period of 3 years (range 1-10 years), when they were all older than 7 years. In this group, 6 of 7 were affected by CCH and only one case by a microMTC. There were not any persistent surgical adverse events and all of them are still in clinical remission. 41 of 90 GC, who are still in active surveillance, were younger than 18 years at time of RET screening: nowadays, 10/41 are older than 18 years and 15/41 are older than 14 years, all with calcitonin still in the normal range. Conclusions: we demonstrated that the calcitonin-based thyroidectomy is a safe approach in GC. Intriguingly, this approach seems to be interesting especially in children in or order to perform still an early and safe surgery but when they are older, possibly adults.

Neuroendocrinology and Pituitary ADVANCES IN NEUROENDOCRINOLOGY Angiotensin II Stimulates Microglia Cell Inflammatory Responses Jean Paul Mailere Véliz, B.S.¹, Dra. Yaritza Inostroza-Nieves, PhD², Jose R. Romero, PhD⁴, Claudia Arenas, B.S.³, Diego Capo, B.S.⁵, ¹SAN JUAN BAUTISTA SCHL OF MED, San Juan, PR, USA, ²Harvard Medical School, Boston, MA, USA.

SUN-254 Angiotensin II Stimulates Microglia Cell Inflammatory Responses Angiotensin II (AngII) is the principal effector molecule of the renin-angiotensin system (RAS). It’s effects on the cardiovascular and renal system are well-documented. AngII acts mainly via interaction with the AngII type-1 receptor (AT1R). Disordered levels of AngII lead to hypertension and cardiovascular disease. Increasing evidence suggests that AngII may also play a role in the pathophysiology of neurodegenerative diseases through unclear mechanisms. We investigated AngII, AT1R and AT2R levels in a mouse model of neurodegenerative disease, the experimentally induced autoimmune encephalomyelitis (EAE) mouse. In EAE mice, AngII and AT1R gene expression in brain tissue were significantly increased when compared to control mice (3.2 folds ±1.9, p<0.05, n=5; and 2.6 folds ±1.1, p<0.01, n=5 respectively). In addition, iNOS mRNA expression by qRT-PCR was likewise upregulated in EAE mice compared to control (3.4 ± 1.4 folds, p<0.01, n=5). We then studied the effects of AngII in human microglial cells (HMC3)-resident innate immune cells of the central nervous system (CNS). In HMC3 cells, treatment with AngII up-regulated the expression IL-6 (3.9 folds ± 1.2, p<0.01, n=4) and increased IL-6 concentration by 83% (p<0.05, n=4) by ELISA; effects that were blocked by the AT1R antagonist, Losartan. Also, AngII induced TNF-α production, increasing its concentration by 90% (p<0.05, n=4), an increase that was blocked by Losartan. We also quantified Nitric Oxide (NO) production by using Griess Reagent and reactive oxygen species (ROS) production by the MUSE Oxidative Stress assay. In these cells, NO and ROS production were significantly increased by AngII (p<0.05, n=4) and treatment with Losartan reduced their production (p<0.05, n=4). In addition, AngII treatment induced iNOS overexpression (2.5 folds ±0.8, p<0.05, n=4); results that are consistent with increases in the EAE mice. These data suggest that AngII can activate microglia cell inflammatory responses and as such may contribute to the pathophysiology of CNS inflammation and neurodegenerative diseases.

Genetics and Development (including Gene Regulation) GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II Single-Cell RNA-Sequencing Deciphers POMC Neuron Destiny Hui Yu, PhD¹, Graham Jones, PhD², Zoe Thompson, PhD³, Marcelo Rubinstein, Ph.D², Malcolm James Lou, MD,PHD⁴. ¹University of Michigan, Ann Arbor, MI, USA, ²Universidad de Buenos Aires, Buenos Aires, Argentina, ³University of Michigan Med School, Ann Arbor, MI, USA.

MON-709 The hypothalamus is one of the critical brain nodes regulating body weight and energy homeostasis. Within this node, POMC neurons sense nutrient and hormonal signals to release melanocortin peptides that induce satiety, whereas AgRP/NPY neurons exert opposite effects by releasing AGRP that promotes feeding. Immature neurons in the hypothalamic ventricular zone start to express POMC at E10.5, reach a maximum number at E14.5 and then decrease to stabilize at E18.5. However, it remains elusive how POMC expressing precursors adopt their final cell fates. Therefore, the goal of this study was to decipher the temporal sequence of transcription factor (TF) expression leading to the terminal differentiation of POMC neurons. Red fluorescent cells collected from dissociated hypothalami of POMC—Dimer—dsRed mice at six critical developmental time points - E11.5, E13.5, E15.5, E17.5, P5 and P12- were FACS sorted for the 10X genomics scRNA-seq pipeline. Unsupervised cell clustering identified 11 distinct clusters based on their transcriptional profiles. Eight of the clusters were highly-enriched for neuronal signature genes and were further characterized based on their transcript levels for POMC (high, medium or low) and other distinct feature genes. Cells in the POMC-high cluster expressed genes identified previously to modulate POMC expression, including Isl1, Nkx2-1, and Tbx3, together with several novel candidate TFs. Unexpectedly, Nr5a1, the ventromedial hypothalamic nucleus marker gene encoding SF1, was highly expressed in the POMC-high cluster at early stages. One of the POMC-low clusters highly expressed Otp, Agrp, Npy, Sst and Calcr while a second was highly enriched with Tac2, Kiss1, Pdyn, Prlr, Ar and Esr1 transcripts. All the clusters showed direct correlations of embryonic stage with the expression of progressively more mature markers of differentiation, thereby extending previous reports of these clusters based on single time points. Moreover, our results uncovered five novel POMC neuron clusters with unique patterns of TF gene expression. For comparison of these data to the adult hypothalamus, we performed a TRAP-Seq study using POMC-CreERT,Rosa26GFP-L10a mice. Prdm12 and Tbx3 were among the most highly differentially expressed TFs in the POMC neuron affinity purified translatome. Similarly, Cited1, Npy2r, and Asb4 were highly expressed in both the POMC-high cluster and the TRAP-Seq derived POMC translatome. This comprehensive molecular

STEROID HORMONE SIGNALING II GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II
characterization of POMC cells during development sheds new light on the molecular diversification of early POMC neuron precursors and provides a valuable resource for elucidating the regulatory mechanisms defining POMC neuron subgroups in the hypothalamus.

