Disorders. Neurotrophic/growth factors such as BDNF, NGF, and NT3 have been linked to these pathological conditions. Carboxypeptidase E (CPE), a proenkephalin/prohormone processing enzyme, also named neurotrophic factor-α1(NFα1) is highly expressed in the stress-vulnerable hippocampal CA3 neurons, and was shown to have neuroprotective activity from in vitro studies. Here we investigated if CPE-NFα1 functions in vivo, independent of its enzymatic activity, and the mechanism underlying its action. We generated knock-in mice expressing a non-enzymatic form of CPE, CPE-E342Q, but not wild-type CPE. The CPE-E342Q mice showed significantly decreased neuropeptide content and exhibited obesity, diabetes and infertility due to lack of prohormone processing activity, similar to CPE-KO mice. However, they showed no hippocampal CA3 degeneration, exhibited neurogenesis in the dentate gyrus, and displayed normal spatial learning and memory, similar to CPE wild-type mice, after weaning stress; unlike CPE-KO mice which showed hippocampal CA3 neuronal degeneration and cognitive deficits. Binding studies showed that radiolabeled CPE bound hippocampal cell membrane specifically, in a saturable manner. Binding of CPE and CPE-E342Q to hippocampal neurons activated Erk signaling and pre-treatment with either of these proteins protected neurons against 

\[ \text{H}_2\text{O}_2 \] 

or glutamate-induced neurotoxicity by increasing BCL2 expression. In vitro and in vivo inhibitor studies demonstrated that this neuroprotective effect was independent of tyrosine kinase receptor signaling. Taken together, the data provide evidence that CPE-NFα1 is a unique neurotrophic factor which acts through a non-tyrosine kinase receptor to activate Erk-BCL2 signaling to protect hippocampal CA3 neurons against stress-induced neurodegeneration and maintaining normal cognitive functions in mice.

**Healthcare Delivery and Education**

**EXPANDING CLINICAL CONSIDERATIONS FOR PATIENT TESTING AND CARE**

**Improving the Accuracy and Reliability of Free Thyroxine (FT4) Measurements Through the CDC Clinical Standardization Programs (CSP)**

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**MON-130**

Reliable free thyroxine (FT4) measurements are essential for assessing thyroid function and for correctly diagnosing and treating thyroid disorders. Thyroid hormones play an important role in normal brain development of the fetus, and abnormal FT4 during pregnancy is associated with adverse pregnancy outcomes. Standardization of FT4 measurements, is critical to improving the accuracy and reliability of current methods and thus to improve diagnosis, treatment and prevention of thyroidal illnesses. Currently, there are no serum-based reference materials available for FT4 to assess the accuracy and reliability of FT4 assays. CDC CSP is collaborating with the International Federation of Clinical Chemistry and Laboratory Medicine, and the Partnership for the Accurate Testing for Hormones to address these issues through development of an accurate and sensitive higher-order Reference Measurement Procedure (RMP) for FT4 that will be used to assign target value to serum-based materials. The CDC CSP FT4 reference method is using equilibrium dialysis in combination with liquid chromatography tandem mass spectrometry (LC-MS/MS). FT4 in serum is isolated from the binding proteins in 1 mL equilibrium dialysis cells for 4 hours at 37°C. FT4 is further isolated by extractions prior to LC-MS/MS analysis. To determine the concentration of FT4 in serum, certified primary reference materials are used to prepare calibration materials. Chromatographic separation is achieved using a C18 reverse phase column with a gradient of methanol and water with 0.1% formic acid. Quantification by selective reaction monitoring is performed in the positive mode using electrospray ionization. Two transitions are monitored

**Neuroendocrinology and Pituitary**

**HYPOTHALAMIC-PITUITARY DEVELOPMENT AND FUNCTION**

**TSH Synthesis and Secretion Are Unperturbed in Male IRS4 Knockout Mice**

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**SAT-286**

It was recently reported that mutations in the insulin receptor substrate 4 (IRS4) gene cause a novel form of X-linked congenital central hypothyroidism (OMIM 300904). To date, four different mutations, three frameshift and one nonsense, have been reported, with two affected male patients showing decreased basal, pulsatile, and total thyroid-stimulation hormone (TSH) secretion (PMID 30061370).

Members of the IRS family canonically act as scaffold proteins between tyrosine kinase receptors and their downstream effectors. IRS4/IRS4 expression is enriched in the pituitary; however, its role in the hypothalamic-pituitary-thyroid (HPT) axis has not been studied in detail.

