Adrenals

Erica L.T. van den Akker\textsuperscript{a} and Evangelia Charmandari\textsuperscript{b, c}
\textsuperscript{a}Department of Pediatric Endocrinology, Erasmus Medical Center, Rotterdam, The Netherlands;  
\textsuperscript{b}Department of Endocrinology, Metabolism and Diabetes, University of Athens Medical School, ‘Aghia Sophia’ Children’s Hospital, Athens and  
\textsuperscript{c}Division of Endocrinology and Metabolism, Clinical Research Center, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

For this year’s chapter we have searched PubMed for articles on ‘adrenal’ or ‘steroidogenesis’ published in English between June 1, 2013 and May 31, 2014. This search yielded more than 5,500 citations. We have examined all citations individually and selected the following collection of basic research and clinical articles. Whenever possible, we have avoided topics that have been discussed in the Yearbook 2013, unless progress in the field has been incremental. Emerging themes for this year’s chapter include: (i) the role of ARMC5 in macronodular adrenal hyperplasia with Cushing’s syndrome; (ii) the role of PRKACA in cortisol-producing adrenal tumors; (iii) the role of CACNA1D in aldosterone-producing adenomas and secondary hypertension; (iv) new therapeutic modalities for the management of patients with classic congenital adrenal hyperplasia and adrenal insufficiency, and (v) new management strategies for pregnancies at risk of congenital adrenal hyperplasia.

Mechanism of the year

**ARMC5 mutations in macronodular adrenal hyperplasia with Cushing’s syndrome**

INSERM Unité 1016, Centre National de la Recherche Scientifique Unité Mixte de Recherche 8104, Institut Cochin, Paris, France


**Background:** Corticotropin-independent macronodular adrenal hyperplasia may be an incidental finding or it may be identified during evaluation for Cushing’s syndrome. Reports of familial cases and the involvement of both adrenal glands suggest a genetic origin of this condition.

**Methods:** Blood and tumor DNA obtained from 33 patients with corticotropin-independent macronodular adrenal hyperplasia was genotyped using single-nucleotide polymorphism arrays, microsatellite markers, and whole-genome and Sanger sequencing. The effects of armadillo repeat containing 5 (ARMC5) inactivation and overexpression were tested in cell-culture models.

**Results:** Loss of heterozygosity at 16p was the most frequent somatic chromosome alteration (24%). The most frequent mutation identified by whole-genome sequencing was in ARMC5, which is located at 16p11.2. ARMC5 gene mutations were detected in tumors obtained from 18 of 33 patients (55%). In all cases, both alleles of ARMC5 carried mutations: one germline and the other somatic. Transcriptome-based classification of corticotropin-independent macronodular adrenal hyperplasia indicated that ARMC5 mutations influenced gene expression. In vitro studies indicated that ARMC5 inactivation decreased steroidogenesis, while its overexpression altered cell survival.

**Conclusions:** Some cases of corticotropin-independent macronodular adrenal hyperplasia appear to be genetic, and are often caused by inactivating mutations of ARMC5, a putative tumor-suppressor gene. Genetic testing for this condition might result in earlier identification and better management of these patients.

Cushing’s syndrome, a challenging disease in which excess cortisol is secondary to diverse tumors with complex molecular mechanisms, is categorized as corticotropin-dependent or corticotropin-indepen-
dent. Approximately 20% of cases are considered corticotropin-independent. Cushing’s syndrome rarely results from primary bilateral nodular hyperplasia. Although bilateral macronodular adrenal hyperplasia was initially considered sporadic, familial autosomal dominant forms are now recognized [1]. The presence of bilateral hyperplasia suggests a pathogenesis that involves either a somatic mutation in adrenal progenitor cells arising during embryogenesis in sporadic cases, or a germline mutation in familial cases [1, 2].

In the present study, Assié et al. identified several inactivating mutations in the armadillo repeat containing protein 5 gene (ARMCS5), which is located at 16p11.2, in 55% of 33 patients with bilateral macronodular adrenal hyperplasia. In each macronodule examined, both alleles carried distinct ARMCS5 mutations: a germline mutation and a distinct somatic mutation. In contrast, in internodular diffuse hyperplasia, only the germline mutation is detected, indicating that the second somatic ARMCS5 mutation is important in the generation of larger nodules and in glucocorticoid excess. This pattern of mutation suggests a ‘two-hit’ model of a tumor suppressor gene, in which a ‘second hit’, in addition to a germline-inactivating mutation, leads to tumor development. In support of this hypothesis, in 4 patients with germline mutations, different nodules from the hyperplastic adrenal glands had different secondary ARMCS5 mutations. In addition, in vitro functional studies of ARMCS demonstrated tumor suppressor protein properties, including effects on steroidogenesis and cell survival. Approximately 50% of first-degree relatives of patients with apparently sporadic cases of Cushing’s syndrome carried the same mutation and had unsuspected subclinical adrenal nodular hyperplasia. Therefore, the above findings indicate that bilateral macronodular adrenal hyperplasia is genetically determined more frequently than previously believed. Furthermore, they provide information that may be helpful in developing diagnostic tests that will lead to earlier diagnosis and management of these patients.

New mechanisms

**Constitutive activation of PKA catalytic subunit in adrenal Cushing’s syndrome**


Medizinische Klinik und Poliklinik IV, Ludwig-Maximilians-Universität München, Munich, Germany


**Background:** Corticotropin-independent Cushing’s syndrome is caused by tumors or hyperplasia of the adrenal cortex. The molecular pathogenesis of cortisol-producing adrenal adenomas is not well understood.

**Methods:** The authors performed exome sequencing of tumor tissue specimens from 10 patients with cortisol-producing adrenal adenomas and evaluated recurrent mutations in candidate genes in an additional 171 patients with adrenocortical tumors. In addition, they performed genome-wide copy-number analysis in 35 patients with cortisol-secreting bilateral adrenal hyperplasias.

**Results:** Exome sequencing revealed somatic mutations in *PRKACA*, which encodes the catalytic subunit of cyclic AMP-dependent protein kinase (protein kinase A (PKA)), in 8 of 10 adenomas. Overall, *PRKACA* somatic mutations were identified in 22 of 59 unilateral adenomas (37%) from patients with overt Cushing’s syndrome. Among 35 patients with cortisol-producing hyperplasias, 5 (including 2 first-degree relatives) carried a germline copy-number gain (duplication) of the genomic region on chromosome 19 that includes *PRKACA*. In vitro studies showed impaired inhibition of both PKA catalytic subunit mutants by the PKA regulatory subunit, while cells from patients with germline chromosomal gains showed increased protein levels of the PKA catalytic subunit; in both instances, basal PKA activity was increased.

**Conclusions:** Genetic alterations of the catalytic subunit of PKA are associated with human disease. Germline duplications of this gene resulted in bilateral adrenal hyperplasias, whereas somatic *PRKACA* mutations resulted in unilateral cortisol-producing adrenal adenomas.
Recurrent activating mutation in PRKACA in cortisol-producing adrenal tumors

Department of Genetics, Yale University School of Medicine, New Haven, CT, USA
Nat Genet 2014;46:613–617

Background: Adrenal tumors autonomously producing cortisol cause Cushing’s syndrome.

