mild autonomous cortisol secretion (MACS) is typically associated with abnormal circadian cortisol production. **Aim:** To characterize the effect of MACS on cognitive performance. **Methods:** We conducted a cross-sectional analysis as part of an ongoing cohort study in patients with MACS compared to age and sex-matched referent subjects without cortisol excess. MACS was defined as serum cortisol concentration >1.8 mcg/dL after the 1 mg overnight dexamethasone suppression test (DST), in the absence of signs and symptoms of overt Cushing syndrome. We used the National Institute of Health Toolbox Cognition Battery to assess cognitive performance. A series of seven IPad-based tests were administered to evaluate five key domains: 1) executive function, 2) episodic memory, 3) working memory, 4) language, and 5) processing speed. Performance was reported using fully corrected T-scores for age, sex, education, and race with a normative mean of 50 and a standard deviation of 10. T-scores were generated for the individual components as well as three summary measures: 1) fluid cognition (includes executive function, episodic memory, working memory, and processing speed), 2) crystallized cognition (includes language), and 3) total cognition (composite of fluid and crystalized cognition). **Results:** A total of 23 patients with MACS and 23 age and sex-matched referent subjects without cortisol excess were enrolled. The median age of diagnosis was 63 years (range, 51–81), and 26 (56%) were women. In the MACS cohort, median cortisol following 1 mg DST was 2.6 ug/dL (range, 1.9–13.0) with median ACTH of 8.5 pg/mL (range, 5.0–38.0) and median DHEA-S of 37 mcg/dL (range, 5.0-141.0). On cognitive assessment, patients with MACS had lower total cognition (T-scores 50 vs. 54, p=0.05) and fluid cognition (T-scores 48 vs. 53, p=0.01) composite scores compared to referent subjects without cortisol excess. In particular, patients with MACS performed worse on tests of executive function (Dimensional Change Card Sort: T-scores 55 vs. 63, p= 0.02 and Flanker Inhibitory Control and Attention: T-scores 45 vs. 52, p=0.01). There were no significant differences observed in the remaining individual domains of language, processing speed, working memory, and episodic memory, or crystallized cognition. **Conclusions:** MACS is associated with impaired total cognition, and in particular, executive function and fluid cognition. These findings suggest that patients with MACS are susceptible to cortisol-mediated changes in the brain. Additional studies should examine the contribution of neuropsychiatric symptoms on cognition in MACS, and possible improvement following treatment for cortisol excess.

Adrenal

ADRENAL – CLINICAL RESEARCH STUDIES

Impaired Muscle Strength and Performance in Patients With Mild Autonomous Cortisol Secretion

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Background: Glucocorticoid-induced myopathy is well-recognized in overt Cushing syndrome (CS), but the impact of mild cortisol secretion on muscle is unclear. Recent data suggest that patients with mild autonomous cortisol secretion (MACS) are frailer and report more weakness than patients with non-functioning adrenal adenomas. We hypothesized that MACS is associated with 1) objective measures of impaired muscle strength and performance and 2) increased tissue accumulation of advanced glycation end products (AGEs), a measure of accelerated aging. **Aim:** To determine the effect of MACS on muscle mass, strength, performance, and tissue accumulation of AGEs. **Methods:** We conducted a cross-sectional analysis as part of an ongoing cohort study in patients with MACS compared to age and sex-matched referent subjects without cortisol excess. MACS was defined as serum cortisol >1.8 mcg/dL after the 1 mg overnight dexamethasone suppression test (DST), in the absence of overt signs and symptoms of CS. We measured hand grip strength with hand grip dynamometer and evaluated functional performance on the timed up and go test, 6 minute walk test, and gait speed assessment. Tissue accumulation of AGEs was measured with point-of-care AGE reader. Appendicular lean mass was calculated and adjusted for height in participants who underwent body composition scan. **Results:** A total of 23 patients with MACS and 23 age and sex-matched referent subjects without cortisol excess were enrolled. The median age of diagnosis was 63 years (range, 51–81), and 26 (56%) were women. In the MACS cohort, median cortisol following 1 mg DST was 2.6 μg/dL (range, 1.9–13.0), median DHEA-S 37 μg/dL (range, 5.0- 141.0), and median ACTH 8.5 pg/mL (range, 5.0–38.0). Patients with MACS had lower hand grip strength (median 29.3 vs. 32.5 kg, p=0.052), slower gait speed (median 1.1 vs. 1.4 m/s, p=0.001), covered less distance during the 6 minute walk test (median 453 vs. 510 m, p=0.001), and took longer to complete the timed up and go test (median 10.1 vs. 8.6 s, p=0.04) than referent subjects without cortisol excess. Accumulation of AGEs was higher in patients with MACS (median 2.9 vs. 2.4, p=0.01). No significant difference was observed in appendicular lean mass (n=19 pairs, 7.8 vs. 7.5 kg/m2, p=0.57). **Conclusions:** MACS is associated with decreased muscle strength and performance without a significant change in muscle mass, suggesting poor muscle quality. We also observed increased tissue accumulation of AGEs in MACS patients, consistent with our hypothesis of MACS-induced accelerated aging. These findings may help explain the increased frailty observed in MACS, and suggest muscle assessment be considered in all patients with autonomous cortisol secretion. Further studies should examine the impact of muscle and functional impairments on morbidity in MACS, and its possible reversal with either a structured exercise intervention or adrenalectomy.

Adrenal

ADRENAL – CLINICAL RESEARCH STUDIES

In Vitro Splicing Assay Proves the Pathogenicity of Intronic Variants in MRAP

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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