

electrolyte disturbance (OR 6.12, $p < 0.001$) and those with a neurologic disorder (OR 2.46, $p = 0.002$). Admitted patients with a concomitant psychological disorder had lower odds of death (OR 0.38, $p = 0.03$).

Conclusions: This study identified clinical characteristics associated with TS-related admission including female gender, age 30-59, Caucasian race, and urban populations. Higher mortality was associated with patients 30-59 years and >60 years of age, electrolyte disturbances, blood disease, weight loss, paralysis, cancer, renal disease, and male gender.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Dio2-CreErt2 Knockin Model to Identify T3-Generating Cells That Express Type 2 Deiodinase in Tissues

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Background: Thyroid hormone promotes many developmental and homeostatic functions. Apart from adequate circulating levels, the concentration of the active hormone T3 within tissues may be amplified by type 2 deiodinase (Dio2) by conversion from the precursor T4. Dio2 is critical in auditory development, bone maturation, brain function and control of the hypothalamic-pituitary-thyroid axis. Despite its crucial role, an obstacle to studying Dio2 is that the protein has a short half-life, is at low levels and is often transiently expressed, making it difficult to identify Dio2 in tissues at cellular resolution. **Methods:** We derived a Dio2-CreERT2 knockin mouse that expresses tamoxifen-dependent Cre recombinase from the endogenous Dio2 gene. When crossed onto Ai6 reporter mice, following tamoxifen treatment, Dio2-CreERT2 expression is detected as fluorescent signal in specific cells in brain regions, pituitary, and other tissues. We showed previously that Dio2 is essential for hearing with rising expression levels in the cochlea prior to onset of hearing. The Dio2-CreERT2 model identified positive cell types in the cochlear spiral ligament, septal divisions and modiolus around the sensory epithelium. Dio2-positive fibrocytes were adjacent to and extended projections around blood capillary networks, the source of T4 supply. Transcriptome analysis of isolated positive cells revealed bone lineage-related origins for many of these cells. **Conclusion:** The Dio2-CreERT2 model detects Dio2 expression sensitively at cellular resolution. In the cochlea, Dio2-positive cell types reside in vascularized support tissues, suggesting combined endocrine and paracrine-like control of the T3 supply. Analysis of cell origins suggests novel interactions between endocrine and skeletal systems in promoting T3 action required for hearing.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

Identification of Cell Types that Express Dio3 Deiodinase, a Thyroid Hormone-Inactivating Enzyme, Using a Dio3-CreERT2 Reporter System

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Background: Thyroid hormone promotes development, growth and metabolism. The level of thyroid hormone ligand (triiodothyronine, T3) in tissues depends not only on circulating levels but also upon tight regulation by activating and inactivating deiodinases within tissues. Type 3 deiodinase (Dio3) inactivates T3 and its precursor thyroxine (T4) and mediates many functions including in neurodevelopmental, sensory and reproductive systems. Dio3 is subject to genomic imprinting. Despite its critical functions, Dio3 is often expressed transiently and at low levels in restricted cell populations making it difficult to detect in natural tissues. **Methods:** To visualize Dio3 expression at cellular resolution, we derived a Dio3-CreERT2 knockin allele that expresses tamoxifen-dependent Cre recombinase from the endogenous Dio3 gene. When crossed with Ai6 reporter mice, Dio3-CreERT2-positive cells display fluorescent signals. When tamoxifen-treated at neonatal ages, Dio3-CreERT2 recapitulates endogenous Dio3 expression as previously reported in brain: in the bed nucleus of the stria terminalis and preoptic nuclei. In addition, we uncovered several positive cell groups in the hypothalamus, brain stem, pituitary and other tissues. Drastic differences were observed for Dio3-CreERT2 as a paternally versus maternally inherited allele, revealing imprinting effect in specific cell types. Dio3-CreERT2 activity is enhanced by T3 administration, in accordance with Dio3 as a T3-inducible gene. **Conclusion:** The Dio3-CreERT2 model sensitively reveals Dio3-expressing cell types in tissues. The model is useful for studying expression patterns, imprinting and lineage tracing of Dio3-positive cells during development and homeostatic challenges.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

iPSC-Derived Human Hepatocytes as a Novel Model to Investigate Thyroid Hormone Action and Signaling

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Thyroid hormone (TH) actions are essential to normal metabolism and neurologic function. Abnormal TH levels can lead to significant morbidity including metabolic abnormalities, cardiac disease, and obesity. TH action is mediated by the thyroid hormone receptor (TR) isoforms and their coregulators. In the disease models of Resistance to Thyroid Hormone (RTH) it is clear that the TR isoforms play tissue-specific roles in humans, however a platform for drug discovery to potentially treat the diseases does not exist. We hypothesize that human hepatocytes (iHeps) derived from induced pluripotent stem cells (iPSCs) will serve as an ideal model to study TH signaling and to delineate mechanisms of TH diseases in humans. To investigate the TH action in humans, we have developed a serum free human iPSC hepatic differentiation protocol that utilizes sequential exposure to growth factors to mimic actual

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 TR β 1 the main TR isoform expressed in liver. we knocked out TR β 1 (THRBKO) 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 Δ 337T mutation found in human TR β 1 that causes RTH. Both THRBKO and Δ 337T 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 TR β 1 deletion as the response to T3 was lost on both positive and negative THRB targets. In the Δ 337T 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

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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 (NCoR1^{lox/lox}) and SMRT (SMRT^{lox/lox}) 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 KO 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/Pcp2-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

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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 μ IU/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 μ IU/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) μ IU/mL. The mean LT4 dose was 105 (36) μ g/day [1.4 (0.5) μ g/kg/day] and LT4 dose was 34 (9) μ g/day [0.4 (0.1) μ g/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