Methods and Results: The authors performed exome sequencing of 25 tumor-normal pairs and identified two subgroups. Eight tumors (including three carcinomas) had many somatic copy number variants (CNVs) with frequent deletion of CDC42 and CDKN2A, amplification of 5q31.2 and protein-altering mutations in TP53 and RB1. Seventeen tumors (all adenomas) had no somatic CNVs or TP53 or RB1 mutations. Six of these had known gain-of-function mutations in CTNNB1 (β-catenin) or GNAS (Gas). Six others had somatic mutations in PRKACA (protein kinase A (PKA) catalytic subunit) resulting in a p.Leu206Arg substitution. PRKACA, GNAS and CTNNB1 mutations were mutually exclusive. Leu206 directly interacts with the regulatory subunit of PKA, PRKAR1A. Leu206Arg PRKACA loses PRKAR1A binding, increasing the phosphorylation of downstream targets. PKA activity induces cortisol production and cell proliferation, providing a mechanism for tumor development.

Conclusions: These findings delineate distinct mechanisms that underlie the pathogenesis of cortisol-producing adrenal tumors.

Activating hotspot L205R mutation in PRKACA and adrenal Cushing’s syndrome

Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Key Laboratory for Endocrine Tumors, Rui-Jin Hospital, Shanghai Jiao-Tong University School of Medicine, Shanghai, China
Science 2014;344:913–917

Background: Adrenal Cushing’s syndrome is caused by excess production of glucocorticoid from adrenocortical tumors and hyperplasias, which leads to metabolic disorders.

Methods and Results: The authors performed whole-exome sequencing of 49 blood-tumor pairs and RNA sequencing of 44 tumors from cortisol-producing adrenocortical adenomas (ACAs), adrenocorticotropin hormone-independent macronodular adrenocortical hyperplasias (AIMAHs), and adrenocortical oncocytes (ADOs). They identified a hotspot in the PRKACA gene with a L205R mutation in 69.2% of ACAs and validated in 65.5% of a total of 87 ACAs. Their data revealed that the activating L205R mutation, which is located in the P+1 loop of the protein kinase A (PKA) catalytic subunit, promoted PKA substrate phosphorylation and target gene expression.

Conclusions: These findings highlight potentially functional mutated genes in adrenal Cushing’s syndrome.
the adrenal cortex leading to corticotropin-independent Cushing’s syndrome. Furthermore, the above findings confirm the hypothesis that all benign adrenocortical lesions have something to do with aberrant cAMP signaling. The importance of cAMP/PKA signaling is known in adrenal hyperplasia, however the activating mutations in oncogenes are particularly valuable for designing cancer-specific molecularly targeted therapies.

New genes

Mutations in CACNA1D cause primary aldosteronism

Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism

Department of Genetics, Yale University School of Medicine, New Haven, CT, USA
Nat Genet 2013;45:1050–1054

Background: Adrenal aldosterone-producing adenomas (APAs) are a common cause of severe hypertension. Mutations in the potassium channel KCNJ5 gene that result in cell depolarization and increased Ca2+ influx account for approximately 40% of these tumors.

Methods and Results: The authors identified 5 somatic mutations (4 altering Gly403 and 1 altering Ile770) in the CACNA1D gene, which encodes a voltage-gated calcium channel, among 43 APAs without mutations in the KCNJ5 gene. Both alterations resulted in channel activation at less depolarized potentials. In addition, Gly403 alterations impaired channel inactivation. These effects are inferred to cause increased Ca2+ influx, which is a sufficient stimulus for aldosterone production and cell proliferation in adrenal glomerulosa. They also identified de novo germline mutations at identical positions in 2 children with a previously undescribed syndrome featuring primary aldosteronism and neuromuscular abnormalities.

Conclusions: Gain-of-function Ca2+ channel mutations are implicated in the pathogenesis of APAs and primary aldosteronism.

Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension

Clinical Pharmacology Unit, Centre for Clinical Investigation, Addenbrooke’s Hospital, University of Cambridge, Cambridge, UK
Nat Genet 2013;45:1055–1060

Background: At least 5% of individuals with hypertension have adrenal aldosterone-producing adenomas (APAs). Gain-of-function mutations in the KCNJ5 gene and apparent loss-of-function mutations in ATP1A1 and ATP2A3 have been implicated in APAs.

Methods and Results: The authors demonstrated that KCNJ5 mutations were common in APAs resembling cortisol-secreting cells of the adrenal zona fasciculata but absent in a subset of APAs resembling the aldosterone-secreting cells of the adrenal zona glomerulosa. They performed exome sequencing of 10 zona glomerulosa-like APAs and identified 9 with somatic mutations in either ATP1A1 gene, which encodes the Na+/K+-ATPase α1 subunit, or CACNA1D gene, which encodes Ca_v1.3. All ATP1A1 mutations caused inward leak currents under physiological conditions, while the CACNA1D mutations induced a shift of voltage-dependent gating to more negative voltages, suppressed inactivation or increased currents.

Conclusions: Given that many APAs with these mutations were <1 cm in diameter and had been overlooked on conventional adrenal imaging, recognition of the distinct genotype and phenotype for this subset of APAs could facilitate the diagnosis.
Aldosterone-producing adenomas (APAs) are often due to specific somatic mutations. Gain-of-function mutations in the potassium channel KCNJ5 gene have been found in approximately 40% of APAs, while mutations in ATP1A1 and ATP2A3 genes, two P-type ATPases regulating Na+, K+ and Ca2+ transport, have been recently discovered in a further 7% of APAs. In the first study, Scholl et al. performed exome sequencing of 18 APAs and matched germline DNA. One gene, CACNA1D, had somatic mutations in 5 of 41 APAs (missense mutations encoding p.Gly403Arg and p.Ile770Met), in which no KCNJ5 or CTNNB1 mutations had been detected. The two mutations were heterozygous, had not been previously described, and were confirmed by direct Sanger sequencing. In the second study, Azizan et al. performed exome sequencing in 10 zona glomerulosa-like APAs (>50% compact zona glomerulosa-like cells and low CYP17A1 mRNA expression) and identified 4 new mutations in 5 of these tumors. Screening of their remaining cohort and two additional independent cohorts of APAs revealed 3 additional new CACNA1D mutations.

The CACNA1D gene encodes Ca1,3, the α1 (pore-forming) subunit of an L-type (long-lasting) voltage-gated calcium channel. The α1 subunits contain four repeated domains (I–IV), each with six transmembrane segments (S1–S6) and a membrane-associated loop between S5 and S6. S5, S6 and the interposed loop line the channel pore. The residues altered by the two CACNA1D mutations occur in similar positions near the cytoplasmic ends of the S6 segments of domains I and II. Electrophysiological studies of mutant Cav1.3 channels implicate increased Ca2+ influx in disease pathogenesis. Increased intracellular Ca2+ provides the normal signal for aldosterone production, and sustained increases lead to glomerulosa cell proliferation, in a way similar to that of KCNJ5 mutations. This similarity suggests that increased intracellular Ca2+ is a final common pathway in APA formation. These findings also have implications for other hormone-secreting tumors and endocrinopathies, in which hormone secretion is regulated by Ca2+.

