been suggested that HPA axis dysregulation is a potential risk factor for the development of depression. In line with this, several studies reported that L-Dopa treatment may alter the serum levels of ACTH, PRL, and glucocorticoids in parkinsonian patients and Parkinson's disease animal models. In the present study, we determined whether the chronic treatment with L-Dopa altered the stress response inducing depressive-like behaviours. Adult male Wistar rats were treated orally during 24 days with LEBOCAR® - commercial formulation of L-Dopa (75 mg/day) and Carbidopa (7.5 mg/day) - in drinking water. Animals were stressed by immobilization during the last 9 days of treatment and depressive-like behaviours were assessed by the sucrose intake and forced swimming tests. Behavioural tests showed no signs of depressive-like behaviours in the LEBOCAR®-treated and/or stressed rats. We next explored the SAM axis reactivity. Circulating noradrenaline and adrenaline increased in rats treated with LEBOCAR® ($p<0.05$; HPLC). Also, the adrenals from stressed animals showed higher content of adrenaline ($p<0.05$). Then, we studied the HPA axis activity. Chronically stressed rats displayed a lower ACTH secretion (ELISA) and a downregulation of POMC expression (qPCR) in the anterior pituitary ($p<0.05$). In addition, LEBOCAR® treatment induced a reduction in serum ACTH and POMC levels ($p<0.05$). As expected, serum corticosterone (ELISA) enhanced under chronic stress, an effect that was inhibited by treatment with LEBOCAR® ($p<0.05$). Finally, pituitary PRL gene expression (qPCR) was downregulated by LEBOCAR® treatment with a more pronounced effect when rats were also stressed ($p<0.05$). Our results suggest that L-Dopa alters the neuroendocrine stress response enhancing SAM axis reactivity and reducing HPA axis activity and PRL expression.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Evaluation of the Molecular Pathogenesis of Adrenocortical Tumors by Whole-Genome Sequencing

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Pathogenesis of autonomous steroid secretion and adrenocortical tumorigenesis remains partially obscure. Our aim was to identify novel genetic alterations in adrenocortical adenomas (ACA) without somatic mutations in known driver genes. Whole-genome sequencing was performed on 26 ACA/blood-derived DNA pairs without driver mutations in *PRKACA*, *GNAS* and *CTNNB1* genes at previous WES (ENSAT study JCEM 2016). These included 12 cortisol-producing adenomas with Cushing syndrome (CS-CPAs), 7 with mild autonomous cortisol secretion (MACS-CPAs), and 7 endocrine-inactive ACAs (EIAs). Seven adrenocortical carcinomas (ACC) were added to the cohort. We developed a bioinformatics pipeline for a comprehensive genome analysis and to reveal differences in variant distribution. Strelka, VarScan2 and ANNOVAR software and an in-house confidence score were used for variant calling and functional annotation. Combined Annotation-Dependent-Depletion (CADD) values were used to prioritize pathogenic variants. Additional focus relied on variants in pathogenically known pathways (Wnt/β-catenin, cAMP/PKA pathway). NovoBreak algorithm was applied to discover structural variations. Two hypermutated CS-CPA samples were excluded from further analysis. Using different filters, we detected variants in driver genes not observed at WES (one p.S45P in *CTNNB1* and one p.R206L in *PRKACA* in two different CS-CPAs). In total, we report 179,830 variations (179,598 SNVs; 232 indels) throughout all samples, being more abundant in ACC (88,954) compared to ACA (CS-CPAs: 31,821; MACS-CPAs: 35,008; EIAs: 29,963). Most alterations were in intergenic (>50%), followed by intronic and ncRNA intronic regions. A total of 32 predicted pathogenic variants were found in both coding (CADD values ≥ 15) and non-coding (CADD values ≥ 5) regions. We found 3,301 possibly damaging and recurrent variants (intergenic mutations removed) (CS-CPAs: 1,463; MACS-CPAs: 1,549; EIAs: 1,268; ACC: 1,660), mostly accumulated in intronic regions. Some of these were detected in members of the Wnt/β-catenin (CS-CPAs: 6; MACS-CPAs: 2; EIA: 1) and cAMP/PKA (CS-CPAs: 6; MACS-CPAs: 7; EIA: 4) pathways (e.g. *ADCY1, ADCY2, GNA13, PDE11A*). We also found a slightly higher number of structural variations in EIA (3,620) and ACC (3,486) compared to CS-CPAs (977) and MACS-CPAs (2,119). In conclusion, still unrevealed genetic alterations, especially in intronic regions, may accompany early adrenal tumorigenesis and/or autonomous cortisol secretion.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Extracellular Vesicles From SDHB Deficient hPheo1 Cells Activate STAT3 in Wild-Type Cells

