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SAT-298

The pituitary gland is a critical regulator of the neuroendocrine system. To further our understanding of the classification, cellular heterogeneity, and regulatory landscape of pituitary cell types, we performed and computationally integrated single cell (SC)/single nucleus (SN) resolution experiments capturing RNA expression, chromatin accessibility, and DNA methylation state from mouse dissociated whole pituitaries. Both SC and SN transcriptome analysis and promoter accessibility identified the five classical hormone-producing cell types (somatotropes, gonadotropes (GT), lactotropes, thyrotropes, and corticotropes). GT cells distinctively expressed transcripts for *Cga*, *Fshb*, *Lhb*, *Nr5a1*, and *Gnrhr* in SC RNA-seq and SN RNA-seq. This was matched in SN ATAC-seq with GTs specifically showing open chromatin at the promoter regions for the same genes. Similarly, the other classically defined anterior pituitary cells displayed transcript expression and chromatin accessibility patterns characteristic of their own cell type. This integrated analysis identified additional cell-types, such as a stem cell cluster expressing transcripts for *Sox2*, *Sox9*, *Mia*, and *Rbpms,* and a broadly accessible chromatin state. In addition, we performed bulk ATAC-seq in the LβT2b gonadotrope-like cell line. While the FSHB promoter region was closed in the cell line, we identified a region upstream of *Fshb* that became accessible by the synergistic actions of GnRH and activin A, and that corresponded to a conserved region identified by a polycystic ovary syndrome (PCOS) single nucleotide polymorphism (SNP). Although this locus appears closed in deep sequencing bulk ATAC-seq of dissociated mouse pituitary cells, SN ATAC-seq of the same preparation showed that this site was specifically open in mouse GT, but closed in 14 other pituitary cell type clusters. This discrepancy highlighted the detection limit of a bulk ATAC-seq experiment in a subpopulation, as GT represented ~5% of this dissociated anterior pituitary sample. These results identified this locus as a candidate for explaining the dual dependence of *Fshb* expression on GnRH and activin/TGFβ signaling, and potential new evidence for upstream regulation of *Fshb*. The pituitary epigenetic landscape provides a resource for improved cell type identification and for the investigation of the regulatory mechanisms driving cell-to-cell heterogeneity.

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Pediatric Endocrinology

PEDIATRIC OBESITY, THYROID, AND CANCER

Utilization of GluCEST, a Novel Neuroimaging Technique, to Characterize the Brain Phenotype in Hyperinsulinism/Hyperammonemia Syndrome

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MON-110

Background: Hyperinsulinism/Hyperammonemia (HI/HA) syndrome is the second most common form of congenital hyperinsulinism. It is caused by gain-of-function mutations in glutamate dehydrogenase (GDH), a mitochondrial enzyme expressed in pancreatic β-cells, liver, kidney, and brain, and is responsible for metabolizing glutamate into α-ketoglutarate and ammonia. In addition to hyperinsulinemic hypoglycemia due to abnormal GDH activity in pancreatic β-cells, ~80% of patients have developmental delays, learning, or behavioral disorders and >60% have atypical absence seizures (Bahi-Buisson, 2008). These neurologic symptoms are not fully explained by hypoglycemia and are hypothesized to result from central nervous system (CNS) glutamate imbalance due to CNS GDH overactivity. Newer magnetic resonance imaging (MRI) techniques have allowed for sensitive estimation of CNS glutamate using Glutamate Chemical Exchange Saturation Transfer (GluCEST). We aimed to comprehensively characterize the biochemical and clinical neurologic phenotype of HI/HA leveraging GluCEST MRI.

Methods: Subjects with confirmed HI/HA diagnosis and without contraindication to MRI had electroencephalogram (EEG), serum ammonia, and the following validated neurodevelopmental assessments: ABAS-3, BRIEF, and ASEBA CBCL (if <18 years) or ASR (if >18 years) completed. GluCEST MRI axial hippocampal and midsagittal slices were acquired on a 7.0T Siemens scanner and reported as GluCEST % contrast. Healthy control GluCEST % contrast data were obtained from a separate study using the same neuroimaging protocol.

Results: 8 HI/HA subjects (4 female; mean age 28 years [range 16-56] years) participated to date. Median serum ammonia was 58 umol/L (IQR 39-89). 50% self-reported learning impairments and 37.5% self-reported prior ADHD diagnosis. Marked unilateral increase in hippocampal GluCEST % contrast was observed in 3/6 subjects (2 L>R; 1 R>L). Overall, median peak GluCEST % contrast level was significantly higher in HI/HA subjects than controls (10.3% [IQR 8.9-11.3] v. 8.0% [IQR 7.8-8.4], p=0.0013, n=6).

Conclusions: This is the first study to evaluate CNS glutamate via GluCEST in HI/HA. Hippocampal glutamate, measured by GluCEST % contrast, was significantly higher in HI/HA subjects than healthy controls. Laterality in hippocampal glutamate was observed in half of subjects. These findings are remarkable given the known role of abnormal glutamate signaling in the development of epilepsy and neurocognitive impairment. Next steps are to complete midsagittal GluCEST image processing, EEG and neurodevelopmental assessment interpretations to explore correlations between CNS phenotype and brain glutamate pattern. GluCEST holds promise for elucidating the pathophysiology of CNS manifestations in HI/HA syndrome.