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**Important for clinical practice**

Multisystem morbidity and mortality in Cushing’s syndrome: a cohort study

Dekkers OM, Horváth-Puhó E, Jørgensen JO, Cannegieter SC, Ehrenstein V, Vandenbroucke JP, Pereira AM, Sørensen HT

Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands

*J Clin Endocrinol Metab 2013;98:2277–2284*

**Background:** Cushing’s syndrome (CS) is characterized by excess cortisol production and is associated with hypercoagulability, insulin resistance, hypertension, osteoporosis and immunosuppression. This study aimed to examine the risks for mortality, cardiovascular disease, fractures, peptic ulcers, and infections in CS patients before and after treatment.

**Patients and Methods:** This population-based cohort study included the entire population of Denmark (1980–2010). Data were obtained from the Danish National Registry of Patients and the Danish Civil Registration System. Benign CS of adrenal or pituitary origin and a matched population comparison cohort were included. Morbidity was investigated in the 3 years before diagnosis, while morbidity and mortality were assessed during complete follow-up after diagnosis and treatment.

**Results:** 343 CS patients and 34,300 controls were included. Mortality was twice as high in CS patients (HR 2.3, 95% CI 1.8–2.9) compared with controls. Patients with CS were at increased risk for venous thromboembolism (HR 2.6, 95% CI 1.5–4.7), myocardial infarction (HR 3.7, 95% CI 2.4–5.5), stroke (HR 2.0, 95% CI 1.3–3.2), peptic ulcers (HR 2.0, 95% CI 1.1–3.6), fractures (HR 1.4, 95% CI 1.0–1.9) and infections (HR 4.9, 95% CI 3.7–6.4). Mortality and risk of myocardial infarction remained elevated during long-term follow-up. Mortality and risks for acute myocardial infarction, venous thromboembolism, stroke, and infections were similarly increased in adrenal and pituitary CS.

**Conclusions:** CS is associated with increased mortality and multisystem morbidity, even before diagnosis and treatment.

Cushing syndrome (CS) is characterized by chronic exposure to cortisol overproduction. The annual incidence of the condition is estimated to be about 2 per million persons and only 10% of these
cases occur in children. The most common cause of CS is an ACTH-secreting pituitary adenoma (Cushing’s disease), which promotes excess cortisol production from the adrenal glands [4]. Patients with CS are at increased risk of mortality, cardiovascular events, peptic ulcers, fractures, and infections. The present study demonstrated that this multisystem risk is already elevated during the 3 years before diagnosis, indicating that it is caused by cortisol overproduction rather than treatment for CS. Mortality, the risk of acute myocardial infarction (AMI) and the risk of infections remained elevated even during long-term follow-up, even in a subgroup of patients that were cured. Whether other factors like pituitary insufficiencies also contribute to mortality risk could not be determined from these data. The risks of venous thromboembolism (VTE) and infection were high during the first 3 months following surgery. In clinical practice, it is important to recognize that the increased risk of some conditions is transient (e.g. VTE, fractures, heart failure, and peptic ulcers), while the risk of AMI and infections is not. The high risk for venous thromboembolism of approximately 1% in the postoperative period warrants the need for adequate perioperative prophylaxis. In addition, the period before the diagnosis of CS in patients with endogenous glucocorticoid excess is characterized by untreated hypercortisolism and can therefore be considered a model for the use of exogenous glucocorticoids.

Increased scalp hair cortisol concentrations in obese children

Veldhorst MA, Noppe G, Jongejan MH, Kok CB, Mekic S, Koper JW, van Rossum EF, van den Akker EL
Department of Pediatrics, Erasmus Medical Center (MC), Sophia Children’s Hospital, Rotterdam, The Netherlands

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**Background:** Significantly increased cortisol exposure induces obesity, however it is not known whether relatively high cortisol concentrations is related to childhood obesity. The aim of this study was to compare hair cortisol concentrations between obese and normal-weight children.

**Methods:** The authors performed an observational case-control study. Twenty obese children (body mass index-SD score (BMI-SDS) >2.3) and 20 age- and sex-matched normal-weight children (BMI-SDS <1.1) aged 8–12 years were studied. Scalp hair samples from the posterior vertex were collected, and hair cortisol concentrations were measured using ELISA. Body weight, height, and waist circumference were determined. From the obese children, additional data on blood pressure and blood lipid concentrations were collected.

**Results:** Five boys and 15 girls were included in both groups (obese vs. normal weight; mean age 10.8 ± 1.3 vs. 10.8 ± 1.2 years). Body weight, BMI, BMI-SDS, and waist circumference were significantly higher in the obese children compared with the normal-weight children (respectively, 69.8 ± 17.2 vs. 35.5 ± 7.2 kg; 29.6 ± 4.9 vs. 16.4 ± 1.6 kg/m²; 3.4 ± 0.5 vs. −0.2 ± 0.8 SDS; 94 ± 13 vs. 62 ± 6 cm; p < 0.001). Hair cortisol concentration was higher in obese than normal-weight children (median (interquartile range), 25 (17, 32) vs. 17 (13, 21) pg/mg; p < 0.05).

**Conclusions:** Hair cortisol concentration, a measure for long-term cortisol exposure, was higher in obese children than normal-weight children. These findings indicate long-term activation of the hypothalamic-pituitary-adrenal axis in obese children and may provide a novel target for treatment of obesity in children.

Determination of hair cortisol concentrations is an interesting tool to study long-term cortisol exposure. This is the first study that describes higher hair cortisol concentration in obese children compared to normal-weight controls. Hair cortisol concentration was positively correlated with BMI-SDS and waist circumference. In adults, an association between hair cortisol concentration and BMI, increased risk of metabolic syndrome and cardiovascular disease have been found. This study further shows that the elevated concentrations of hair cortisol are already present early in the course of obesity. Future studies are needed to determine whether the HPA-axis activation precedes or is a result of obesity. Obese children experience more psychological stress but it is also possible that the metabolism of obese children differs from healthy controls. Answering these key questions will improve our understanding of childhood obesity and may change the way we treat it.
Recent reports have questioned the rationale for neonatal screening for congenital adrenal hyperplasia (CAH) owing to low sensitivity in salt-wasting forms and a high rate of recall in preterm infants. This study aimed to determine the efficiency of the neonatal screening program for CAH in Sweden.

Methods: In a longitudinal prospective population-based study in Sweden, the authors assessed the neonatal screening for CAH from January 1, 1986, through December 31, 2011. During this period of time 2,737,932 infants (99.8%) underwent testing. The \( \text{CYP21A2} \) genotype was investigated in 219 cases with true-positive findings (94.8%). They investigated the screening outcomes for 231 patients who had true-positive findings, 43 with late diagnosis, and 1,497 infants with false-positive findings.

Results: 143 patients with salt-wasting CAH were identified; none were missed by screening. The sensitivity was lower for milder forms of the disorder \((p=0.04)\), including 79.7% for the simple virilizing and 32.4% for the non-classic forms. The positive predictive value was higher in full-term \((25.1\%)\) than preterm \((1.4\%)\) infants and correlated with gestational age \((r=0.98; p<0.001)\). The recall rate in full-term infants \((0.03\%)\) was lower than that in preterm infants \((0.57\%)\) \((p<0.001)\).

