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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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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