in neurosarcoidosis is usually associated with permanent endocrine defects. Hence, hypothalamic-pituitary axis evaluation should be performed promptly and long-term management is required.

Neuroendocrinology and Pituitary

HYPOTHALAMIC-PITUITARY DEVELOPMENT AND FUNCTION

The Expression of the Homeodomain Transcription Factor SIX3 within Kisspeptin Neurons Is Necessary for Reproduction in Mice

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The homeodomain transcription factor SIX3 is necessary for normal reproductive processes in mice. The loss of a single allele results in haploinsufficient phenotypes that are reflective of individuals with Kallmann Syndrome, such as hypogonadism, reduced GnRH neurons, and anosmia. *Six3* heterozygous mice have reproductive impairments including an increased time to first litter, increased estrous cycle lengths in females, and fewer litters in males compared to wildtype controls. Prior studies found that deleting SIX3 specifically from GnRH neurons did not recapitulate the same phenotypes produced from a global knockout of the *Six3* allele and imposed no reproductive abnormalities. This suggests that the role of SIX3 in reproduction is due to SIX3 populations outside of GnRH neurons. Using qPCR and RNA-seq, we have verified that *Six3* is expressed in arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) populations of kisspeptin neurons and can regulate the kisspeptin promoter *in vitro*. **This led us to hypothesize that SIX3 in kisspeptin neurons is required for *Kiss1* regulation and maximal fertility.** We selectively deleted *Six3* from kisspeptin neurons by crossing a Six3-floxed mouse with a Kiss1-Cre mouse to produce Six3 flox/flox; Kiss1-Cre positive mice (cKO) and Six3 flox/flox, Kiss-Cre negative littermates (controls). In female mice, we found that the loss of SIX3 from kisspeptin neurons disrupted the estrous cycle and reduced fertility. Compared to controls, cKO mice had fewer cycles in a 16-day period (0.38 vs 1.44; p=0.004; n=8-9), and spent a greater percentage of time in diestrus (80.5 vs 61.8; p=0.002; n=8-9) and a smaller percentage of time in estrus (15.6 vs 29.2; p=0.030; n=8-9). The loss of kisspeptin-specific SIX3 also resulted in reduced fecundity, with the cKO female mice having fewer pups within 90 days (15.0 vs 34.4; p=0.048; n=5-6) compared to controls. In addition to its importance in female mice, kisspeptin-specific SIX3 also plays a role in male fertility. We found that male cKO mice had reduced motile sperm (52.2% vs 72.5%; p=0.012; n=7-9) compared to controls. We also assayed levels of *Kiss1* mRNA in the male arcuate nucleus and observed decreased *Kiss1* levels compared to controls (0.34 vs 1.01; p=0.042; n=3-4). These results support our hypothesis that SIX3 in kisspeptin neurons is necessary for critical reproductive processes in both females and males, including progression through the estrous cycle, fecundity, and sperm motility, potentially through *Kiss1* regulation.

Thyroid

THYROID HORMONE ACTION AND SIGNALING

Human Type 1 Iodothyronine Deiodinase (DIO1) Mutations Cause Abnormal Thyroid Hormone Metabolism

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Iodothyronine deiodinases (Ds) mediate thyroid hormone (TH) action. They catalyze triiodothyronine (T_3) production and degradation via, respectively; outer (ORD) and inner (IRD) ring deiodination. Type 1 D (D1) has a relatively high Km for substrates thyroxine (T_4) and reverse T_3 (rT_3), catalyzes both ORD and IRD and is inhibited by propylthiouracil (PTU). Although no mutations in *DIO1* gene have been reported so far in humans, based on the D1-deficient C3H mouse and the heterozygous *Dio1* knockout mice, the expected phenotype of D1 loss-of-function is higher serum rT_3 and slightly elevated T_4. Our objective was to identify new gene defects causing unexplained and discrepant thyroid function tests using whole-exome sequencing (WES). Exons and splice sites are captured with Agilent SureSelectXT and sequenced in Illumina HiSeq2500. Data are analyzed with BWA, GATK, and ANNOVAR applications for alignment, variant calling and annotation, respectively. Sanger sequencing is used to confirm and segregate WES variants in families. A heterozygous pathogenic missense variant in the *DIO1* gene (c.282C>A:p.N94K; N-linker) was identified in four family members with relatively higher serum rT_3, T_4, and free T_4 than unaffected relatives, and normal TSH. We identified a second heterozygous pathogenic mutation in the *DIO1* (c.603G>A:p.M201I; thioredoxin-fold) in a second family. Two affected individuals presented slightly elevated TSH, higher serum rT_3, normal T_4 levels, while the T_3/T_4 ratio was lower compared to unaffected members. To assess the functional activity of the mutant D1 protein, human embryonic kidney epithelial cells (HEK293) were transiently co-transfected with pcDNA3 plasmids expressing pD1-WT, pD1-N94K or pD1-M201I, and pGFP as transfection control. In assays performed with 1μM ^{125}I-T_4, the catalytic activities of pD1-N94K and pD1-M201I were 44.7% and 54.1% lower as compared to pD1-WT, respectively. To study the enzyme kinetics, the D1 assay was repeated in the presence of increasing ^{125}I-T_4 concentrations (0.1-20μM). The enzymatic activity assays revealed similar Vmax for pD1-N94K and pD1-M201I mutants compared to pD1-WT ($Vmax_{pD1\text{-}N94K}$=53.7 vs $Vmax_{pD1\text{-}WT}$=40.9 and $Vmax_{pD1\text{-}M201I}$=58.8 vs $Vmax_{pD1\text{-}WT}$=42), but higher Michaelis constant (Km) than pD1-WT ($Km_{pD1\text{-}N94K}$=16.4 vs $Km_{pD1\text{-}WT}$=6 and $Km_{pD1\text{-}M201I}$=21.4 vs $Km_{pD1\text{-}WT}$=6.9), which demonstrates a reduced