Conclusions: Screening for CAH was highly effective in detecting the salt-wasting form and thereby reducing mortality. Additional late-onset cases of CAH were detected in childhood and adolescence, reducing the sensitivity for milder forms. The positive predictive value was high despite a low recall rate in full-term infants. Further improvements are necessary to increase the effectiveness of screening among preterm infants.

The primary aim of neonatal CAH screening programs is to prevent salt loss and enable correct sex assignment within a short period of time. This Swedish study is unique in its long follow-up duration (26 years), it covers the screening of 99.8% of all newborns in Sweden, and has regular genotyping confirmation and assessment of disease severity. Therefore, accurate diagnosis was possible for the different forms of CAH, including the milder ones. The overall recall rate was low and the positive predictive value in this study was high compared with other studies. Most previously published studies analyzed smaller series of patients over a shorter follow-up period. This is important to determine the true sensitivity of the CAH neonatal screening in milder cases, which can only be evaluated when enough time has passed to allow their diagnosis. The high rate of false-positive results in preterm infants remains a problem, as cut-off levels correlate with birth weight and/or gestational age. Tandem mass spectroscopy and second-tier procedures may be beneficial to increase its effectiveness among preterm infants.

Clinical trials

New treatments for CAH and adrenal insufficiency

An oral multiparticulate, modified-release, hydrocortisone replacement therapy that provides physiological cortisol exposure

Academic Unit of Diabetes, Endocrinology and Reproduction, University of Sheffield, Sheffield, UK
Clin Endocrinol (Oxf) 2014;80:554–561

Background: Current hydrocortisone replacement therapy is unable to mimic the diurnal cortisol profile in patients with adrenal insufficiency. Previous attempts with modified-release technology were unsuccessful. This study aimed to develop hydrocortisone formulations that recreate the diurnal cortisol profile using multiparticulate technology.
Methods: The authors performed screening by in vitro dissolution profiles, pharmacokinetic testing in dexamethasone-suppressed dogs and humans, and comparison with a reference population.

Results: Formulations were generated using an enteric (delayed-release) design configuration with an extended (sustained-release) dissolution profile. In vitro dissolution confirmed delayed and sustained hydrocortisone release. However, in dogs and humans, sustained release resulted in reduced bioavailability. A formulation, DIURF-006, was developed that maintained delayed release but omitted the sustained-release functionality. Pharmacokinetic characterization of DIURF-006 showed that, despite the absence of a sustained-release component, absorption was sufficiently sustained to deliver extended hydrocortisone absorption. In dexamethasone-suppressed volunteers (n = 16) receiving a twice-daily regimen (20 mg at 23:00 h and 10 mg at 07:00 h), DIURF-006 gave a similar cortisol profile to physiological cortisol concentrations. The relative bioavailability of DIURF-006 vs. hydrocortisone was 89%. Cortisol concentrations increased linearly with doses between 5 and 30 mg.

Conclusions: A multiparticulate oral hydrocortisone formulation with only an enteric coat provides delayed and sustained absorption, and when given twice daily provides physiological cortisol exposure.

Classic CAH is characterized by a defect in cortisol and aldosterone secretion, impaired development and function of the adrenal medulla, and adrenal hyperandrogenism. Treatment aims to provide adequate glucocorticoid and, when necessary, mineralocorticoid replacement to prevent adrenal crises, and to suppress the excess secretion of androgens and steroid precursors from the adrenal cortex. However, the currently available formulations of hydrocortisone are unable to simulate the normal cortisol circadian rhythm, and patients are often at risk for developing in tandem iatrogenic Cushing’s syndrome and hyperandrogenism [5]. The use of a delayed- and extended-release formulation of hydrocortisone represents a new treatment approach to CAH that offers the prospect of a more physiologic cortisol replacement.

In this study, the authors report the development of Chronocort formulations using a scalable technology based on multiparticulates. The design configuration for this dosage form comprises an inert microcrystalline core coated with a drug layer and then further coated with polymeric layers that modify drug release. They screened a number of multiparticulate formulations, both in vitro and in vivo, and chose an optimal Chronocort formulation, the DIURF-006, which reproduces the early morning rise in cortisol. The relative bioavailability of DIURF-006 to hydrocortisone was 89%, the $C_{\text{max}}$ on twice-daily treatment was 665 nmol/l (24 μg/dl) compared with a physiological peak cortisol of 594 nmol/l (22 μg/dl) and the median $T_{\text{max}}$ equated to a clock time of 07:30 h similar to the time of the normal physiological cortisol peak. When given as a twice-daily ‘toothbrush’ regimen, 20 mg at night (23:00 h) and 10 mg in the morning (07:00 h), DIURF-006 provided cortisol exposure similar to that seen in physiological cortisol concentrations in a healthy reference population and also to that seen in dexamethasone-suppressed healthy volunteers after a single dose of 30 mg hydrocortisone.

Abiraterone acetate to lower androgens in women with classic 21-hydroxylase deficiency

Division of Metabolism, Diabetes, and Endocrinology, University of Michigan, Ann Arbor, MI, USA
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Background: In classic congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency, supra-physiologic doses of glucocorticoid are often necessary to suppress increased androgen production, however they contribute to the high prevalence of obesity, glucose intolerance and reduced bone mass in these patients. Abiraterone acetate (AA) is a prodrug for abiraterone, a potent CYP17A1 inhibitor used to suppress androgen concentrations in patients with prostate cancer. The objective of this study was to investigate whether AA added to physiologic hydrocortisone and 9α-fludrocortisone acetate corrects androgen excess in women with 21-OHD without causing hypertension or hypokalemia.

Methods: The authors screened 14 women with classic CAH receiving hydrocortisone 12.5–20 mg/day and enrolled 6 participants with serum androstenedione concentrations >345 ng/dl. AA was administered for 6 days at 100 or 250 mg every morning with 20 mg/day hydrocortisone and 9α-fludrocortisone acetate.

Results: Using 100 mg/day of AA, mean predose androstenedione fell from 764 to 254 ng/dl. At 250 mg/day AA, mean androstenedione normalized in 5 participants (83%) and decreased from 664 to 126
ng/dl. Mean androstenedione concentrations declined further during day 6 to 66 and 38 ng/dl at 100 and 250 mg/day, respectively. Serum testosterone and urinary metabolites declined similarly. Hypertension and hypokalemia were not observed. 

**Conclusions:** AA at a dose of 100–250 mg/day added to replacement hydrocortisone normalized several measures of androgen excess in women with classic CAH.

Abiraterone acetate is a prodrug which is metabolized to abiraterone, a potent active site-directed inhibitor of CYP17A1. Given that all androgen biosynthesis requires CYP17A1 activities, Auchus et al. examined whether abiraterone acetate controls the androgen excess in patients with classic CAH, thereby obviating the need for administering supraphysiologic doses of glucocorticoids. They tested this hypothesis in a phase 1 study of adult women with inadequately controlled classic CAH. Their findings provide compelling proof of concept that CYP17A1 inhibition is a feasible method to control androgen excess in classic CAH without using supraphysiologic doses of hydrocortisone or more potent synthetic glucocorticoids. Given the challenges of conducting phase 1 studies, this study included a limited number of participants, a single treatment regimen, and two cycles of 6 days each. Abiraterone acetate consistently and reproducibly lowered serum and urine androgens concentrations in all 6 adult women with classic CAH. These data encourage extended trials using potent inhibitors of androgen biosynthesis and action, in selected adult women with classic and non-classic CAH, as well as in prepubertal children with inadequately controlled classic CAH.

