

Methods: We conducted a retrospective review of patients (n = 645) who had histopathologically confirmed diagnosis of CD from 2005-2019 (NCT NCT00060541). We selected patients that had ≥ 3 plasma ACTH values over a 7 day span prior to surgical or medical intervention. We grouped the ACTH measurements into morning (AM) and midnight (PM) values to account for diurnal variation in ACTH secretion. We then analyzed post-operative hormone measurements performed every 6 hours prior to administration of replacement corticosteroids. Remission was assigned to patients with nadir serum cortisol level ≤ 5 mcg/dL within ten days post-operatively^{3,4}.

Results: We found 54 patients with multiple PM (n = 27) and AM (n = 41) ACTH measurements within a 7 day span. We found that the median coefficient of variation (CV) of intra-patient variability was 19.7% (N=41) (95% CI:12.5-27.5) for the AM and was 24% (N=27) (95% CI: 9.6-31.8) for the PM. Age, the number of tests, or the length of test period were not correlated with CV or absolute levels of ACTH. The intraclass correlation coefficient (ICC) of the AM data set was 0.59 and the PM data set was 0.79 which demonstrates a good and excellent reliability respectively. We found that that, in general, 30-60% decrease from pre-operative ACTH levels predicted remission from CD. ACTH decrease $>50\%$ on POD2 and 3 had 100% specificity and sensitivity in predicting remission. The decrease in ACTH preceded cortisol nadir in 3/10 patients by 24 hours.

Conclusion: We found significant intra-patient variability in plasma levels of ACTH at individual diurnal timepoints in CD patients. We also found that the change in ACTH $>50\%$ on POD2 or 3 is an excellent predictor of remission from CD.

Reproductive Endocrinology

OVARIAN FUNCTION — FROM OLIGOMENORRHEA TO AMENORRHEA

Emergence of Ovarian Hyperandrogenism and Luteal Insufficiency Following ESR1 Knockdown in the Mediobasal Hypothalamus of Adult Female Rhesus Monkeys

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Diminished estradiol (E2) negative feedback action by neuronal ESR1 in the arcuate nucleus (ARC) of the mediobasal hypothalamus (MBH) is hypothesized to cause gonadotropin-releasing hormone (GnRH) hypersecretion, and thus LH excess, contributing to ovarian

hyperandrogenism in polycystic ovary syndrome (PCOS). In primates, including humans, however, the mediating estrogen receptor is unknown. Thus, to test the hypothesis that diminished E2 action on ARC ESR1 contributes to female primate ovarian hyperandrogenism, eleven, ovary intact, adult female rhesus macaques, pair housed with female peers, received five 12 μ l MRI-guided MBH infusions into the rostral-to-caudal extent of both right and left ARC. Each infusion comprised gadolinium contrast agent and $\sim 3\text{-}4 \times 10^{10}$ adeno-associated virus 8 (AAV8) particles containing either a shRNA specific for ESR1 (n=6, ERaKD) or scrambled shRNA (n=5, control). Mid-surgery MRI scans identified targeting accuracy. 2-2.5 years following AAV8 infusion, EIA-determined P4 values were obtained from twice weekly serum samples; samples obtained during the follicular phase of menstrual cycles or anovulatory periods were submitted to liquid chromatography, tandem mass spectrometry (LCMS) for additional steroid hormones. LCMS-determined values were also obtained 0 hours (h) and 24 h following an IM injection of 200IU hCG. Both ERaKD (28.5 ± 1.3 days, mean \pm SEM) and control (34.0 ± 3.3 days) female groups exhibited comparably regular menstrual cycles. ERaKD exhibited higher circulating levels of LH (2.8 ± 0.2 ng/ml, p=0.03), androstenedione (A4, 0.43 ± 0.03 ng/ml, p=0.03) and testosterone (T, 0.23 ± 0.03 ng/ml, p=0.09), and LH/FSH ratio (1.7 ± 0.2 , p=0.05) compared to controls (LH, 2.1 ± 0.4 ; A4, 0.30 ± 0.05 ; T, 0.18 ± 0.01 ng/ml; LH/FSH 1.3 ± 0.2). Following an ovarian androgen-stimulating hCG injection, ERaKD 24-h peak levels for T (0.28 ± 0.01 ng/ml) were higher (p=0.03) compared to controls (0.21 ± 0.01 ng/ml). In addition, luteal insufficiency emerged in ERaKD females, with mean (2.4 ± 0.3 ng/ml), peak (3.6 ± 0.4 ng/ml) and area-under-the-curve (AUC, 23.2 ± 4.2 ng/ml/days) P4 values diminished compared to controls (mean, 3.6 ± 0.1 , p=0.01; peak 5.7 ± 0.1 ng/ml, p=0.01; AUC, 43.7 ± 6.7 ng/ml/days, p=0.03). Taken together, these results suggest that knockdown of ARC ESR1 disrupts Gn stimulation of ovarian function, contributing to female monkey ovarian hyperandrogenism and menstrual cycle impairment emulating PCOS in women.

