human development. iHeps are robustly responsive to TH with over 1000 regulated genes. Importantly, they allow us to use CRISPR/Cas9 to determine molecular physiology and mechanism of disease. To demonstrate the role of TRs in TH signaling in human liver cells we have focused on TRβ1 as the main TR isoform expressed in liver. We knocked out TRβ1 (θTRβ1KO) using guide RNAs (gRNAs) we engineered to specific genomic loci to truncate the expression of this isoform at the DNA binding domain. Also, to define mechanisms of TR action in the context of RTH, we used this same approach to introduce the Δ337T mutation found in human TRβ1 that causes RTH. Both θTRβ1KO and Δ337T lines are karyotypically normal and flow cytometry analysis in both lines demonstrated that these lines differentiated normally into hepatocytes after gene editing with CRISPR/Cas9. Once clones were identified the edited iHeps were treated with PBS (vehicle) or T3 (10nM) for 24 hours. Real-time quantitative PCR (RT qPCR) was done to assess mRNA expression of T3 target genes. RT qPCR analysis confirmed the success of θTRβ1 deletion as the response to T3 was lost on both positive and negative TRβ1 targets. In the Δ337T iHeps, the response to TH was diminished indicating the successful generation of this line. These preliminary results confirm our ability to edit IPSCs and then to differentiate into hepatocytes, allowing us to further study the action of TH and the mutations involved in RTH.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Nuclear Receptor Corepressors NCoR1 and SMRT Plays Unique Roles in Central Nervous System

Izuki Amano, MD PhD³, Ayane Ninomiya, MS², Megan Ritter, MD⁴, Kristen R. Vella, PhD³, Anthony Neil Hollenberg, MD⁴, Noriyuki Kobuchi, MD,PhD³.

¹GUNMA UNIVERSITY, Maebashi, Japan, ²GUNMA UNIVERSITY, Gunma, Japan, ³NEW YORK PRESBYTERIAN HOSPITAL, New York, NY, USA, ⁴Weill Cornell Medicine, New York, NY, USA, ⁵Gunma University Grad School of Medical, Maebashi, Japan.

The nuclear corepressor 1 (NCoR1) and the silencing mediator of retinoid and thyroid hormone receptors (SMRT) are critical coregulators of the thyroid hormone receptor (TR), mediating transcriptional repression via histone deacetylation. Thyroid hormone (TH) plays an essential role in many physiological processes via the TR. How the corepressors regulate TR signaling is not fully understood, especially in central nervous system (CNS). To determine the role of NCoR1 and SMRT in the CNS, we used mice with conditional NCoR1 (NCoR1αlox/lox) and SMRT (SMRTfllox/fllox) alleles in combination with mice that express Cre recombinase in a neuronal specific fashion (Snap25-Cre). Global deletion of NCoR1 or SMRT during embryogenesis results in lethality. We also showed that NCoR1/SMRT double knock-out mice die within two weeks after induction of Cre activity in adult mice. Now, we found that neuronal specific NCoR1 or SMRT mice survive without obvious impairment of neuronal development. However, NCoR1/SMRT double knock-out mice die within postnatal 1-2 weeks and have impaired body growth. Thus, both NCoR1 and SMRT have important roles in maintaining normal neuronal function. Recently, cases of mutations in NCoR1 and SMRT in humans have been reported. These cases report phenotypes including Autism Spectrum Disorder (ASD) and intellectual disability. The cerebellum has been thought to contribute to motor control and learning. Surprisingly, it has also been shown to be a key brain structure involved in social cognition and its dysfunction may play a role in ASD. The Purkinje cell is the main neuron in the cerebellum. Thus, we generated cerebellar Purkinje cell specific NCoR1/SMRT knock-out mice using L7/Pep2-Cre mice. In contrast to neuronal specific KO mice, both NCoR1 or SMRT single or double knock-out mice survive until adulthood. SMRT Purkinje cell knock-out mice showed abnormalities in 3ch social interaction test indicating impaired social functioning, similar to some ASD symptoms. Electrophysiological testing showed current injection evoked more action potentials in SMRT KO mice. These results suggest Purkinje cell dysfunction caused by SMRT deletion may result in social disability. Our data demonstrate for the first time that NCoR1 and SMRT have separate functions in different areas of the brain but also have some redundant function when knocked out together in all neurons.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Physiologic Effects of Levothyroxine and Liothyronine in the in Older Individuals With Persistent Subclinical Hypothyroidism: A Randomized, Double-Blind, Cross-Over Study

Paige Meizlik, MD, MSTR, Andrew Cucchiara, PhD², Lakshmi Kannan, MD MSc³, Jarvis Mays, BA⁴, Jennifer Jolivert, BA⁴, Theresa Scatteredood, MSN, RN⁵, Anne Rentoumis Cappola, MD, ScM⁶.