**Continuous subcutaneous hydrocortisone infusion versus oral hydrocortisone replacement for treatment of Addison’s disease: a randomized clinical trial**


Department of Clinical Science, University of Bergen, Bergen, Norway

*J Clin Endocrinol Metab* 2014;99:1665–1674

**Background:** Conventional glucocorticoid replacement therapy fails to mimic the physiological cortisol secretion, which may have implications for morbidity and mortality in patients with Addison’s disease. The objective of this study was to compare the effects of continuous subcutaneous hydrocortisone infusion (CSHI) with conventional oral hydrocortisone (OHC) replacement therapy.

**Methods:** This prospective crossover, randomized, multicenter clinical trial compared 3 months of treatment with three times daily OHC vs. CSHI. 33 patients with adrenal insufficiency were recruited. All patients were assessed at baseline and after 8 and 12 weeks in each treatment arm.

**Results:** CSHI resulted in normalization of morning ACTH and cortisol concentrations, while 24-hour salivary cortisol curves resembled the normal circadian variation. Urinary concentrations of glucocorticoid metabolites displayed a normal pattern with CSHI but were clearly altered with OHC. Several health-related quality of life (HRQoL) indices in the vitality domain improved over time with CSHI. No benefit was found for either treatment for any subjective or objective sleep parameters.

**Conclusions:** Compared with OHC, CSHI safely attained ACTH and cortisol concentrations closer to normal circadian secretion without adversely affecting glucocorticoid metabolism, and improved HRQoL. These findings indicate that CSHI might become a treatment option for patients poorly controlled on conventional therapy.

**Subcutaneous pulsatile glucocorticoid replacement therapy**


Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol, UK

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**Background:** The glucocorticoid hormone cortisol is released in pulses resulting in a complex and dynamic ultradian rhythm of plasma cortisol that underlies the circadian rhythm. These oscillating levels are also seen at the level of tissues, such as the brain and trigger pulses of gene activation and downstream signaling. Different patterns of glucocorticoid presentation (constant vs. pulsatile) result not only in different patterns of gene regulation but also in different neuroendocrine and behavioural responses. Current
‘optimal’ glucocorticoid replacement therapy does not replicate physiological pulsatile cortisol secretion.

Methods: The authors performed a validation study of a novel portable pulsatile continuous subcutaneous delivery system in healthy volunteers under dexamethasone and metyrapone suppression. Preliminary studies were carried out on 23 healthy subjects and included: (1) dose ranging of individual pulses; (2) testing for optimal frequency of pulses to ensure appropriate peak and nadir levels throughout the day, and (3) combination of three different pulse doses over the 24 h to reproduce normal circadian and ultradian rhythmicity.

Results: During the 24-hour profile of plasma cortisol concentrations achieved following pulsatile infusion of hydrocortisone under dexamethasone suppression, a circadian peak >500 nmol/l and trough <100 nmol/l of cortisol were achieved. These results were confirmed in a separate study in which instead of dexamethasone inhibition of ACTH secretion, secretion of cortisol was suppressed with metyrapone.

Conclusions: Pulsatile subcutaneous hydrocortisone more closely replicates physiological circadian and ultradian rhythmicity.

Although glucocorticoid replacement has been available for over a half century, there have been few new developments in the oral preparations for treatment of patients with adrenal insufficiency (AI). Oral hydrocortisone in daily divided doses is the most widely used glucocorticoid in cortisol replacement therapy. Studies in patients with AI have shown a more than double the standardized mortality rate despite optimal glucocorticoid replacement therapy by contemporary standards [6]. Also, patients with hypopituitarism have a doubled standardized mortality rate, and young adults with AI as part of their hypopituitarism have a 7-fold excessive mortality rate [7]. Likely explanations include the supra-physiological maintenance doses, poor diurnal glucocorticoid exposure-time profile and inadequate rescue therapy in response to intercurrent illnesses. Patients with AI also have increased cardiovascular risk factors, reduced health-related quality of life (QoL) and decreased bone mineral density.

In an attempt to improve patient outcome, studies in which both the dose and the dosing strategies were adjusted have been performed and demonstrated that the pattern of hydrocortisone delivery and the serum cortisol exposure-time profile may be as crucial for patient outcome as the total daily dose. The above studies demonstrated that, compared with standard oral hydrocortisone (OHC) treatment, continuous subcutaneous hydrocortisone infusion (CSHI) can safely re-establish the circadian cortisol rhythm and normalize morning ACTH concentrations in patients with AI. Furthermore, although OHC resulted in major alterations in the pattern of glucocorticoid metabolites and metabolic enzyme activities, CSHI restored glucocorticoid metabolism close to normal. Future studies are required to replicate the full ultradian rhythm and to assess the long-term cognitive, metabolic, cardiovascular and immunological consequences of these new pulsatile CSHI therapies.

New hope


Laboratoire d’Endocrinologie Moléculaire et Maladies Rares, Centre de Biologie et de Pathologie Est, Hospices civils de Lyon, Bron, France
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Background: Prenatal dexamethasone (DEX) treatment has been proposed to prevent genital virilization in girls with congenital adrenal hyperplasia (CAH). DEX is effective in CAH females if initiated before the 6th week of gestation (WG), but its safety in children treated in utero remains controversial. The aim of this study was to avoid prenatal DEX in males and initiate DEX in due time in CAH females by using a protocol that is based on fetal sex determination in the maternal serum (SRY test).
Methods: The authors conducted a retrospective study of the management of 258 fetuses at risk for CAH (134 males and 124 females). DEX was offered to pregnant women following informed consent. The sensitivity of an early SRY test was evaluated following data collection.

Results: The SRY test was sensitive from 4 weeks and 5 days of gestation and avoided prenatal DEX in 68% of males. DEX was maintained until prenatal diagnosis in non-CAH females. Virilization was prevented in 12 CAH girls treated by 6 WG and was minimized in 3 girls treated between 6 and 7 WG. No fetal malformations were noted in the 154 children treated in utero.

Conclusions: The SRY test is reliable to avoid prenatal DEX in males, however its application must be improved. Prenatal DEX should be maintained to prevent virilization and surgery in CAH girls. Information should be provided to families about the benefits and risks of this treatment and written informed consent should be obtained in all cases.

Noninvasive prenatal diagnosis of congenital adrenal hyperplasia using cell-free fetal DNA in maternal plasma

Departments of Pediatrics and of Medicine, Mount Sinai School of Medicine, New York, NY, USA
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Background: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is an autosomal recessive condition that arises from mutations in CYP21A2 gene. To prevent genital ambiguity in affected female fetuses, prenatal treatment with dexamethasone must begin on or before the 9th week of gestation (WG). Currently employed chorionic villus sampling and amniocentesis provide genetic results at 14 WG at the earliest. Therefore, mothers who wish to undergo prenatal dexamethasone treatment will be unnecessarily treating 7 out of 8 fetuses (males and 3 of 4 unaffected females). This underlies the importance of earlier genetic diagnosis in utero. This study aimed to develop a noninvasive method for early prenatal diagnosis of fetuses at risk for CAH.