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Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors that originate from the adrenal medulla and extra-adrenal paraganglia, respectively. Inactivating mutations in succinate dehydrogenase (*SDHx*) genes leads to succinate accumulation, increased HIF1-α levels, and uncontrollable growth of PPGLs. We hypothesized that small extracellular vesicles (EVs) released from progenitor cells derived from pheochromocytoma (hPheo1) with a shRNA mediated knockdown of *SDHB* are enriched in succinate metabolites that play a key role in the activation of various tyrosine dependent

signaling pathways that are involved in turmorigenesis and proliferation. We isolated EVs from the conditioned media of human wild-type hPheo1 cells and hPheo1 cells with shRNA *SDHB* knockdown. The EVs from three separate preparations of each group were characterized by nanoparticle tracking analysis, transmission electron microscopy, and Western blotting using antibodies against different types of EV and one non-EV marker. Our results show small EVs from the *SDHB* knockdown hPheo1 cells increased the activation of phosphotyrosine residues in wild-type cells compared to cells treated with control EVs from the same cell type. Additionally, our data show these EVs increase phospho-STAT3 compared to the control EVs (3843.10 +/- 1138.89 vs. 213.65+/- 40.75; p<0.05; n=3) in cultured wild-type hPheo1 cells. Protein tyrosine kinases (PTKs) control various cellular processes including growth, differentiation, and metabolism by activating various signaling pathways including STAT3. The significance of these findings is that in some cancers, elevated succinate from a *SDHx* mutation has been shown to activate STAT3 which may explain a possible pathway for tumorigenesis. Studies from other investigators have shown that STAT3 expression is elevated in malignant PPGL tissues. Through enriched EV analysis our findings have confirmed the role of STAT3 in *SDHB* deficient cells. Additional studies are needed to identify other metabolites that are enriched in EVs that regulate phosphorylation of tyrosine residues and STAT3 activation.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Human Adrenal Cortical Zone Changes With Aging

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Background: Previous adrenal morphological studies have shown that the zona reticularis (ZR) and the zona glomerulosa (ZG) decrease in size with aging. Although several lines of evidence indicate that the hypothalamic-pituitary-adrenal axis becomes hyperactive in elderly, little is known about age-related transformations of the adrenal zona fasciculata (ZF). **Objectives:** To investigate the morphological and functional changes of the adrenal cortex across the adult life span, with emphasis on: 1) the understudied ZF, and 2) potential sexual dimorphisms. **Methods:** We used immunohistochemistry to evaluate the expression of several cortical proteins: aldosterone synthase (CYP11B2), visinin-like protein 1 (VSNL1), 3β-hydroxysteroid dehydrogenase type II (HSD3B2), 11β-hydroxylase (CYP11B1) and cytochrome b_5 type A (CYB5A). The ZF area was estimated by subtracting the VSNL1-positive (a ZG marker) area from the HSD3B2-expressing area (ZG and ZF). All captured images were quantitated by ImageJ. In addition, we employed liquid chromatography-tandem mass spectrometry to quantify the morning serum concentrations of 6 steroids: cortisol, 11-deoxycortisol (11dF), 17α-hydroxyprogesterone (17OHP4), 11-deoxycorticosterone (DOC), corticosterone, and androstenedione (A4). The Mann-Whitney *U* test and Spearman's rank correlation coefficients were used for statistical analysis, as appropriate. **Results:** We included 60 adrenal glands from 30 men and 30 women, with ages between 18–86 years. The total cortical area was positively correlated with age (r=0.34, p=0.008), and this association was significant only in men (p=0.02). Both the total (VSNL1-positive) and functional ZG (CYP11B2-positive) areas declined abruptly with aging in men (r=-0.57 and -0.76, p=0.001 and p<0.0001, respectively), but not women (p=0.06 and 0.27, respectively). The CYB5A-positive area, marking the ZR, correlated negatively with age (r=-0.76, p<0.0001) in both sexes. In contrast, the estimated ZF area showed a strong positive correlation with age both in men (r=0.59, p=0.0006) and women (r=0.49, p=0.007), while CYP11B1-positive area remained stable across ages (p=0.86). Finally, we measured morning levels of 6 steroids in 149 men and 149 women, with ages between 21–95 years, matched for age and body mass index. Serum cortisol, corticosterone, and DOC levels remained relatively stable across ages (p=0.38, 0.64 and 0.25, respectively), while 11dF levels increased slightly with age (r=0.16 and p=0.007), particularly so in men (p=0.005). Expectedly, 17OHP4 and A4 declined with aging (r=-0.37 and -0.37, p<0.0001 for both). **Conclusions:** In contrast with the ZG and ZR, the ZF and the total adrenal cortex area enlarge with aging. An abrupt decline of the ZG occurs with age in men, but not in women, possibly contributing to sexual dimorphism in cardiovascular risk.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Intracellular Cholesterol Metabolism in Aldosterone-Producing Adenoma.~A Possible Association With Cellular Morphometry and Genotype~

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Introduction: Primary aldosteronism (PA) is the most common cause of secondary hypertension. More than 70% of APAs have been reported to have KCNJ5 somatic mutation in Asian countiries. Patients with KCNJ5 mutated APAs generally harbor high plasma aldosterone concentration (PAC), and are mainly composed of clear tumor cells containing abundant lipid droplets. However, an association among intracellular cholesterol metabolism, morphological features and genotypes in tumor cells has