vs hypophosphatemic rickets. Ga-DOTATE scan and PET scan were negative, so the patient subsequently underwent genetic testing. She was found to have a phosphate regulating endopeptidase homologue (PHEX) gene mutation and was finally diagnosed with XLHR Her PHEX mutation was caused by a novel variant, c.1366 T>C or W456R, which has only been documented once in the literature. The patient was treated with 2 gm per day of phosphate supplementation in divided doses and calcitriol 0.25 mcg once daily which normalized her phosphate and 1,25 vitamin D levels. 1 month later after treatment, she reported significant improvements in bone pain, and her DEXA scans were stable for the following 4 years. **Discussion:** XLHR is a heterogeneous group of inherited disorders characterized by hypophosphatemia and impaired bone mineralization leading to rickets. It results from mutations affecting the PHEX gene of which more than 300 pathogenic variants have been described. The mutation causes excess FGF-23 which leads to osteomalacia and chronic hypophosphatemia. This condition can be difficult to distinguish from TIO as both present with low phosphate and elevated FGF-23 but can be differentiated with genetic testing. Recognition of the correct diagnosis is prudent to providing correct treatment. The current treatment for XLH is calcitriol and phosphorus replacement. Recently, burosumab was FDA approved in 2018 for treatment in adults.

Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II

Restoration of Growth and Fertility in Zebrafish (Danio Rerio) Model with PROP1 Knockout Generated by CRISPR/Cas9 Genomic Editing.

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MON-712

Introduction: Hypopituitarism is defined as the deficiency of one or more pituitary hormones and can occur due to pathogenic allelic variants in transcription factors involved in pituitary development. *PROP1* gene is responsible for progenitor cell migration from the marginal zone to the anterior lobe, and its terminal differentiation into corticotropes and gonadotropes cell lines besides somatotropes, lactotropes and thyrotropes due to *POU1F1* (also known as *PIT1*) activation. In humans, mutations in the *PROP1* gene are the most common cause of congenital hypopituitarism with GH, TSH, LH/FSH, and progressive ACTH deficiencies. A dwarf phenotype with short stature, pituitary hormone deficiency, and infertility has been described in humans and Ames mice lineage harboring mutations in the *PROP1/Prop1* gene. Another valuable animal model used in basic research is the zebrafish (*Danio rerio*) due to a high homology in neuroendocrine functioning. To test the potential of this model, in our previous study, a 32bp insertion carrying a stop codon was directed into the second exon of *prop1* with CRISPR/Cas9, establishing a homozygous mutant strain ($prop1^{mut}$). **Objective**: To characterize the phenotype and expression patterns of transcription factors and hormones in the zebrafish $prop1^{mut}$ lineage. **Methods**: *prop1, pit1,* and *gh1* mRNA levels were analyzed during embryonic development at 24 and 72 hours post-fertilization (hpf). RNA from 30 pooled embryos was extracted using DirectZol RNA Miniprep. cDNA was synthesized from 1ug of total RNA using High-Capacity cDNA Reverse Transcription Kit and qPCR was performed using SYBR Green PCR Master Mix. Gene expression was normalized to *ef1a* and the $prop1^{mut}$ group was compared with the control wild type group (WT). Animals were kept in the tanks at a density of 15 animals/liter and images were acquired at 13 and 20 days post fertilization (dpf) after brief anesthetization using a stereomicroscope and measured in ImageJ software to determine the larval standard length from nose to the end of the spinal cord. **Results:** At 24 and 72hpf, $prop1^{mut}$ embryos expressed the altered *prop1* mRNA at similar levels to the *prop1* expression observed in WT. Lower *pit1* expression in $prop1^{mut}$ embryos was observed at both periods ($p<0.01$). Albeit in low levels, similar *gh1* expression was observed in both lineages at 24hpf, and $prop1^{mut}$ embryos presented lower *gh1* expression at 72hpf ($p<0.001$). $prop1^{mut}$ larvae presented a significant decrease in size at 13dpf ($p<0.001$) but not at 20dpf. **Conclusion:** In this study, the $prop1^{mut}$ zebrafish model exhibited a dwarf phenotype during larval development associated with diminished *pit1* and *gh1* expression during the embryonic stage. Additionally, in the juvenile stage, the development rate in $prop1^{mut}$ animals was restored, presenting similar standard lengths observed in WT animals.

Adipose Tissue, Appetite, and Obesity

MECHANISMS AND TREATMENT OF OBESITY IN HUMANS

ARNT2: A Potential Novel Candidate Gene for Monogenic Obesity in Humans

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OR33-07

Introduction: Aryl hydrocarbon nuclear translocator 2 (*ARNT2*) is a basic helix-loop-helix (bHLH)-PAS (Per/Arnt/Sim) transcription factor shown to be critical to the development of paraventricular nucleus of the hypothalamus (PVN), key region for energy homeostasis and feeding response. *In vivo* and *in vitro* studies have shown that *ARNT2* is an obligate heterodimer for *SIM1*, known cause of monogenic obesity. Null mutations in *Arnt2* in animals are not viable, but hypomorphic mutation results in hyperphagic

obesity and its associated consequences (1). Due to the critical role of *ARNT2* in the development of PVN, we hypothesize that hypomorphic mutations may result in early onset obesity in humans.

