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Introduction: Familial glucocorticoid deficiency (FGD) is characterised by isolated glucocorticoid deficiency in a patient who retains normal mineralocorticoid production. FGD causing mutations in the MC2R accessory protein, *MRAP*, are often splice-site or nonsense mutations resulting in a truncated protein. Many of these mutations occur at the canonical donor splice-site of intron 3, where it has been shown previously that c.106 + 2_3dupTA, for example, results in skipping of the first coding exon with unknown consequences at the protein level. Patients and methods: DNA was isolated from three consanguineous individuals diagnosed with early onset FGD (0 - 13 months) with high ACTH and/or low cortisol levels and underwent whole exome sequencing. The proband in family 1 (P1) presented at 13 months and had a hyperpigmented sibling who died in neonatal period due to adrenal failure. Patient 2 (P2), who also had a family history of adrenal insufficiency, was noted to be hyperpigmented at birth with markedly raised ACTH, patient 3 (P3) was noted to have diffuse hyperpigmentation in the early neonatal period and on formal testing at 16m was found to have low serum cortisol. Variants were confirmed using Sanger sequencing and predicted splice-site mutations were investigated using an *in vitro* splicing assay. **Results:** Homozygous mutations in *MRAP* were identified in all three cases which were heterozygous in their parents. Previously described mutations, c.106 + 1delG (chr21:33671388delG; rs1476574441; CD050155) in P1 and c.106 + 2dupT (Chr21: 33671390_91insT; rs761576317; CI118288) in P2 at the canonical donor splice-site of intron 3, were identified, with the former predicted to destroy the splice site and the latter to weaken it. These mutations *in vitro* resulted in the complete skipping of exon 3, which contains the translational start site, and presumably result in no protein product. A novel homozygous mutation in intron 4, c.206 + 5G>T; (chr21:33679055G>T rs1064796398) was identified in P3, but was not predicted to alter splicing. In vitro, this mutation negates the canonical donor splice site and creates two different alternative sites, both resulting in frameshifts and predicted early termination of the protein (p.Val44fs*50, p.Pro72fs*90). **Conclusion:** All mutations reported here are predicted to produce no protein, either because the start site is excluded (for c.106 + 1delG and c.106 + 2dupT) or because the transcripts are likely to undergo nonsense mediated decay (for c.206 + 5G>T), resulting in the early onset FGD seen in the patients. Splice prediction protocols, although effective for variants within 2bp of exon/intron boundaries may not predict the true outcome of a base change whereas the splice assay conclusively revealed the effect of all three variants allowing us to assign pathogenicity to them.

Adrenal

ADRENAL – CLINICAL RESEARCH STUDIES

Incidence of Venous Thromboembolic Events in Patients With Endogenous Cushing Syndrome

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Background: Hypercortisolemia is a hypercoagulable state associated with increased risk of venous thromboembolic events (VTE). The reported incidence of VTE in patients with ACTH-dependent or independent Cushing Syndrome (CS) is variable, ranging from 3 to 14%. Our aim was to assess the incidence of clinically significant VTE among patients with endogenous CS and to identify risk factors for the development of VTE.
Methods: We conducted a single center retrospective longitudinal study of adult patients diagnosed with endogenous CS between 2010 and 2020. Patients with a known prothrombotic disease (e.g. Factor V Leiden), insufficient data, or non-neoplastic hypercortisolism were excluded. Data collected included patient demographics, presenting symptoms, biochemical and radiological workup, treatment details, and incidence of clinically significant VTE.
Results: A total of 114 patients (mean age of 45.55 ± 14.78 years, 79.8% women) followed for mean of 3.26 ± 2.9 years were included. Of the 114 patients, 58 (50.9%) had Cushing disease (CD), 40 (35.1%) had CS due to adrenal adenoma/hyperplasia, 6 (3.5%) had adrenocortical carcinoma (ACC), and 10 (8.8%) had ectopic Cushing syndrome (eCS). The overall incidence of VTE at any time point was 14/114 (12.3%); 11 (79%) VTEs were associated with presence of an additional VTE risk factor (8 surgery and 3 malignancy). Prior to any intervention for CS, 3 of 114 (2.6%) patients had a VTE. Surgery for CS (adrenalectomy, transsphenoidal surgery, tumor resection) was performed in 97 patients (85.1%) whereas 17 were treated medically (n=10), died before treatment (n=1) or observed (n=6). VTE occurred in 2 patients receiving medical therapy for CS. The post-operative incidence of VTE was 9 (9.3%; 4 in CD, 1 in adrenal CS, 3 in ACC, and 1 in eCS). VTE occurred ≤ 3-month post-operative in 4 patients (44.4%). Among the 5 patients in whom VTE occurred >3 months post-operative, 3 had recurrent metastatic ACC with hypercortisolemia and 2 were in remission (1 with CS and 1 with eCS). The median time from surgery to VTE occurrence was 315 days (8-1006). Compared to those who did not develop VTE, those who developed VTE had higher mean 24-hour urine free cortisol (4663.6 vs 558.21 mcg/dL; n = 100, $P < 0.0001$) and mean 1 mg overnight dexamethasone suppression test (36.3 vs 11.8 mcg/dL; n = 69, $P = 0.0003$), but similar mean late-night salivary cortisol (0.591 vs 0.790 ng/dL, n = 84, $P = 0.71$) at diagnosis of CS.
Discussion: Among those with CS, the overall incidence of VTE was 12.3% and the majority of VTE were provoked (surgery, malignancy). Moreover, VTE was more likely in those with higher UFC and 1 mg overnight dexamethasone suppression test in our cohort. This suggests that in

patients with CS who have an active malignancy, severe CS or those undergoing a surgical procedure may be at increased risk of VTE. Future studies should investigate the optimal type and duration of the VTE prophylaxis.