Methods: Fourteen families, each with a proband affected by classic CAH, were recruited. Cell-free fetal DNA was obtained from 3.6 ml of maternal plasma. Using hybridization probes designed to capture a 6-Mb region flanking CYP21A2, targeted massively parallel sequencing (MPS) was performed to analyze genomic DNA samples from parents and proband to determine parental haplotypes. Plasma DNA from pregnant mothers also underwent targeted MPS to deduce fetal inheritance of parental haplotypes.

Results: In all 14 families, the fetal CAH status was correctly deduced by targeted MPS of DNA in maternal plasma as early as 5 weeks and 6 days of gestation.

Conclusions: MPS on 3.6 ml plasma from pregnant mothers could potentially provide a noninvasive diagnostic method for CAH before the 9th WG. Therefore, only affected female fetuses will be treated. This strategy represents a generic approach for noninvasive prenatal testing for other autosomal recessive disorders.
fusion and clitoromegaly in CAH females. Virilization was prevented in 12 CAH girls treated by 6 WG and was minimized in 3 girls treated between 6 and 7 WG. No malformations were noted in the 154 children treated in utero.

In the second study, New et al. used massively parallel sequencing (MPS) of cell-free fetal DNA obtained from maternal plasma. The authors made the diagnosis of CAH in utero by using 3.6 ml maternal plasma obtained as early as 6 WG for targeted MPS of the genomic region flanking and including CYP21A2 gene. They first mapped single nucleotide polymorphisms (SNPs) linked to the CYP21A2 gene in the parents and proband, and then looked for representation of the respective haplotype maps in the plasma of pregnant mothers. This allowed them to elucidate paternal and maternal inheritance of the fetus at the CYP21A2 locus. They noted complete concordance of the CAH diagnosis between invasive diagnosis and non-invasive MPS in all 14 cases. This approach allows the diagnosis of CAH before genital development begins, thereby restricting dexamethasone therapy to mothers bearing affected females only.

New concerns

Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation

Gee DG, Gabard-Durnam LJ, Flannery J, Goff B, Humphreys KL, Telzer EH, Hare TA, Bookheimer SY, Tottenham N
Departments of Psychology and Psychiatry and Biobehavioral Services, University of California, Los Angeles, CA, USA

Background: Under typical conditions, medial prefrontal cortex (mPFC) connections with the amygdala are immature during childhood and become adult-like during adolescence. Studies in rodent models showed that maternal deprivation accelerates this development. The aim of the present study was to examine the amygdala-mPFC phenotypes in humans following maternal deprivation.

Methods and Results: Previously institutionalized youths, who experienced early maternal deprivation, exhibited atypical amygdala-mPFC connectivity. In contradistinction to the immature connectivity (positive amygdala-mPFC coupling) of comparison children, children with a history of early adversity evidenced mature connectivity (negative amygdala-mPFC coupling), thereby resembling the adolescent phenotype. This connectivity pattern was mediated by cortisol, indicating that stress-induced modifications of the hypothalamic-pituitary-adrenal (HPA) axis shape amygdala-mPFC circuitry.

Conclusion: These findings suggest that accelerated amygdala-mPFC development is an ontogenetic adaptation in response to early adversity.

Several studies have reported that even brief exposure to stressful experiences early in life can have lifelong impact on brain development and socioemotional functioning. In animal models, maternal deprivation has long-term effects on socioemotional and brain development, with particular influences on amygdala-medial prefrontal cortex (mPFC) circuitry. Amygdala is highly susceptible to early environmental adversity owing to its early structural development and readiness to respond to stressors. In humans, early adverse caregiving leads to structural volume abnormalities in the amygdala, which are associated with increased trait anxiety and emotional dysregulation, as well as increased amygdala reactivity to emotional stimuli [11, 12]. Abnormally rapid brain development following early adversity may be a response that reprioritizes developmental goals to match the demands of an adverse early environment. Maternal absence accelerates amygdala-mPFC functional development via premature elevations of glucocorticoids, suggesting that maternal deprivation acts on amygdala-related circuitry through alterations of the hypothalamic-pituitary-adrenal (HPA) axis.

The aim of the present study was to investigate whether a similar neurohormonal process explains affective development in humans following early maternal deprivation. The authors examined the age-related amygdala-prefrontal functional phenotypes in a cross-sectional sample of previously institutionalized children and adolescents who experienced maternal deprivation and a typically developing control group. The maternally deprived youth were subsequently adopted into stable
The authors demonstrated an aberrant frontoamygdala development following maternal deprivation, such that previously institutionalized children and adolescents exhibited amygdala hyperreactivity and an altered trajectory of amygdala-prefrontal connectivity. More specifically, children in the control group showed an immature pattern of connectivity (positively coupled amygdala-mPFC activity), whereas children with a history of maternal deprivation displayed the mature pattern of connectivity (negatively coupled amygdala-mPFC activity), such that they resembled the adolescent phenotype. The findings of this study confirm earlier findings of amygdala hyperactivity in previously institutionalized children, and provide support for the hypothesis that maternal deprivation initiates earlier maturation of amygdala-mPFC connectivity, leading to alterations in HPA-axis activity.

Hypothalamo-pituitary and immune-dependent adrenal regulation during systemic inflammation


Department of Medicine III, Technical University Dresden, Dresden, Germany


Background: Inflammation-related dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is central to the course of systemic inflammatory response syndrome or sepsis. The underlying mechanisms, however, are not well understood. Initial activation of the HPA axis during early sepsis depends on the stimulation of hypothalamus and pituitary by cytokines; in late sepsis, there is a shift from neuroendocrine to local immune-adrenal regulation of glucocorticoid production. Therefore, the modulation of the local immune-adrenal cross-talk may be more promising than the neuroendocrine circuits involved in ACTH production in the prevention of the adrenal insufficiency associated with prolonged sepsis.

Methods: The authors investigated the function of the crucial Toll-like receptor (TLR) adaptor protein myeloid differentiation factor 88 (MyD88) in systemic and local activation of adrenal gland inflammation and glucocorticoid production mediated by lipopolysaccharides (LPSs). They used mice with a conditional MyD88 allele. These mice either were interbred with Mx1 Cre mice, resulting in systemic MyD88 deletion, or were crossed with Akr1b7 Cre transgenic mice, resulting thereby in deletion of MyD88, which was adrenocortical-specific.

Results: Although reduced adrenal inflammation and HPA-axis activation mediated by LPS were found in Mx1(Cre+)-MyD88(+/−) mice, adrenocortical-specific MyD88 deletion did not alter the adrenal inflammation or HPA-axis activity under systemic inflammatory response syndrome conditions.

Conclusions: These findings suggest an important role of immune cell rather than adrenocortical MyD88 for adrenal inflammation and HPA-axis activation mediated by LPS.