Methods: The Genetics of Early Childhood Obesity (GECO) study recruits children with severe obesity (BMI > 120% of 95th percentile) of early onset (< 6 years). Whole exome sequencing (WES) was performed in a subset of proband-parent trios. The functional validation of the mutation(s) in *ARNT2* is ongoing with co-transfection of tagged *Arnt2* and *Sim1* in HEK293 cells, with the induction of a luciferase reporter gene under the control of 6 repeats of bHLH-PAS core binding element by the *Arnt2-Sim1* complex.

Results: Two adolescents from unrelated families were found to have genetic variants in *ARNT2*. Subject 1 has a novel *de novo* heterozygous coding variant in *ARNT2*, c.388 C>G (p.P130A, CADD 25), predicted to be deleterious by 8/12 *in silico* algorithms. She is a 14-year old Caucasian girl with severe early onset obesity, BMI 28.1 kg/m^2 (BMIz +4.72) at 2.5 years of age that has increased to 53.54 kg/m^2 (BMIz + 3.25) at 14-years, and height > 95th %tile. She is non-dysmorphic, has developmental delay, absence seizures, behavior abnormalities & glucose intolerance/dyslipidemia secondary to obesity. Using genematcher, we identified another proband with the phenotype of obesity: an African American girl (BMIz +1.9) with biallelic inherited heterozygous variants in *ARNT2*, c.1228T>A (p.W410R, CADD 29) and c.916G>A (p.G306S, CADD 22). An only child conceived by IVF, she is non-dysmorphic and on treatment for bilateral focal epilepsy. All 3 variants are rare, with mean allele frequency < 0.005 in population-based databases such as gNOMAD. Both the patients have early onset obesity and a significant neurological phenotype. *ARNT2* is a highly constrained gene of 717 amino acids with a significant depletion of missense variants in the N-terminus (1-244 aa) and overall fewer loss of function variants in ~282,644 alleles sequenced in gNOMAD.

Conclusions: We propose that hypomorphic mutations in *ARNT2* could be a potential novel cause of monogenic obesity in humans. Future studies will investigate the molecular mechanisms causing weight dysregulation in patient specific disease relevant hypothalamic neurons.

Reference: (1) Turer et al., Dis Model Mech. 2018; 11(12)

Adipose Tissue, Appetite, and Obesity

NEURAL MECHANISMS OF OBESITY

Shared Signaling Profile Between Human MRAPα-Induced Human MC4R Constitutive Activity and Obesity-Associated Human MC4R Constitutive Activity

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SAT-598

The human melanocortin 4 receptor (hMC4R) plays a critical role in the regulation of energy balance with more than 150 distinct human obesity-associated mutations. Most exhibit defective MC4R functionality but six have been reported to associate with constitutive activity. This represents a conundrum since a lean phenotype is expected for enhanced MC4R signaling. Human melanocortin 2 receptor accessory protein alpha (hMRAPα) induces hMC4R constitutive activity in transfected HEK293 cells (1,2). We do not know whether the hMRAPα-induced gain-in-function for hMC4R would cause, or prevent, obesity because of this conundrum. Here, we hypothesize that wild-type hMC4R, obesity-associated constitutively active hMC4R and hMRAPα-induced constitutive active hMC4R can exist in distinct conformational states and elicit distinct signaling profiles. To test this, we compared transiently expressed HA-hMC4R in HEK293 cells for basal and agonist activation for adenylyl cyclase, Cre driven β-galactosidase reporter transcription, and receptor protein expression. Six previously reported obesity-associated hMC4R constitutively active variants were compared with two hMC4R constitutively active mutations not associated with obesity, two hMC4R variants associated with protection from development of obesity, five non-constitutively active hMC4R mutations associated with obesity, hMRAPα co-expressed with hMC4R, and wild-type hMC4R. Our data confirm hMC4R constitutive activity coupling to both adenylyl cyclase and Cre β-galactosidase reporter for only two hMC4R variants associated with obesity (H76R & L250Q), one hMC4R mutation (H158R) not associated with obesity, and hMRAPα co-expressed with hMC4R. We show α-MSH stimulated concentration curves for wild-type hMC4R, H76R, L250Q & H158R hMC4R variants and hMRAPα co-expressed with hMC4R coupling to adenylyl cyclase. Surprisingly, out of these, only wild-type hMC4R and H158R hMC4R variant exhibited α-MSH-stimulated Cre β-galactosidase reporter concentration curves. Western blotting and ELISA showed ~70% reduced cell surface and total receptor protein expression for hMC4R co-expressed with hMRAPα and obesity-associated constitutively active hMC4R variants, compared to wild-type hMC4R. To summarize, two constitutively active hMC4R variants (H76R and L250Q) associated with obesity, and hMC4R co-expressed with hMRAPα, share a signaling profile comprising protein expression and α-MSH
stimulated functional coupling to adenylyl cyclase and Cre-reporter gene expression. We conclude (1) if hMC4R is co-expressed with hMRAPα *in vivo* it would likely contribute to human obesity, and (2) obesity-associated constitutively active hMC4R variants exhibit a signaling anomaly that may underpin development of anti-obesity therapeutics.

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Thyroid

THYROID NEOPLASIA AND CANCER

Institutional Experience with Cytologically Indeterminate Thyroid Nodules: No Molecular Testing Versus Afirma Gene Expression Classifier or Genomic Sequencing Classifier

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