One patient started at 1.3 mg/kg exhibited an anti-Xa level of 1.7 IU/ml, with appropriate anti-Xa levels at a dose of 0.6 mg/kg. 3 patients started at reduced treatment doses (0.68, 0.78 and 0.87 mg/kg) exhibited therapeutic anti-Xa levels. In summary, therapeutic anti-Xa levels were achieved at a dose of approximately 1 mg/kg in only three patients (0.87, 0.96, 1.12 mg/kg); others required reduction of the usual recommended dose to between 0.36 and 0.78 mg/kg. CYP3A4 inhibitors were used in 5/8 patients with elevated anti-Xa levels.

Conclusions: Patients with Cushing's syndrome appear to require lower than standard dose of enoxaparin; which may be only partly explained by concomitant CYP3A4 inhibitors. We suggest that anti-Xa levels be more closely monitored in CS patients to avoid morbidity and mortality caused by PE or bleeding. Further studies are needed to determine if this risk is present in patients receiving supraphysiologic doses of exogenous glucocorticoids.

1. Wagner J et al. *Front Endocrinol (Lausanne)*. 9: 805, 2018

Thyroid

BENIGN THYROID DISEASE AND HEALTH DISPARITIES IN THYROID I

Interaction Between Gene Polymorphisms and Urine Iodine Levels on Susceptibility to TPOAb Positivity in the Chinese Population

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SAT-420

Objective: Hashimoto thyroiditis, characterized by positive thyroid peroxidase antibodies (TPOAbs), is caused by the interaction of genetic and environment factors. The aim of this study was to clarify the interaction of gene polymorphisms and iodine intake in the incidence of TPOAb positivity. **Methods:** 1733 subjects were included in this study. Genomic DNA was extracted from peripheral blood white cells. Seven SNPs (rs10944479, rs11675434, rs1230666, rs3094228, rs653178, rs9277555 and rs301799) were selected for genotyping. Thyroid hormones and autoimmune antibodies (TPOAb and TGAb) were determined using the electrochemiluminescence immunoassay method. **Results:** The mean TSH level in TPOAb-positive subjects was higher than in TPOAb-negative subjects (P<0.001). There were no significant differences in urine iodine and blood iodine between these two groups. Genotype GG of rs9277555 and genotype TT of rs11675434 were associated with an increased risk of TPOAb positivity. Logistic regression analysis showed rs9277555 was associated with TPOAb positivity in all models. Furthermore, rs9277555 was also associated with TPOAb levels in linear regression analysis. The cross-validation consistency and the testing accuracy indicated that there were no significant differences between SNPs and urine iodine interaction. **Conclusion:** rs9277555 was associated with an increased risk of TPOAb positivity in a Chinese Han population. Furthermore, there was no gene polymorphisms-iodine intake interactions in our cohort.

Neuroendocrinology and Pituitary

RESEARCH ADVANCES IN PITUITARY TUMORS

Pituitary Tumors and Immortalized Cell Lines Generated by Cre-Inducible Expression of SV40 T-Antigen

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OR06-06

Pituitary and hypothalamic cell lines have been developed by targeted oncogenesis. This involved using cell-specific transcriptional regulatory sequences to drive expression of large and small SV40 T-antigens in transgenic mice. Invariably, tumors develop in some of the mice, and the cells in these tumors can sometimes be adapted to grow in culture into stable, immortalized cell lines that maintain some of the features of differentiated cells. Cell lines that represent pregonadotropes (αT3-1), gonadotropes (LβT2), precursors to the POU1F1 lineage (GHFT1, Pit1-zero), differentiated cells of the POU1F1 lineage (Pit1-triple, TaT1, and Pit1-PRL), and GnRH neurons (GT1-1) have been made by this approach. Tumors often develop early and cause infertility or death. To increase the opportunity for generating cell lines and to make it feasible to follow the process of tumorigenesis, we developed a mouse strain that expresses SV40 T-antigens in response to cre-recombinase. Using CRISPR/Cas9 we inserted an 8 kb cassette with coding sequences for SV40 T-antigens and IRES-GFP into the *Rosa26* locus, downstream from a stop sequence flanked by loxP sites: *Rosa26*$^{\text{LSL-SV40-GFP}}$. 30% of the progeny born from hybrid zygotes injected with template DNA, CRISPR/Cas9, and sgRNA had correctly targeted the *Rosa26* locus. These mice were mated with previously established *Prop1-cre* and *Tshb-cre* transgenic lines. The majority of *Rosa26*$^{\text{LSL-SV40-GFP/+}}$; *Prop1-cre* and *Rosa26*$^{\text{LSL-SV40-GFP/+}}$; *Tshb-cre* mice developed dwarfism and large tumors by 4 wks. The pituitaries of *Rosa26*$^{\text{LSL-SV40-GFP/+}}$; *Tshb-cre* mice appear grossly normal at birth, but they are enlarged and showing evidence of increased vascularization by 2 wks. Flow-sorted GFP-positive cells from *Rosa26*$^{\text{LSL-SV40-GFP/+}}$; *Prop1-cre* and *Rosa26*$^{\text{LSL-SV40-GFP/+}}$; *Tshb-cre* mice express *Prop1* and TSH, respectively. Tumors from *Rosa26*$^{\text{LSL-SV40-GFP/+}}$; *Tshb-cre* mice were adapted to growth in cell culture. We have established a thyrotrope-like cell line that expresses *Cga* and *Pou1f1*. These studies demonstrate the utility of the novel, *Rosa26*$^{\text{LSL-SV40-GFP}}$ mouse line for reliable targeted oncogenesis and development of unique cell lines.

