oxidative stress are associated with development of diabetes mellitus and B-1 lymphocytes have been shown to be protective against obesity associated inflammation, glucose intolerance, and insulin resistance. We tested the hypothesis that overexpression of E06-scFv could attenuate age-related bone loss and glucose intolerance. Serial BMD measurements by DXA of both female and male C57BL/6 E06-scFv transgenic mice (and their WT littermates) up to 22 and 24 months, respectively, showed that E06-scFv attenuated age-related bone loss at the spine and femur in both sexes. As revealed by microCT analysis, this effect was due to the attenuation of the age-associated decline in cancellous bone in both sexes. Additionally, both male and female E06-scFv transgenic mice accumulated less fat mass than WT littermates during aging. Intraperitoneal glucose tolerance test, at 15 months of age, revealed that glucose tolerance was greater in both male and female E06-ScFv mice than in respective WT littermates and did not differ from the glucose tolerance of young mice, indicating that E06-scFv improves glucose metabolism. These data suggest that OxPL impair both age-related bone loss and age-related glucose intolerance. Therefore, targeting OxPL with a neutralizing antibody such as E06, represents a prototypic therapeutic intervention that may simultaneously ameliorate important age-associated diseases.

Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II

The Chromatin Landscape of Glucocorticoid Regulated Genes in Mouse Embryonic Neural Stem / Progenitor Cells

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MON-727

The Chromatin Landscape of Glucocorticoid Regulated Genes in Mouse Embryonic Neural
Stem/Progenitor Cells
Antenatal administration of Dexamethasone (Dex), a synthetic glucocorticoid (GC), is a common clinical intervention for women at risk for preterm birth or in preterm labor that effectively
reduces fetal risk of mortality and bronchopulmonary-related comorbidities. Despite the
therapeutic potential of Dex, excess GC act adversely in the developing central nervous system
to reprogram distinct neural circuits in the brain by acting through the glucocorticoid receptor
(GR). For example, prenatal exposure to excess GCs can impact neural stem and progenitor cell
(NSPC) proliferation leading to long-term alterations in prefrontal cortical neuronal complexity,
which could contribute to behavioral and cognitive impairments later in life. The GR is a
member of the nuclear receptor superfamily that, when bound by a ligand, translocates from
the cytoplasm to the nucleus and associates indirectly or directly with DNA elements (e.g.
glucocorticoid responsive elements or GREs) resulting in the activation and/or repression of
target genes. While GR-regulated transcriptomes have been identified in many NSPC models,
the mechanisms responsible for programming these cells for GC-responsiveness remain largely
unknown. We therefore used transposase accessible chromatin followed by genome-wide
sequencing (Omni ATAC-seq) to characterize the chromatin landscape of primary embryonic
mouse NSPCs in response to an acute *in vitro* treatment with Dex. We identified a small, yet
distinct fraction (0.002%, $p<0.05$) of open chromatin sites that were Dex-inducible. 95% of
these Dex-induced changes in chromatin accessibility occur within intronic or intergenic
regions, suggesting the presence of long-range enhancer-promoter contacts that mediate NSPC
transcriptional responses to Dex. Motif enrichment analysis revealed putative GRE sites located
in Dex-inducible open chromatin within -5kb/+2kb of a Dex-induced gene, providing possible
DNA targets of GR for further validation. A number of other transcription factors implicated in
neurodevelopmental processes were found to underlie both Dex-inducible and constitutively
open chromatin regions. Characterization of the precise epigenetic and transcriptional response
to excess GC *in-utero,* and its influence on acute and chronic neurological outcomes, will
encourage the development of alternative GC treatment regimens that could protect the
developing brain from insult while providing optimal health outcomes in neonates.

Tumor Biology

TUMOR BIOLOGY: GENERAL, TUMORIGENESIS, PROGRESSION, AND METASTASIS

An Uncommon Case of Squamous Cell Carcinoma of the Vulva with Metastasis to the Thyroid Gland

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