Sepsis and septic shock are major causes of death in intensive care units worldwide. In sepsis, excessive, uncontrolled activation of the immune system is harmful to the host and leads to multi-organ failure and death. Adrenal glucocorticoid production plays a beneficial role in response to systemic inflammation by counteracting the hyperactivation of the immune system. However, in many critically ill patients, this homeostatic activation of glucocorticoid secretion is impaired, given that 60% of critically ill patients display an abnormal glucocorticoid response to administration of exogenous ACTH [13]. Glucocorticoid production in sepsis regulated by cytokines that increase hypothalamic CRH and pituitary ACTH secretion. Pattern-recognition receptors, such as Toll-like receptors (TLRs), play a substantial role in the HPA-axis activation induced by pathogens. Furthermore, besides activation of immune cells, bacterially derived TLR ligands, such as lipopolysaccharides (LPSs), can directly affect both neuronal cells and steroid-producing cells in the adrenal gland. Adrenocortical cells express several TLRs and secrete multiple proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-6 in response to bacterial endotoxin stimulation.
Here, Kanczkowski et al. investigated the role of systemic and local adrenal TLR signaling in the activation of the adrenal glucocorticoid response to stress and the regulation of the immune-adrenal cross-talk during the systemic inflammatory response syndrome (SIRS) phenomenon. They used mice with conditional deletion of a crucial TLR adapter protein, myeloid differentiation factor 88 (MyD88), which promotes nuclear factor-κB (NF-κB) activation in response to LPS or IL-1β. They found that adrenocortical-specific MyD88 deletion did not alter the adrenal inflammation or HPA-axis activity under systemic inflammatory response syndrome conditions. These results suggest that immune cells, rather than adrenal cells, are major regulators of the systemic and intra-adrenal inflammatory response to LPS.

**Alterations in DNA methylation of Fkbp5 as a determinant of blood-brain correlation of glucocorticoid exposure**

Johns Hopkins Mood Disorders Center, Johns Hopkins School of Medicine, Baltimore, MD, USA
Psychoneuroendocrinology 2014;44:112–122

**Background:** Epigenetic studies that utilize peripheral tissues to identify molecular substrates of neuropsychiatric disorders rely on the assumption that disease-relevant, cellular alterations that occur in the brain are mirrored and detectable in peripheral tissues such as blood. The objective of this study was to test this assumption by using a mouse model of Cushing’s disease and investigate whether epigenetic changes induced by glucocorticoids can be correlated between these tissue types.

**Methods:** Mice were treated with different doses of glucocorticoids in their drinking water for 4 weeks to assess gene expression and DNA methylation (DNAm) changes in the stress response gene Fkbp5.

**Results:** Linear relationships were observed between DNAm and 4-week mean plasma corticosterone concentrations for both blood ($R^2 = 0.68, p = 7.1 \times 10^{-10}$) and brain ($R^2 = 0.33, p = 0.001$). In addition, the degree of methylation change in blood correlated with both methylation ($R^2 = 0.49, p = 2.7 \times 10^{-3}$) and expression ($R^2 = 0.43, p = 3.5 \times 10^{-5}$) changes in the hippocampus, with methylation changes having occurred at different intronic regions between blood and brain tissues.

**Conclusions:** Although these findings are limited to several intronic CpGs in a single gene, they demonstrate that DNA from blood can be used to assess dynamic, glucocorticoid-induced changes occurring in the brain.

Epigenetic studies that employ DNA from peripheral sources, such as blood, are based on the assumption that non-genetic alterations that have occurred in target CNS regions are mirrored in the periphery. This assumption is especially prevalent in epigenetic studies of neuropsychiatric disorders, where brain tissues are virtually inaccessible in patients, and researchers must rely on other sources of DNA, such as from blood or buccal swabs. Only a few studies have tested this assumption by capturing disease-relevant, dynamic changes in the brain and associating them to alterations occurring in peripheral tissues. Here, Ewald et al. examined whether there is a correlation between brain and blood by measuring dose-dependent DNA methylation changes that occur following exposure to glucocorticoids. They searched the glucocorticoid responsive Fkbp5 gene for candidate loci that could account for the expression changes observed in both blood and brain of corticosterone-treated mice. Pyrosequencing revealed significant DNAm changes in a highly conserved GRE in intron 5 of the gene. They demonstrated a significant linear relationship between DNAm and 4-week mean plasma corticosterone concentrations for both blood and brain. Furthermore, the degree of methylation change in the blood correlated significantly with both methylation and expression changes in the hippocampus. Since DNAm changes in blood were also significantly correlated with expression changes of Fkbp5 in the hippocampus, it may be possible to assess sensitivity and resistance of this tissue to glucocorticoids. Similar studies with additional glucocorticoid-responsive genes, such as GR, Fkbp4 and Hsp90, are necessary to provide a more complete picture of glucocorticoid signaling in the brain, however the idea that blood is representative of brain epigenetics will open new horizons in epigenetic research.
Intra-adrenal corticotropin in bilateral macronodular adrenal hyperplasia

INSERM Unité 982, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, Institute for Research and Innovation in Biomedicine, and Rouen University, Mont-Saint-Aignan, Paris, France

Background: Bilateral macronodular adrenal hyperplasia is a rare cause of primary adrenal Cushing’s syndrome characterized by increased secretion of cortisol and suppression of ACTH release by the pituitary corticotrophs. Therefore, the disease has been termed corticotropin-independent macronodular adrenal hyperplasia. This study aimed to examine the abnormal production of ACTH in these hyperplastic adrenal glands.

Methods: The authors obtained specimens of hyperplastic macronodular adrenal tissue from 30 patients with primary adrenal disease. Pro-opiomelanocortin (POMC) and ACTH expression were assessed by PCR and immunohistochemical analysis. The production of ACTH and cortisol was assessed in 11 specimens with the use of incubated explants and cell cultures coupled with hormone assays. ACTH concentrations were determined in adrenal and peripheral venous blood samples from 2 patients.

Results: The expression of POMC messenger RNA (mRNA) was detected in all samples of hyperplastic adrenal tissue. ACTH was detected in steroidogenic cells arranged in clusters that were disseminated throughout the adrenal specimens. Adrenal ACTH concentrations were higher in adrenal venous blood samples than in peripheral venous samples, a finding that was consistent with local production of the peptide within the hyperplastic adrenals. The release of adrenal ACTH was stimulated by ligands of aberrant membrane receptors but not by CRH or dexamethasone. A semiquantitative score for corticotropin immunostaining in the samples correlated with basal plasma cortisol levels. Corticotropin-receptor antagonists significantly inhibited in vitro cortisol secretion.

Conclusions: Cortisol secretion by the adrenals in patients with macronodular hyperplasia and Cushing’s syndrome appears to be regulated by ACTH, which is produced by a subpopulation of steroidogenic cells in the hyperplastic adrenals. Thus, the hypercortisolism associated with bilateral macronodular adrenal hyperplasia appears to be ACTH-dependent.

