Neuroendocrinology and Pituitary

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Potential Role for the RASD1 Glucocorticoid-Responsive Gene in Corticotroph Tumorigenesis

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Introduction: Originally identified due to its dexamethasone inducibility in mouse corticotropinoma AtT20 cells, RASD1 is a receptor-independent activator of G-proteins, via guanine nucleotide exchange factor (GEF) activity. It remains unclear, however, whether, and if so, how RASD1 mediates the effects of glucocorticoids on corticotroph cells. We identified a rare germline *RASD1* variant and investigated its functional effects *in vitro*. **Methods:** We screened 209 CD patients (94.3% pediatric) studied at the at the National Institutes of Health Clinical Research Center between 1997 and 2018 by germline whole-exome sequencing (WES) only (n=157), germline and tumor WES (n=27), and/or *RASD1* droplet digital PCR germline copy number variant (CNV) analysis (n=201). Corticotropinoma DNA was available in 72 patients to screen for *USP8* hotspot variants by Sanger sequencing. A *RASD1* variant was identified and functionally characterized. **Results:** We studied 119 female (56.9%) and 90 (43.1%) male CD cases, including 197 pediatric (≤18 years at disease onset) and 12 adult patients. *USP8* defects were present in 19.4% (14/72) of cases. No *RASD1* CNVs were found. A rare (with a minor allele frequency of 0.0022% in gnomAD v3) heterozygous germline missense *RASD1* variant, c.580A>C, p.M194L was detected in one male sporadic case. Neither *USP8* variants nor loss of heterozygosity at the *RASD1* variant position were observed in the patient's microadenoma. The wild type and p.M194L RASD1 transiently overexpressed proteins displayed similar short half-lives (<1 h) by cycloheximide chase in HEK293 cells, as well as cytoplasmic localization by immunocytofluorescence in AtT20 cells. A CRISPR/Cas9 *Rasd1* knockout AtT20 cell line displayed reduced *Pomc* expression compared with the parental cell line at the mRNA level (*Actb*-normalized absolute quantification 5.80±0.92 vs 9.62±0.7, P=0.005). Viability of the cell lines did not differ significantly by MTT assay. Overexpression of p.M194L resulted in increased accumulation of phospho-CREB S133 (1.83±0.8 vs 1±0.2 in empty vector control, P=0.0390) as well as a non-significant increase in *Pomc* expression in wild type, but not in *Rasd1* knockout AtT20 cells by immunoblot band densitometry. **Conclusions:** We found an infrequent *RASD1* variant in one CD patient. *Rasd1* seems to have a role within the intracellular signaling pathways controlling *Pomc* expression. Overexpression of the p.M194L variant caused phospho-CREB S133 activation, suggesting increased GEF activity for this variant. Interestingly, another variant at the same position, p.M194I, was found in the COSMIC database (COSS2121715) as a somatic change in cutaneous malignant melanoma. Further studies are required to better define the role of *RASD1* in corticotroph physiology and its possible involvement in tumorigenesis.

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Prenatal Androgen Excess Impairs Sexual Behavior in Adult Female Mice: Perspective on Sexual Dysfunction in PCOS

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders worldwide, affecting 5-20% of reproductive aged women [1]. PCOS is characterised by androgen excess, oligo- or anovulation, and polycystic ovarian morphology [1]. PCOS patients also experience sexual dysfunction, including decreased sexual desire, increased sexual dissatisfaction and gender dysphoria [2-4]. The origins of PCOS-related sexual difficulties remain unidentified, but may be related to impaired central mechanisms regulating sexual behaviours. Prenatally androgenized (PNA) mice recapitulate the PCOS phenotype and exhibit alterations in the neuronal network regulating reproductive function [5], providing a powerful, pathology-based model to unravel the biological origins of sexual dysfunction in PCOS. Here, we aimed to determine whether female sexual behaviours are impaired in the PNA mouse model of PCOS. To model PCOS, female dams received injections of dihydrotestosterone (PNA) or oil vehicle (VEH) daily from gestational day 16-18. Adult female offspring were ovariectomized and implanted with a silastic capsule of estradiol to examine the female-typical sexual behaviour: lordosis as well as partner preference. We also examined a potential masculinisation of the brain by replacing the estradiol implant by a testosterone implant then testing the female for male-like sexual behaviours. PNA females exhibited significantly reduced lordosis behaviour compared to VEH females ($p<0.01$). In contrast, partner preference and male-like sexual behaviour were not different between PNA and VEH females. In addition, using Open-field test and elevated-plus maze, we observed no effect of prenatal androgen exposure on locomotion and anxiety. These results highlight, for the first time, that prenatal exposure to the non-aromatisable androgen, DHT, impairs female receptivity only without masculinisation. These findings support the use of the PNA mouse model of PCOS to identify the neuronal targets of prenatal androgen action and to determine the mechanisms by which prenatal androgen excess

impairs lordosis. Taken together, this study introduce a novel perspective on the origins of sexual dysfunction in women with PCOS and indicate the need for further investigation into the mechanisms of androgen excess on the female brain and sexual function. [1] Lizneva D *et al*, Fertil Steril. 2016;106:6-15. [2] Fliegner M *et al*, Geburtshilfe Frauenheilkd. 2019;79:498-509.[3] Kowalczyk R *et al*, Acta Obstet Gynecol Scand. 2012;91:710-4.[4] Mansson M *et al*, Eur J Obstet Gynecol Reprod Biol. 2011;155:161-5. [5] Ruddenklau A, Campbell RE. Endocrinology. 2019 Oct 1;160(10):2230-2242.