Diabetes Mellitus and Glucose Metabolism
CLINICAL AND TRANSLATIONAL STUDIES IN DIABETES

Genetic Knockout of Intestinal Hexokinase Domain-Containing Protein 1 Affects Whole-Body Glycemic Control and Triglyceride Metabolism
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MON-642
Hexokinase domain-containing protein 1 (HKDC1) is a recently discovered putative fifth hexokinase that is widely expressed in a variety of human and mouse tissues. Previous work indicates that HKDC1 is important for whole-body glucose homeostasis and utilization in pregnancy and aging, and suggest roles for HKDC1 in nonalcoholic fatty liver disease development and progression of hepatocellular carcinoma. Prior work in the letter further showed that global heterozygous-deleted HKDC1 mice exhibit blunted uptake of triglycerides following an olive oil bolus compared to wild-type mice, suggesting a role for intestinal HKDC1 expression in intestinal lipid metabolism (unpublished results). To specifically study the significance of intestinal HKDC1 on whole-body glucose and lipid homeostasis, we utilized Cre-mediated recombination of HKDC1 in which Cre was expressed under the control of the villin gene promoter, creating a mouse model in which HKDC1 expression is specifically deleted in the intestinal epithelium. Quantitative RT-PCR data confirmed the knockout of HKDC1 within the mouse intestine in young and aged mice, while HKDC1 expression in other tissues was comparable to wild-type mice. Next, intestinal HKDC1 knockout mice and their wild-type littermate controls were either maintained on a normal diet or were switched to a high fat diet at 6 weeks of age to simulate the state of impaired glucose tolerance, and the effects of intestinal HKDC1 on glucose and lipid homeostasis were analyzed between 28-34 weeks of age. Mice fed a normal diet did not exhibit any differences in serum glucose or triglyceride during oral/intraperitoneal glucose tolerance tests or oral olive oil bolus, respectively, regardless of intestinal HKDC1 status. Interestingly, mice lacking intestinal HKDC1 that were on a high fat diet demonstrated improved overall glycemic control compared to wild-type mice after the administration of an oral glucose load, all while there were no changes in insulin levels, gluconeogenesis or insulin tolerance related to HKDC1 status. Additionally, introduction of an intraperitoneal glucose load to mice fed a high fat diet did not alter glucose control in the presence or absence of intestinal HKDC1. However, high fat diet-fed mice lacking intestinal HKDC1 did not have a significant increase in serum triglyceride following an oral olive oil bolus, while their stool fat and triglyceride content were comparable to wild-type. Collectively, these data indicate that intestinal HKDC1 has important roles in glucose and triglyceride metabolism within the intestinal epithelium, and further suggest a role in whole-body glucose homeostasis and in the development of insulin resistance and diabetes.

Thyroid
THYROID NEOPLASIA AND CANCER

Deep-Machine Learning for Objective Quantification of Nerves in Immunohistochemistry Specimens of Thyroid Cancer
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MON-535
Introduction: Nerves in the cancer microenvironment have prognostic significance, and nerve-cancer crosstalk may contribute to tumour progression, but the role of nerves in thyroid cancer is not known (1). Reproducible techniques to quantify innervation are lacking, with reliance on manual counting or basic single-parameter digital quantification.

Aims: To determine if a deep machine learning algorithm could objectively quantify nerves in a digital histological dataset of thyroid cancers immunostained for the specific pan-neuronal marker PGP9.5.

Methods: A training dataset of 30 digitised papillary thyroid cancer immunohistochemistry slides were manually screened for PGP9.5 positive nerves, annotated using QuPath (2). 1500 true positive nerves were identified. This dataset was used to train the deep-learning algorithm. First, a colour filter identified pixels positive for PGP9.5 (Model 1). Then, a manually tuned colour filter and clustering method identified Regions of Interest (ROIs): clusters of PGP9.5 positive pixels that may represent nerves (Model 2). These ROIs were classified by the deep learning model (Model 3), based on a Convolutional Neural Network with approximately 2.7 million trainable parameters. The full model was run on a testing dataset of thyroid cancer slides (n=5), containing 7-35 manually identified nerves per slide. Model predictions were validated by human assessment of a random subset of 100 ROIs. The code was written in Python and the model was developed in Keras.

Results: Model 2 (colour filter + clustering only) identified median 2247 ROIs per slide (range 349-4748), which included 94% of the manually identified nerves. However, most Model 2 ROIs were false positives (FP) (median 85% FP, range 68-95%), indicating that Model 2 was sensitive but poorly specific for nerve identification. Model 3 (deep learning) identified fewer ROIs per slide (median 1068, range 150-3091), but still correctly identified 94% of manually annotated nerves. Of the additionally detected ROIs in Model 3, median FP rate was 35%. However, in slides where higher non-specific immunostaining was present, then the number of FP ROIs was >90%.

Conclusion: Simple image analysis based on colour filtration/cluster analysis does not accurately identify immunohistochemically labelled nerves in thyroid cancers.