¹Teva Pharmaceuticals, Dresher, PA, USA, ²Perelman SOM, University of Pennsylvania, Philadelphia, PA, USA, ³COORDINATED HEALTH, Allentown, PA, USA.

Background: Subclinical hypothyroidism is common in older individuals, but the physiologic responses to treatment with levothyroxine (LT4) and liothyronine (LT3) are not well defined in this age group. Methods: We conducted a randomized, double-blind, cross-over study of LT4 and LT3 treatment in men and women aged 70 years and over without anti-thyroid peroxidase antibodies with persistent subclinical hypothyroidism, defined as having a TSH level between 4.5 and 19.9 µIU/mL with a normal free thyroxine (FT4) level at two consecutive time points. Physiologic outcome measures assessed after achieving a TSH level of 0.5-1.5 µIU/mL with each therapy included vital signs, weight and body composition, bone mineral content and bone density, lipids, resting energy expenditure (REE), cognitive function, quality of life, and thyroid symptoms. Results: Thirteen participants [mean (SD) age 77 (5) years], 4 women and 9 men, completed the study. Baseline mean TSH was 4.84 (1.29) µIU/mL. The mean LT4 dose was 105 (36) µg/day [1.4 (0.5) µg/kg/day] and LT4 dose was 34 (9) µg/day [0.4 (0.1) µg/kg/day]. Mean time on LT4 was 200 days and on LT3 was 231 days, with a 28 day washout period. Compared with baseline, participants had an average weight loss of 1.1 kg on LT4 (p<0.02) and 2.5 kg on LT3 (p<0.001), which was significantly different between
the two treatments (p=0.01). Fat mass decreased by an average of 0.7 kg on LT4 (p=0.03 vs. baseline) and 1.5 kg on LT3 (p<0.01 vs. baseline) and differed between treatments (p=0.01). There was a significant difference in total cholesterol of 13.3 mg/dL (p<0.001) and in low-density lipoprotein cholesterol (LDL) of 10.8 mg/dL (p<0.001) between LT4 and LT3 treatment arms; for both, the levels were lower on LT3 than LT4. No differences were seen in the other assessed outcomes. **Conclusions:** In a cross-over study of treatment of LT4 or LT3 in persistent subclinical hypothyroidism, participants lost fat mass and weight after each treatment, with a greater decrease after treatment with LT3. These findings support different physiologic responses to LT4 compared with LT3.

**Thyroid**

**THYROID HORMONE METABOLISM AND ACTION**

**Postnatal Hypothyroidism Permanently Disrupts Neural Stem Cell Fate in the Murine Subventricular Zone**

Pieter Vancamp, PhD, Barbara Demeneix, Professor, Sylvie Remaud, PhD.
Centre National de la Recherche Scientifique, Paris, France.

The subventricular zone (SVZ) of the adult mammalian brain harbors neural stem cells (NSCs) that generate neurons and oligodendrocytes throughout life. Single-cell RNA-Seq analysis on mouse SVZ-NSCs isolated at different developmental stages established they gradually acquire their adult neurogliogenic identity between postnatal day 7 and 20. However, the factors governing this transition remain elusive. As a key factor driving transcriptional responses during brain development, as well as NSC lineage commitment in the adult SVZ, we hypothesized that thyroid hormone (TH) could fulfill this role. TH serum levels rise postnatally and peak around P15. Re-analysis of single-cell data from the P2 and P20 SVZ revealed a dynamically increased expression of the TH transporters Mct8 and Oatp1c1, as well as the TH-(in)activating deiodinases Dio2 and Dio3 in NSCs, signs that local TH action is promoted. Immunostainings showed a concomitant burst in SVZ-neurogenesis between P4 and P21. Then, to study what occurs if TH synthesis is blocked, we fed dams a 0.15% propylthiouracil-enriched diet from embryonic day 15 to P21. Postnatal hypothyroidism decreased Ph3-positive mitotic cell numbers at P4 and P21, whereas increased Sox2 expression coincided with a larger proportion SOX2-positive SVZ-NSCs and progenitors. In the dorsal SVZ, the main site of neurogenesis, less neuroblasts were detected at P21, while numbers of OLG2-positive oligodendroglia precursors did not change significantly. Next, we prepared in vitro neurospheres from dissected SVZs of control and PTU-treated mice, and allowed them differentiate with or without exogenous T3. The neuro/glia balance in neurosphere cultures prepared from P4 animals of either condition did not change when T3 was added, suggesting perinatal NSCs are irresponsive to TH. The balance did change in T3-treated neurospheres prepared from control P21 animals, however, not in those from P21 PTU-exposed mice, suggesting hypothyroid NSCs are irresponsible to T3. Lastly, we examined 3-month-old mice that regained a normal diet following developmental PTU exposure. Fewer oligodendroglia precursors in the SVZ resulted in a lasting altered neuro/glia output. A reduced ability to remember earlier-presented odors indicates impaired olfaction, a behavior strongly depending on SVZ-neurogenesis. Taken together the data indicate that developmental hypothyroidism affects postnatal SVZ organization and permanently alters NSC lineage commitment. Our study allows to determine relevant new read-outs to identify adverse outcome events on brain development and will permit comparison with events following exposure to endocrine diuraptors.