Adrenal

ADRENAL – CLINICAL RESEARCH STUDIES

Incident Cardiometabolic Outcomes in Adrenal Adenomas: A Population-Based Cohort Study of 1,004 Patients

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Background: Adrenal adenomas have been linked with cardiovascular morbidity in selected patient populations from specialized referral centers. Population-based data examining the association of adrenal adenomas with cardiometabolic outcomes are lacking. **Aim:** To determine the incidence of cardiometabolic outcomes in a population-based cohort of patients with adrenal adenomas. **Methods:** We conducted a population-based cohort study of patients diagnosed with adrenal adenomas while living in a defined community from 1995–2017. Eligible patients were retrospectively identified by a medical records linkage system and confirmed on chart review. Adenomas were classified as mild autonomous cortisol secretion (MACS) if the serum cortisol concentration was >1.8 mcg/dL after 1 mg overnight dexamethasone suppression test (DST), nonfunctioning adrenal tumor (NFAT) if serum cortisol after DST was ≤1.8 mcg/dL, and adenoma with unknown cortisol secretion (AUCS) if DST was not performed. Patients with overt hormone excess were excluded. Cardiometabolic outcomes were assessed at the time of adrenal adenoma diagnosis. Patients were then followed until death, migration out of the community, or through December 31, 2017. Incident outcomes were assessed starting at 1 year following the diagnosis and excluded those with the outcome of interest at baseline, except for myocardial infarction (MI) and coronary intervention, which were adjusted for in the analysis. **Results:** were compared to 1:1 age and sex-matched referent subjects without adrenal adenomas from the same community. **Results:** Adrenal adenomas were diagnosed in 1004 patients with 141 (14%) NFAT, 81 (8%) MACS, and 782 (78%) AUCS. The median age of diagnosis was 63 years (range, 20–96), and 582 (58%) were women. The baseline data was previously presented and showed higher prevalence of hypertension, diabetes, peripheral vascular disease (PVD), and heart failure (HF) in the adenoma group, after adjusting for BMI and tobacco use. During a median follow-up of 6.8 years (range, 0–22), patients with adrenal adenomas were more likely than referent subjects to develop new-onset dyslipidemia (HR 1.31, 95% CI 1.03–1.67), diabetes (HR 1.68, 95% CI 1.28–2.22), chronic kidney disease (HR 1.77, 95% CI 1.39–2.25), atrial fibrillation (HR 1.32 (1.03–1.70), PVD (HR 1.61, 95% CI 1.24–2.09), and HF (HR 1.46, 95% CI 1.15–1.85). In addition, the adenoma group had higher risk for incident MI (HR 1.62, 95% CI 1.16–2.25) and coronary intervention (HR 1.70, 95% CI 1.25–2.31). **Conclusions:** Adrenal adenomas are associated with increased incidence of adverse cardiometabolic outcomes in this population-based cohort study. While these results are potentially explained by different degrees of cortisol excess, the majority of patients received suboptimal hormone evaluation, suggesting a knowledge gap in the workup of adrenal adenomas in the broader medical community.

Adrenal

ADRENAL – CLINICAL RESEARCH STUDIES

Low Protein Expression of ATRX and ZNRF3 as a Novel Prognostic Marker of Adult Adrenocortical Carcinoma

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Adrenocortical carcinoma (ACC) is a rare malignant neoplasia that is usually associated with a dismal prognosis. ACC overall survival (OS) depends on the particular biology of the tumor. Pan-genomic studies have demonstrated the involvement of *ATRX* and *ZNRF3* genes in adrenocortical tumorigenesis. **Aim**: To evaluate ATRX and ZNRF3 protein expression in a large cohort of adults with ACC followed at a single tertiary referral center to establish the prognostic value of these genes. **Methods:** Two pathologists analyzed immunohistochemically-stained slides for ATRX and ZNRF3 (blinded assessment) proteins using tissue microarrays comprising tissue samples from 82 ACC (kappa 0.854; $P<0.001$). Cohort: female 76.8%; median age 38.2 (15.3–85.4) years; 67.5% of patients presented with hypercortisolism; ENSAT stage 1, 11.12%; 2, 44.44%; 3 and 4: (44.44%). The median follow-up time was 39.58 (1.4–406.8) months; 39 patients died during this period. Low protein expression was defined as follows: ATRX, when < 25% of tumor cells were immunoreacted; ZNRF3, when a score < 3 (extension + intensity) was established. **Results:** Low ATRX protein expression was positively associated with the age at diagnosis ($P< 0.001$), and it was negatively correlated with tumor size ($P=0.020$) and with Weiss score ($P=0.033$). Low ZNRF3 protein expression correlated negatively with tumor weight ($P=0.026$), with tumor size ($P= 0.005$), and with Weiss score ($P=0.002$). Regarding OS, the low protein expression of ATRX and ZNRF3 was associated