Bilateral macronodular adrenal hyperplasia is characterized by increased secretion of cortisol and suppression of ACTH release by the pituitary corticotrophs. Therefore, the term ‘corticotropin-independent macronodular adrenal hyperplasia’ is often used. This study examined the abnormal production of ACTH in these hyperplastic adrenal glands. It showed that in patients with ‘corticotropin-independent bilateral macronodular adrenal hyperplasia’, cortisol production is controlled both by aberrant membrane receptors and by corticotropin produced within the adrenocortical tissue; therefore, the term ‘bilateral macronodular adrenal hyperplasia’ appears to be more appropriate for the disease. Analysis of cell types of the corticotropin producing cells by immunohistochemistry showed that the production did not arise from adrenal chromaffin-like, lymphocyte-like, or pituitary corticotroph-like cells. The corticotropin-containing cells exhibited the morphologic characteristics of steroidogenic cells with gonadal-like differentiation. The authors speculate that the presence of gonadal-like cells in the adrenal glands may represent the counterpart of adrenal rests in the gonads. The suggestion that corticotropin produced by steroidogenic cells in adrenal macronodular hyperplasia stimulates cortisol secretion through a paracrine mechanism may have important implications for future treatment strategies targeting the ACTH receptor instead of cortisol production.
**Higher crash and near-crash rates in teenaged drivers with lower cortisol response. An 18-month longitudinal, naturalistic study**

Faculty of Medicine and Health Sciences, University of Sherbrooke, Longueuil, QC, Canada  
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**Background:** Road traffic crashes are one of the leading causes of injury and death among teenagers worldwide. Better understanding of the individual pathways to driving risk may lead to better-targeted intervention in this vulnerable group. The aim of the study was to analyze the association between cortisol and driving risk.

**Methods:** The study was part of the US Naturalistic Teenage Driving Study, designed to continuously monitor the driving behavior of teenagers by instrumenting vehicles with kinematic sensors, cameras, and a global positioning system. In a community sample of 42 newly licensed 16-year-old volunteers, the driving behavior was monitored. Cortisol response during a stress-inducing task was assessed at baseline, followed by measurement of their involvement in crash and near-crash and driving exposure during their first 18 months of licensure.

**Results:** In teenagers, higher cortisol response to stress was associated with lower crash and near-crash rates during their first 18 months of licensure and with faster reduction in crash and near-crash rates over time.

**Conclusion:** Cortisol stress response is associated with teenaged-driving risk. Objective markers of teenaged-driving risk are necessary for the development of more personalized intervention approaches.

Teenaged drivers have high crash rates early in licensure that decline rapidly in the first 6 months of driving. After the first 6 months, the crash rates decline more slowly for a period of years until reaching the level of experienced adult drivers. The decline of crash rates in the first 6 months has high interindividual variation, which is influenced by unknown factors. This study demonstrated that teenaged drivers with higher cortisol response exhibited lower crash or near-crash rates and showed faster decrease rates over time compared to those with lower cortisol response. No difference was found between males and females. These findings are in line with the previously found link between low cortisol response and risky behaviors, e.g. alcohol abuse and aggressive behavior. As in other problem-behavior fields, identification of an objective marker of a specific pathway to teenaged driving risk promises the development of more personalized intervention approaches. However, the precise mechanisms underlying the link between cortisol response and behavior are unknown. Therefore, the question still remains whether the cortisol stress response is blunted in risk-seeking teenagers or the cortisol stress response is exaggerated in risk-averse teenagers.

**Reviews**

**Treatment and health outcomes in adults with congenital adrenal hyperplasia**

Han TS, Walker BR, Arlt W, Ross RJ  
Academic Unit of Diabetes, Endocrinology and Metabolism, Medical School, University of Sheffield, Sheffield, UK  
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Congenital adrenal hyperplasia (CAH) is a genetic disorder characterized by defective steroidogenesis that results in glucocorticoid deficiency. The most common cause of the disease is 21-hydroxylase deficiency. Although life-saving glucocorticoid treatment has been introduced since the 1950s, treatment is still far from optimal and no consensus has been reached on the management of patients with CAH. Adult patients are prescribed a variety of glucocorticoids, including hydrocortisone, prednisone,
Adrenals

prednisolone and dexamethasone. Despite these personalized treatments, biochemical control of CAH is only achieved in approximately one third of patients. Some patients have a poor health status, with an increased incidence of obesity and osteoporosis, and impaired fertility and quality of life. Patients receiving high doses of glucocorticoids and the more potent synthetic long-acting glucocorticoids are at an increased risk of obesity, insulin resistance and a compromised quality of life. Further research studies are required to optimize the treatment of adult patients with CAH and to improve health outcomes.

Congenital adrenal hyperplasia (CAH) is the most common genetic endocrine disorder. Guidelines have been agreed for the management of children with CAH. However, no consensus exists on the optimal management of adult patients because of a paucity of data from cohorts of a meaningful size. To address this issue, the congenital adrenal hyperplasia adult study executive (CaHASE) was formed in 2003 to study the health status of patients with CAH in adulthood. Around the UK, 17 specialist endocrinology centres recruited a cohort of 203 adult patients under their care and collected information on medical treatment, fertility, genetic analysis and quality of life. Similar studies have been performed in Europe and the USA. They all demonstrate that adult patients with CAH have a higher morbidity than the general population. This review summarizes the pathophysiology of CAH owing to 21-hydroxylase deficiency, the classification and current treatment approaches, as well as the latest data on the health status of adult patients with CAH.

The ‘omics’ of adrenocortical tumors for personalized medicine

Assié G, Jouinot A, Bertherat J
Department of Endocrinology, Referral Centre for Rare Adrenal Diseases, Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Cochin, Paris, France
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Pangenomic analyses of genetic and epigenetic alterations and gene expression profiles provide important new insights into the pathogenesis and molecular classification of cancers. The technologies and methods used for these studies are rapidly improving. The use of such methodologies for the analysis of adrenocortical tumors has revealed clear transcriptomic (mRNA and microRNA expression profiles), epigenomic (DNA methylation profiles) and genomic (DNA mutations and chromosomal alterations) differences between benign and malignant tumors. Interestingly, genomic studies of adrenal cancers have also identified subtypes of malignant tumors, which are associated with different clinical outcomes. Following these genomic studies, efforts to develop new molecular tools that improve diagnosis and prognostication of patients with adrenocortical tumors have also been made. This review describes the progress that has been made towards classification of adrenocortical tumors based on key genomic approaches. Furthermore, the potential for the development and use of various molecular tools to personalize the management of patients with adrenocortical tumors is discussed.

Methodological advances now enable gene expression profiles, and genetic and epigenetic alterations in cancers to be studied at the pangenomic level. Genomics studies in adrenocortical tumors, specifically, have been reported in several publications [14, 15]. The potential of pangenomic analytical approaches has been demonstrated with respect to the classification of a number of common cancers and hematological malignancies. One interesting aspect of cancer genetics that has arisen due to this advance is the close concordance between transcriptome-based classifications and the major molecular events that are thought to be key contributors to tumorigenesis. Such associations demonstrate the relevance of transcriptome-based classifications that use unsupervised clustering algorithms in determining and understanding tumor biology.

Among the most important discoveries that have been made to date is the development of specific molecular classifications that can identify subgroups of cancers not currently distinguishable using conventional pathology techniques. Genomic studies have also investigated rare tumors. Specifically in adrenocortical tumors, subgroups of tumors with different biology and outcomes have been described. Moreover, this progress has stimulated the development of molecular markers aimed at improving diagnosis and prognostication of adrenocortical tumors. This review discusses the advances in our understanding of adrenocortical tumors that have resulted from various ‘genomics’ studies, including those concerning the epigenome, transcriptome and miRNome that have been reported.
during the past decade. The authors focus mainly on how the results of these studies might be used to develop diagnostic, classifying and/or prognostic molecular markers, which could improve provision of personalized medicine in patients with adrenocortical tumors.

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