We generated novel whole-body Irs4-knockout mouse lines using CRISPR-Cas9. A specific guide RNA was used to target the Cas9 enzyme to the 5’ end of the single exon Irs4 gene. A two-nucleotide deletion was introduced into Irs4, resulting in a frameshift and premature stop codon. We hypothesized that like IRS4 deficient patients, these mice would exhibit central hypothyroidism. Given that Irs4 is X-linked, we focused our initial characterization on males. Under normal laboratory conditions, Irs4 knockout mice do not exhibit differences in pituitary expression of Tshb, which encodes one of the subunits of the TSH heterodimer. Expression of the gene encoding the thyrotropin-releasing hormone (TRH) receptor, Thr1, is also unperturbed in these knockout mice. Additionally, there are no differences in their serum thyroid hormones, T3 (triiodothyronine) and T4 (thyroxine). When Irs4 knockout males were placed on a low-iodine diet supplemented with propylthiouracil (PTU) for 3 weeks and rendered hypothyroid, their serum TSH increased similarly to wild-type males. Overall, Irs4 knockout males do not exhibit central hypothyroidism or phenocopy IRS4 deficient patients. Compensation by another IRS protein may explain euthyroidism in these mice.
Adipose Tissue, Appetite, and Obesity
RARE CAUSES AND CONDITIONS OF OBESITY: PRADER WILLI SYNDROME, LIPODYSTROPHY

Variants in Known Monogenic Causal Genes of Hypertriglyceridemia Are Not Major Contributors for Hypertriglyceridemia in Lipodystrophy Due to a LMNA Mutation

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SUN-593

Background: Lipodystrophy is a heterogeneous disorder of adiposity, and one common lipid manifestation is hypertriglyceridemia (HTG). The LMNA gene, which encodes for nuclear envelope proteins, is a known causal gene for heritable lipodystrophy. At present, underlying mechanisms for each clinical manifestation of lipodystrophy due to a LMNA mutation are unknown.

Hypothesis: A likely explanation for HTG in lipodystrophy is the paucity of adipose tissue where excess triglycerides (TGs) are normally stored, thus it may not be due to a specific defect in lipoprotein metabolism. Consequently, rare variants in HTG-associated genes would not be expected to be major contributors for HTG in lipodystrophy with LMNA mutations.

Method: A proband and her father with a clinical diagnosis of lipodystrophy were recruited into an IRB-approved study investigating molecular etiologies of dyslipidemia at the University of Pennsylvania. Next-generation sequencing (NGS) with the LipidSeq panel, targeting causal genes for lipodystrophy, and monogenic HTG was performed, and confirmed by Sanger sequencing. Also, unweighted TG-polygenic scores by summing the number of TG-raising alleles from 14 single nucleotide polymorphisms (SNPs) associated with TG levels were assessed.

Results: The proband and her father were diagnosed with lipodystrophy of two different subtypes, generalized in the daughter and partial in the father. The proband reported a gradual loss of subcutaneous fat starting around age 10. A highest reported TG in the proband was 19,000 mg/dL with eruptive xanthomas, whereas TG in the father was never >500 mg/dL. Their BMI’s and DEXA body fat% were 12.9 kg/m² and 7% in the proband, and 25.7 kg/m² and 25% in the father, corresponding to their fat storage capacities. The molecular analyses revealed only a lipodystrophy causal mutation in LMNA, c.29G>T, T101I with no other significant findings in 18 other lipodystrophy-related genes. No deletion or duplication was identified by a targeted array CGH of LMNA.

As predicted, no rare monogenic variants in HTG-causal genes (LPL, GPIHBP1, APOA5, APOC2, LMF1, GPD1) were identified in either subject. However, TG-polygenic scores were 17/28 (95th %ile) in the proband, and 13/28 (50th %ile) in the father, the same trend as the level of HTG levels seen in them. Apolipoprotein E genotypes were non-contributory, (3/3) in the proband, and (3/4) in the father.

Conclusion: Our findings suggest that the pathophysiology of HTG in lipodystrophy is likely to be due to lack of TG-storage space (adipose tissues), and is unlikely due to a defect in lipoprotein metabolism seen in patients with rare monogenic HTG-variants. Although the HTG-polygenic score was higher in the proband, and the accumulative effects of the at-risk alleles may be contributory to the HTG phenotype, it is unlikely to be the leading cause of severe HTG seen in the proband.

Adrenal

ADRENAL CASE REPORTS II

Case Report: Mifepristone Taper in an Individual with Equivocal Cushing’s Syndrome Screening Tests

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SUN-186

We present a 75-year-old male evaluated by the inpatient endocrine service during an admission for hemorrhagic stroke. Approximately 1 year prior to this admission he was started on mifepristone therapy for presumed Cushing’s Syndrome. Initial Cushing’s work-up was equivocal: 1 mg dexamethasone suppression cortisol level of 1.9 and midnight salivary cortisol 167 ng/dl. Random ACTH measurement was not obtained as part of this initial evaluation. Review of prior imaging studies did not demonstrate obvious culprit pituitary nor adrenal lesions. Mifepristone induced hyperaldosteronism, thyroid dysfunction and adrenal insufficiency were demonstrated presumably secondary to cortisol receptor antagonist induced up-regulation of adrenocorticotropic hormone and cortisol. We describe our experience stopping mifepristone and performing re-evaluation.