The authors have nothing to disclose.

Steroid Hormones and Receptors

STEROID BIOLOGY AND ACTION

Salivary Cortisol and Cortisone Measurement Provide a Novel and Non-Invasive Method of Monitoring Medical Therapy in Cushing's Syndrome

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SAT-741

Introduction:
Salivary glucocorticoids (cortisol [SalF], cortisone [SalE]) are collected non-invasively, unaffected by variation in cortisol binding globulin (CBG) and an established tool in the investigation of Cushing's syndrome (CS). We have previously shown a strong correlation between salivary glucocorticoids and circulating free serum cortisol, better than that with total serum cortisol. Measurement by liquid chromatography with tandem mass spectrometry (LC-MS/MS) permits lower limits of quantification, eliminates cross-reactivity both between cortisol and cortisone, and by metyrapone-induced elevations in 11-deoxycortisol.
Previous studies in CS have demonstrated that a mean (based on 5 samples during a single day) serum cortisol (serumF) of 150-300 nmol/l equates to a normal isotopically calculated cortisol production rate. We report the use of salivary glucocorticoid measurement in metyrapone-treated CS patients undergoing dose titration to achieve a mean serumF of 150-300 nmol/L.
Methods:
Seventeen (11 females; age-range 24-74 years) patients with CS undergoing dose titration with metyrapone were studied on 44 occasions: 15 ACTH-dependent (5 ectopic) and 2 adrenal.
24 healthy male volunteers (HV) were also studied.
Both cohorts had paired serumF and SalE and SalF samples collected at 5 time-points (10:00; 11:30; 13:00; 14:30; 16:00). Serum and salivary glucocorticoids were measured using LC-MS/MS.
The TR for salivary glucocorticoids was determined from the mean SalE and SalF in HV whose mean serumF (n=20) was in the desired range (150-300 nmol/L). In CS patients the metyrapone dose had been titrated to achieve a mean serumF of 150-300 nmol/L on 14 (out of 44) occasions.
Results:
Mean SalE (r=0.70; p<0.001) and mean SalF (r=0.51; p<0.001) showed a significant correlation with serumF. The TR from HV for mean SalE and mean SalF were 5.8–24.7 nmol/L and 0.6-5.4 nmol/L respectively.
In CS patients, SalE had greater sensitivity (79% vs. 75%) and specificity (80% vs. 57%) in predicting a mean serumF in the TR than SalF.
Conclusion:
We have demonstrated a close correlation between mean SalE and mean serumF in metyrapone treated CS patients. Salivary glucocorticoids within the derived TR were highly predictive for a target serumF. It is unsurprising, due to the effect of CBG on serumF measurements, that the sensitivity and specificity of SalE and SalF are not greater than reported. Consequently, SalE and SalF, as surrogates for free serum F, have the potential to be superior than serumF when assessing adequacy of medical therapy in CS and may permit out-of-hospital monitoring. Further work is required to validate these findings.

Pediatric Endocrinology

SEXUAL AND GENDER DEVELOPMENT IN THE PEDIATRIC POPULATION

Integrative and Analytical Review of the 5 Alpha Reductase Type 2 Deficiency Worldwide

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OR15-06

Introduction: The conversion of testosterone into dyhidrotestosterone is catalyzed by the 5 α reductase type 2 enzyme which plays a crucial role in the external genitalia virilization. It is encoded by the *SRD5A2* gene. Allelic variants (AV) in this gene cause a 46,XY DSD with no genotype-phenotype relationship. It was firstly reported at early 70's from isolated clusters. Since then, several cases have been reported. Putting together, it will expand the knowledge about the molecular bases of androgen regulation. **Methods:** We searched for *SRD5A2* AV in the literature (Pubmed, EMBASE, Medline) and websites (ensemble, HGMD, ClinVar). Only cases with AV in both alleles, either in homozygous (HM) or compound heterozygous state (CH) and 5ARD2 phenotype were included. The AV were analyzed according to ethnicity, exon, domain, aminoacid (aa) conservation, age at diagnosis, sex assignment, gender change, external genitalia virilization and functional studies. External genitalia virilization was scored using Sinnecker scale. Conservation analysis was carried out using CONSURF platform. For categorical variables we used X2 test and Cramer's *V*. Continuous variables were analyzed by t test or ANOVA. Concordance was estimated by Kappa. **Results:** We identified 434 cases of 5ARD2 deficiencies from 40 countries. Most came from Turkey (23%), China (17%), Italy (9%), and Brazil (7%). 69% were assigned as female. There were 70% of AV in HM and 30% in CH state. Most were *missense* variants (76%). However, small indels (11%), splicing (5%) and large deletions (4%) were all reported. They were distributed along all exons with exon 1 (33%) and exon 4 (25%) predominance. AV in the exon 4 (NADPH-binding domain) resulted in lower virilization (F=10.5; p<.0001). The positions 55, 65, 196, 235 and 246 are hotspots making up, 25% of all AV. Most AV (76%) were located at conserved aa. However, AV at non-conserved aa were more frequently indels (28% vs 6%; p<.01). The overall rate of gender change from female to male ranged from 16% to 70%. The lowest rate of gender change occurred in Turkey and the highest in Brazil. Virilization was similar between those who changed and kept their gender. The gender change rate was significantly different across the countries (*V*=0.44; p<.001) even with similar virilization scores. There was no concordance between genotype and phenotype in all recurrent AV (196, 235 and 246; *k* 0.6, 0.12 and 0.19). **Conclusion:** 5ARD2