**Thyroid**

**THYROID HORMONE METABOLISM AND ACTION**

**Reverse T3 Level and T3 to Reverse T3 Ratio in Dried Blood Spot Samples at Birth May Facilitate Early Diagnosis of MCT8 Deficiency**

Hideyuki Iisayama, MD, PhD1, Hiroki Kabita, MD, PhD1, Masumi Iwasa, BE1, Shinsuke Adachi, MD, PhD1, Kyoko Takano, MD, PhD1, Masahiro Kikuchi, MD, PhD1, Yasuko Fujisawa, MD, PhD1, Hitoshi Osaka, MD, PhD1, Yasumasa Yamada, MD, PhD1, Akihisa Okumura, MD, PhD1, Samuel Refetoff, MD2.

1Aichi Medical University, Nagakute, Japan, 2Fukuyama City Hospital, Fukuyama, Japan, 3Shinshu University Hospital, Matsumoto, Japan, 4Hitachi General Hospital, Hitachi, Japan, 5Hamamatsu University School of Medicine, Hamamatsu, Japan, 6Shizouka University, Jichi Medical School, Shimotsuke, Japan, 7University of Chicago, Chicago, IL, USA.

**Background:** Monocarboxylate transporter 8 (MCT8) deficiency is an X-chromosome-linked neurodevelopmental disorder resulting from impaired thyroid hormone transporter across cell membrane. The diagnosis of MCT8 deficiency is typically delayed owing to the late appearance of signs and symptoms as well as inability of standard biomarkers of neonatal screening to make an early diagnosis. Here, we report for the first time the ability to identify MCT8 deficiency at birth using dried blood spot (DBS) samples.

**Methods:** We measured T3, T4, and reverse T3 (rT3) levels in DBS samples obtained at birth in healthy neonates (n = 42) and neonates with genetically confirmed diagnosis of MCT8 deficiency (n = 6). T3, rT3 and T4 levels were measured in 8 mm diameter DBS samples using liquid chromatography-tandem mass spectrometry.

**Results:** Mean ± SD level of T3 tended to be higher in the MCT8 group than that in healthy neonates (0.941 ± 0.183 ng/mL vs. 0.742 ± 0.195 ng/mL, p = 0.0525). More importantly rT3 level in the MCT8 group was significantly lower than that in healthy neonates (0.317 ± 0.065 ng/mL vs. 0.768 ± 0.196 ng/mL, p < 0.0001) and the T3/rT3 ratio in the MCT8 group was significantly higher (3.04 ± 0.67 vs. 1.01 ± 0.34, p < 0.0001) with no overlap of values. T4 was lower than that in healthy neonates (0.941 ± 0.183 ng/mL vs. 0.742 ± 0.195 ng/mL, p = 0.0525). More importantly rT3 level in the MCT8 group than that in healthy neonates (0.941 ± 0.183 ng/mL vs. 0.742 ± 0.195 ng/mL, p < 0.0001) and the T3/rT3 ratio in the MCT8 group was significantly higher (3.04 ± 0.67 vs. 1.01 ± 0.34, p < 0.0001) with no overlap of values. T4 was lower than that in healthy neonates (0.941 ± 0.183 ng/mL vs. 0.742 ± 0.195 ng/mL, p < 0.0001) and the T3/rT3 ratio in the MCT8 deficient group was higher (0.105 ± 0.0029 vs. 0.0051 ± 0.0010, p< 0.0001).

**Conclusion:** rT3 and T3/rT3 ratio measured in the DBS obtained from neonates can serve as biomarkers for diagnosis of MCT8 deficiency at birth.