Bone and Mineral Metabolism

NEW INSIGHTS INTO PTH AND CALCIUM RECEPTOR SIGNALING

The Roles of GNAQ and GNA11 in Calcium-Sensing Receptor (CaSR) Signalling

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The G-protein subunits $G\alpha_{11}$ and $G\alpha_q$, which share $>90\%$ peptide sequence identity and are encoded by the *GNA11* and *GNAQ* genes, respectively, mediate signalling by the calcium-sensing receptor (CaSR), a class C G-protein coupled receptor (GPCR) that regulates extracellular

calcium (Ca^{2+}) homeostasis. Germline $\text{G}\alpha_{11}$ inactivating and activating mutations cause familial hypocalciuric hypercalcaemia type-2 (FHH2) and autosomal dominant hypocalcaemia type-2 (ADH2), respectively, but such $\text{G}\alpha_q$ mutations have not been reported. We therefore investigated the DiscovEHR cohort database, which has exomes from 51,289 patients with matched phenotyping data, for such *GNAQ* mutations. The DiscovEHR cohort was examined for rare *GNAQ* variants, which were transiently expressed in CaSR-expressing HEK293A $\text{G}\alpha_{q/11}$ knockout cells, and their effects on CaSR-mediated intracellular calcium (Ca^{2+}) release and MAPK activity, in response to increasing concentrations of extracellular calcium were assessed using a nuclear factor of activated T-cells response element (NFAT-RE) luciferase reporter construct and a serum response element (SRE) luciferase reporter construct, respectively. Responses were compared to those of wild-type (WT), inactivating FHH2-associated *GNA11* mutations (Leu135Gln and Phe220Ser), and engineered *GNAQ* mutations that were equivalent to the FHH2-causing *GNA11* mutations. $\text{G}\alpha_{q/11}$ protein expression was confirmed by Western blot analysis. Six rare missense *GNAQ* variants (Arg19Trp, Ala110Val, Gln299His, Ala302Ser, Ala331Thr, Val344Ile) were identified in DiscovEHR individuals, all of whom had mean plasma calcium values in the normal range (8.30–10.00 mg/dL). Functional characterisation of all six $\text{G}\alpha_q$ variants showed no significant difference to WT $\text{G}\alpha_q$ responses, thereby indicating that these variants are unlikely to be disease-causing mutations. In addition, the FHH2-causing *GNA11* mutations (Leu135Gln and Phe220Ser) had significantly reduced responses, compared to WT $\text{G}\alpha_{11}$; however, this could be compensated by WT $\text{G}\alpha_q$. *GNAQ* Leu135Gln and Phe220Ser, in contrast to their $\text{G}\alpha_{11}$ counterparts, showed no differences in protein expression or signalling responses when compared to WT $\text{G}\alpha_q$. Our study, which provides mechanistic insights into the differences between $\text{G}\alpha_q$ and $\text{G}\alpha_{11}$, indicates that $\text{G}\alpha_q$, unlike $\text{G}\alpha_{11}$, does not play a major role in the pathogenesis of FHH2 or ADH2.

Adrenal

ADRENAL CASE REPORTS II

Giant Bilateral Myelolipomas in a Patient with Congenital Adrenal Hyperplasia

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INTRODUCTION Adrenal myelolipomas are rare, benign adrenal tumors composed of adipose and hematopoietic tissue. The first mass was identified by Gierke in 1905 and named by Oberling in 1929. Myelolipomas often coexist with CAH and other hormonal disorders although this relationship is still unclear. We present a rare case of giant bilateral myelolipomas in a patient with CAH. **CASE REPORT** A 28-year-old female with a history of CAH due to 21-hydroxylase deficiency diagnosed at birth presented to our endocrinology clinic after being lost to follow up for 4 years and a history of poor follow up for

several years before that. She reported being on varying doses of hydrocortisone throughout her life with the most recent being 10mg TID with poor adherence. Over the past 4 years, the patient noticed increased hair growth on her face, abdomen, inner thighs and back, abdominal striae, and weight gain of 80-100lbs. She also reported a history of bilateral flank pain for the last several years, requiring several ER visits where she was told she had adrenal nodules based on scans that were done. A few months prior to her first visit to our clinic she developed increasing flank pain, which prompted a visit to a local ER. A CT of the abdomen revealed bilateral adrenal septated complex cystic masses measuring 19x12x20 cm on the left and 12x11x11 cm on the right. Initial biochemical workup was consistent with poorly managed CAH: ACTH 45.2 pg/mL (n 7.2 - 63.3), total testosterone 401 ng/dl (n 8-48), androstenedione 2085 ng/dl (n 41-262), 17-OH progesterone 18880 ng/dl, (n 15-290), DHEA-sulfate 423.5 ug/dl, (n 84.8-378), and estradiol 72.0 pg/dl, (n 12.5-498). Plasma renin activity was 13.904 ng/mL/hr (n 0.167-5.380 ng/mL/hr). Based on laboratory results hydrocortisone was decreased to 10mg BID and dexamethasone was added. Due to severe recurrent pain the patient underwent bilateral adrenalectomy. On pathology the bilateral masses were found to be myelolipomas with the left diameter measuring 22.3 cm and the right measuring 16.5 cm. Post-operatively her lab values showed significant improvement compared to her initial workup: ACTH 4.1 pg/mL, total testosterone 6.7 ng/dl, androstenedione 73 ng/dl, 17-OH progesterone 29 ng/dl, DHEA sulfate 7.2 ug/dl, and estradiol 228.3 pg/dl. Plasma renin was also within normal range at 1.776 ng/mL/hr. On follow up, the patient had recovered well and reported improvement in her flank pain. **CONCLUSION** This is a rare case of giant bilateral adrenal myelolipomas. Despite these tumors being benign and often asymptomatic, they are clinically relevant due to their role in the differential diagnosis of an adrenal mass. The presence of megakaryocytes in a biopsy specimen of a fatty adrenal mass is pathognomonic for myelolipoma. Treatment is guided by the tumor size and patient presentation; masses >7 cm, hormonally active, or causing abdominal pain should be surgically removed as demonstrated in this case

Neuroendocrinology and Pituitary

NEUROENDOCRINE & PITUITARY PATHOLOGIES

Depression, Subjective Stress and Serum Osteocalcin Concentrations in People with Type 2 Diabetes Mellitus

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