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Progesterone Might Be an Active Component of the Sleep-Wake Homeostatic Mechanism

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Background: Using a randomized, double blind, placebo-controlled, crossover protocol, we have shown previously that progesterone may prevent sleep disturbances but has no action on undisturbed sleep. In that study, 8 healthy postmenopausal women took daily at 2300 h for 3 wk a capsule of either 300 mg of progesterone or placebo. Sleep was polygraphically recorded during the last two nights and, during the second night, blood samples were collected at 15-min intervals using an iv catheter,. During the first night, sleep was normal under placebo and progesterone had no effect. During the second night, blood sampling procedure was associated under placebo with marked sleep disturbances, which were considerably reduced under progesterone: at the group level, mean ± SEM duration of wake after sleep onset (WASO) dropped from 152 ± 37 min to 71 ± 19 min (P = 0.01), and slow-wave sleep (SWS) duration increased from 53 ± 6 min to 79 ± 10 min (P = 0.04). **Objective:** To submit individual data collected in that study to new analyses designed to further investigate possible mechanism(s) of progesterone actions on sleep architecture. **Results:** Among individual subjects, no relation could be evidenced between progesterone levels and sleep variables, or between individual progesterone-associated improvements and absolute values of corresponding sleep variables under placebo. By contrast, for WASO and SWS, significant positive correlations (Spearman test) were evidenced between individual responses to progesterone (i.e. the difference, during night 2, between value under progesterone and value under placebo) and corresponding individual alterations caused under placebo by the blood sampling procedure (i.e. the difference, under placebo, between value during night 1 and value during night 2): WASO: $r_s = 0.74$, P = 0.037, n = 8; SWS: $r_s = 0.86$, P = 0.014, n = 7. (Pearson test yielded similar results: WASO: r = 0.85, P = 0.008; SWS: r = 0.76, P = 0.047). **Conclusions:** Although they obviously need to be confirmed by larger studies performed in a variety of clinical conditions, the present findings suggest that progesterone action on sleep architecture is specifically tailored to restore individual normality rather than group normality. Since SWS is mainly regulated by the sleep-wake homeostatic mechanism relating sleep pressure to the duration of prior wakefulness, it is tempting to speculate that progesterone might be an active component of this mechanism.

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Prolonged, Controllable Protein Production of cDNA-Encoded hGH Maintained at Therapeutic Serum Levels Following One Systemic Administration of a Non-Viral, Non-Integrating, DNA-and Liposome-Based* *in vivo* *Gene Therapy Platform in Immunocompetent Mice

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Recombinant human growth hormone (rhGH), the mainstay of hGH replacement therapy, is injected daily for years to enable children to achieve normal stature. Daily rhGH injections are required because its serum-$T^1/_2$ is <20 minutes. Human trials testing $T^1/_2$-extending rhGH proteins are promising, but even weekly rhGH administration poses potential short and long-term toxicity risks, including exacerbation of diabetes or hypertension and potential for oncogenesis. Frequent hGH injections produce non-physiologic IGF-I profiles, (peak serum IGF-I frequently supra-normal, trough IGF-I concentrations at baseline). Therefore, a safe, effective, single administration hGH approach that maintains hGH and IGF-I serum concentrations within their respective therapeutic ranges for approximately one year could offer significant advantages. Accordingly, DNARx tested HEDGES™, it's non-viral, non-integrating, DNA-and liposome-based systemic, *in vivo* gene therapy approach to produce long-term hGH serum levels within the therapeutic range (1 -10 ng/ml) in immunocompetent mice. Previously, one systemic HEDGES-human granulocyte colony stimulating factor (hG-CSF, serum $T^1/_2$ <3 hours) cDNA administration produced durable therapeutic hG-CSF serum levels (Science advances, 2019, **5**, 2019). Importantly, simply by modifying selected aspects of HEDGES's DNA vector and liposomal components, the duration of serum hG-CSF protein production was controllable over a broad temporal range. Long-term assessment of serum hGH in immunocompetent mice was assessed by injecting either one HEDGES administration or one administration followed by one re-dose of DNA vectors expressing either A) wildtype hGH cDNA (HEDGES-1) or B) wildtype hGH fused to selected serum protein $T^1/_2$-extending DNA sequences (HEDGES-2), with these results: 1) One HEDGES-1 administration produced slowly-increasing, but sub-therapeutic hGH serum levels from day 7 to day 99 after injection. From day 99 on, serum hGH levels remained within the therapeutic range for > 230 days; 2) Concurrently, hGH-induced elevation of endogenous mouse serum IGF-I levels remained between 1 to 3 fold above baseline. 3) One HEDGES-1 re-injection was administered 35 days after initial injection, a time