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Studies sought epidemiological profiles to establish risk groups for Covid-19's evolution and discovered that the age group above 65 years old is one of the most severely affected, due to the presence of other comorbidities. In the USA approximately 55 million individuals are above that age range and more than 23 million are elderly obese individuals, alongside the high mortality rate in this population and lack of proven effective medicine against Covid-19, therapeutic alternatives against this disease are sought. With the occurrence of inflammatory pulmonary conditions increased by SARS-Cov-2 infection, irisin was indicated as having a potential preventive action. This peptide is secreted endogenously by striated skeletal muscles with fibronectin type III domain-containing protein 5 (FNDC5) proteolysis, when preceded by continued exercise of low to moderate intensity for more than 20 uninterrupted minutes. Regarding the weight variant, previous studies showed low levels of FNDC5 in people with excess fatty acids (in humans, adipose tissue divides into white, favoring the deposit of lipids, triglycerides and fat, more easily inflamed, and brown that stimulates thermogenesis). Among other functions, FNDC5 also stimulates the coactivator 1-alpha of PPAR gamma (PGC-1α) which corroborates with the uncoupling between the Uncoupling Protein 1 (UCP1) and the mitochondria. When UCP1 is disincorporated from this organelle, it becomes active and inhibits ATP synthesis, releasing heat, as well as favoring fatty acid oxidation and provides the mechanism of browning of white fat. After this conversion, irisin reduces ¼ of lipid accumulation present in the adipocyte by lipolysis, significantly increasing the levels of serum triglycerides and glycerols, as well as considerably reducing oxidative stress and DNA damage. Other genes expressed by irisin also assist in the fat browning process, such as TMEM26, ELOVL3, CIDEa and COX7a. Regarding the age variant, the SARS-CoV-2 Spike protein benefits from the high concentration of angiotensin-converting enzyme 2 (ACE2) receptors, present in elderly pulmonary alveoli, which bind to the airway and enable contagion. However, ACE2 is negatively regulated by Tribbles homolog 3 (TRIB3) protein from its connection to the nucleocapsid protein of the virus, generating some protection for the individual when TRIB3 is at satisfactory levels. In elderly men, this protein is below normal levels, making this population more vulnerable to SARS-CoV-2 infection. Moreover, it was verified that irisin's weekly synthesis promoted by regular physical exercises triples TRIB3 levels in pulmonary alveoli. In short, this hormone was able to negatively modulate two important risk factors related to the inflammatory profile of this elderly population (weight and age), and thus irisin should be considered a potential molecule in the prevention against Covid-19.

## Genetics and Development (including Gene Regulation)

### FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

### *Genetic Study in a Cohort of Children With ROHHAD Syndrome*

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**Introduction:** Rapid-onset obesity, hypoventilation, hypothalamic dysfunction and autonomic dysregulation (ROHHAD) is a rare syndrome beginning at 3-6 years of age with approximately 150 cases described. Additional features include eye abnormalities, neurobehavioral dysfunction and paraneoplastic tumors. The etiology of the complex phenotype remains unknown.
**Methods:** This study aims to investigate the genetic landscape of this complex phenotype by whole exome sequencing (WES) and copy number variation (CNV) analysis. We recruited 33 families (27 trios, 1 duo and 5 singletons) with a proband with ROHHAD syndrome (Ize-Ludlow 2007, Pediatrics). WES of 89 individuals was performed at the Center for Mendelian Genomics, Broad Institute. The Illumina platform with a mean coverage of ~100X (> 90% targets 20x) and Infinium Global Screening Array BeadChip 24v1.0 were used.
**Results:** This report includes 28 probands (female = 18, 64%) with rapid onset obesity (100%), hypoventilation (88%), hypothalamic dysfunction (69%), eye disorders (62%) and neurobehavioral abnormalities (76%). Neuroendocrine tumor, ganglioneuroblastoma, was present in 38% (n=13). No unifying causative single gene or CNV was identified, but a number of sequence variants are prioritized.
*ARNT2*, which encodes for a helix-loop-helix transcription factor, plays a role in the development of the hypothalamic-pituitary axis, postnatal brain growth, and visual and renal function. The de novo monoallelic missense variant was found in a 14-year old white girl (BMIz +3.25) with extreme obesity and a neurobehavioral phenotype.
*OCRL1*, a multi-domain protein involved in cytoskeleton-plasma membrane adhesion, endosomal trafficking and in primary cilium assembly. Mutations in this gene have also been known to cause Lowe syndrome. A hemizygous X-linked frameshift variant in a 5-year old white boy with extreme obesity (BMIz +5.48), central hypoventilation neurobehavioral dysfunction and ganglioneuroblastoma.
A monoallelic missense variant in *NSD1*, a transcriptional intermediary factor acting as a histone methyltransferase, was identified in a 8-year old Hispanic girl with severe obesity (BMIz +2.91), neurobehavioral disorder, pituitary and eye dysfunction and ganglioneuroblastoma. *NSD1* is known to cause Sotos and Beckwith-Wiedemann.
Compound heterozygous variants in *KIF7*, a key component of the Hedgehog signaling pathway, were identified in a

14-year old white girl with severe obesity (BMIz +3.00), autistic behavior, pituitary dysfunction and central hypoventilation. This gene is known to cause autosomal recessive hydrolethalis and acroscallosal syndromes with mutations also noted in Bardet-Biedl, Meckel and Joubert syndromes. **Conclusion:** While no unifying genetic cause has been identified in ROHHAD syndrome, it is possible that the phenotype represents a collection of complex genetic syndromes.

## Genetics and Development (including Gene Regulation)

### FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

***Heterodimerization and Subcellular Distribution of Melatonin and Cannabinoid Type 1 Receptors***

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Membrane receptors belonging to the G proteins coupled receptors (GPCRs) form the largest family of proteins in the human genome with more than 800 members. Until recently GPCRs functions were thought to occur only at the plasma membrane after activation upon binding of their cognate ligand. However evidences show that many functional GPCRs are found in intracellular compartments opening new direction of research to understand their roles in a cellular context. Among these intracellular compartments mitochondria are the latest organelle in which some GPCRs were identified. Melatonin receptor type 1 (MT1) and cannabinoid receptor type 1 (CB1) were identified in mouse neuronal mitochondria where they were shown to exert an inhibitory action on cytochrome c release (MT1) or on the respiratory chain (CB1). Using several techniques my current results describe a new cross-talk between MT1 and CB1 receptors. Confocal analysis of immunofluorescence experiments of cells coexpressing both receptors showed a high degree of colocalisation. A combination of coimmunoprecipitation experiments performed on extracts of transfected HEK293T or HeLa cell lines and immunodetection of receptors by Western-blot revealed that MT1 and CB1 receptors can physically interact to form heterodimers in absence of ligand. Heterodimers formation was also confirmed by Proximity Ligation Assay (PLA) experiments in live HEK293T and HeLa cells. Confocal analysis revealed a colocalisation of PLA staining with the mitochondrial marker TOMM20. Experiments in relation with the functional role of MT1 and CB1 in mitochondria are ongoing.

## Genetics and Development (including Gene Regulation)

### FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

***Histone Lysine Demethylase 1A Is a Master Regulator of Genes Necessary for Trophoblast Cell Proliferation***

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Histone lysine demethylase 1A is a master regulator of genes necessary for trophoblast cell proliferation.

A proper functioning placenta is critical for pregnancy, fetal growth and development and postnatal health. Trophoblast cell proliferation and differentiation is critical for placental development and function. Recently we demonstrated that the histone lysine demethylase KDM1A binds to androgen receptor (AR) in human and sheep trophoblast cells, and targets the same promoter region of vascular endothelial growth factor A (VEGFA), suggesting a role for KDM1A and AR in early placental angiogenesis. The goal of this study was to determine the function of KDM1A during early placental development. We hypothesized that KDM1A regulates genes that are necessary for trophoblast cell proliferation, and early placental development. To this end, both in vitro and in vivo approaches were used in this study. ACH-3P cells (human first trimester trophoblast cells (CT and EVT) fused with the choriocarcinoma cell line AC1-1) were used, and a KDM1A knock out (KO) cell line was generated using CRISPR-Cas 9 based genome editing. KDM1A KO in ACH-3P cells led to significant ($P<0.05$) reduction in AR and VEGFA. Furthermore, factors important for cell proliferation and trophoblast cell development high mobility group AT-hook 1 (HMGA1), LIN28, and MYC protooncogene (cMYC) were significantly ($P<0.05$) lower in KDM1A KO ACH-3P cells. Cell proliferation assays revealed a significant ($P<0.05$) reduction in KDM1A KO ACH-3P cells compared to scramble controls. An *in vivo* experiment was conducted to demonstrate a role for KDM1A in placental development, using the sheep as a model. Day 9 hatched blastocysts were flushed and infected with a Lenti-CRISPRv2 KDM1A target construct (n=4) to knockout KDM1A specifically in the trophectoderm, or with SC (n=5). Infected embryos were transferred to recipient ewes and embryos were collected at gestational day 16. Data suggests that KDM1A KO in trophoblast cells is necessary for conceptus elongation. Current experiments are ongoing to determine the effects of KDM1A and AR knockdown using shRNA lentiviral target vectors on conceptus elongation and pregnancy. Collectively these results indicate that KDM1A plays a central role in regulating genes necessary for trophoblast cell proliferation.

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## Genetics and Development (including Gene Regulation)

### FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY