Adrenals

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For this year's chapter on 'Adrenals', we have searched the PubMed for articles on 'adrenal' and 'steroidogenesis' published in English between June 1, 2011 and May 31, 2012. Our search yielded more than 6500 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 2011, unless progress in the field has been incremental. Emerging themes for this year's chapter include the role of minichromosome maintenance 4 (MCM4) gene in adrenal function, novel mechanisms that control adrenal steroidogenesis, and a novel, dual-release formulation of hydrocortisone for the treatment of adrenal insufficiency and congenital adrenal hyperplasia.

Mechanism of the Year

MCM4 mutations are a cause of adrenal insufficiency, growth failure and natural killer cell deficiency in humans

MCM4 mutation causes adrenal failure, short stature and natural killer cell deficiency in humans

Hughes CR, Guasti L, Meimaridou E, Chuang CH, Schimenti JC, King PJ, Costigan C, Clark AJ, Metherell LA
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J Clin Invest 2012;122:814–820

Background: A variant of familial glucocorticoid deficiency (FGD) is characterized by adrenal insufficiency, growth failure, increased chromosomal breakage and natural killer (NK) cell deficiency.

Methods and Results: Targeted exome sequencing in 8 patients with this condition identified a variant (c.71-1insG) in minichromosome maintenance-deficient 4 (MCM4) gene that was predicted to result in a severely truncated protein (p.Pro24ArgfsX4). Western blotting of patient samples revealed that the major 96-kDa isoform present in unaffected human controls was absent, while the presence of the minor 85-kDa isoform was preserved. Histological studies with Mcm4 knock-out mice showed grossly abnormal adrenal morphology. Given that MCM4 is one part of a MCM2-7 complex recently confirmed as the replicative helicase essential for normal DNA replication and genome stability in all eukaryotes, it is possible that these patients may have an increased risk of neoplastic change.

Conclusions: This study identified the first human mutation in MCM4, which is associated with adrenal insufficiency, short stature and NK cell deficiency.

Familial glucocorticoid deficiency (FGD) is an autosomal recessive condition characterized by adrenocorticotrophic hormone (ACTH) resistant glucocorticoid deficiency [1]. The condition is relatively common in the Irish Traveler community, a genetically isolated population with increased rates of consanguinity. In addition to adrenal insufficiency, a subgroup of patients have evidence of increased chromosomal breakage, growth failure and NK cell deficiency. In this study, Hughes and colleagues investigated children with adrenal insufficiency from three kindreds within the Irish Traveler community. Known causes of adrenal insufficiency were excluded clinically and biochemically, and mutations in MC2R, MRAP, and STAR were not detected. Since the clinical features cosegregated and inheritance patterns were suggestive of an autosomal recessive mode of transmission, the authors sought common areas of homozygosity and subsequently interrogated these areas using exon cap-

ture and high-throughput sequencing. They demonstrated a mutation in the MCM4 gene, which leads to adrenal insufficiency, short stature and NK cell deficiency. Patients demonstrate a phenotype similar to other DNA repair and replication disorders, including increased chromosomal fragility, pre- and postnatal growth retardation, and variable immune deficiency. In addition, this disorder includes adrenal insufficiency. MCM4, a component of the MCM2-7 complex, is part of the pre-replicative complex, which licenses origins for DNA synthesis in the S phase. These findings indicate that defects in replication licensing might lead to disorders with similar growth retardation phenotypes but distinct developmental abnormalities. Therefore, other components of the MCM complex represent prime potential candidates for other undiagnosed cases of chromosomal instability or adrenal insufficiency.

Partial MCM4 deficiency in patients with growth retardation, adrenal insufficiency and natural killer cell deficiency

Gineau L, Cognet C, Kara N, Lach FP, Dunne J, Veturi U, Picard C, Trouillet C, Eidenschenk C, Aoufouchi S, Alcaïs A, Smith O, Geissmann F, Feighery C, Abel L, Smogorzewska A, Stillman B, Vivier E, Casanova JL, Jouanguy E
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J Clin Invest 2012;122:821–832

Background: Natural killer (NK) cells are circulating cytotoxic lymphocytes that exert potent and non-redundant antiviral activity and anti-tumoral activity in the mouse; however, their function in host defense in humans is not clear. In the present study, the authors investigated 6 related patients with autosomal recessive adrenal insufficiency, growth retardation and a selective NK cell deficiency characterized by a lack of the CD56(dim) NK subset.

Methods and Results: Using linkage analysis and fine mapping, they identified the disease-causing gene, MCM4, which encodes a component of the MCM2-7 helicase complex required for DNA replication. A splice-site mutation in the patients produced a frameshift, but the mutation was hypomorphic due to the creation of two new translation initiation methionine codons downstream of the premature termination codon. The patients' fibroblasts exhibited genomic instability, which was rescued by expression of the wild-type MCM4.

Conclusions: Partial MCM4 deficiency results in a genetic syndrome of growth retardation, adrenal insufficiency and selective NK deficiency. The clinical manifestations of growth retardation and adrenal insufficiency likely reflect the ubiquitous but heterogeneous impact of the MCM4 gene mutation in various tissues.

Natural killer (NK) cells are circulating cytotoxic lymphocytes lacking antigen-specific Tcell and Bcell receptors. They exert potent and non-redundant antiviral activity and anti-tumoral activity in the mouse model; however, their function in host defense in humans remains unclear. Several children with a specific quantitative circulating NK cell defect but normal Tcell counts have been reported. In the present study, Gineau and colleagues investigated six related patients with autosomal recessive adrenal insufficiency, growth retardation and a selective NK cell deficiency characterized by a lack of the CD56dim NK subset. Using linkage analysis and fine mapping, they confirmed that the MCM4 was the disease-causing gene. Partial MCM4 deficiency is the first genetic etiology of a human disorder associated with selective NK cell deficiency to be described. In humans, the MCM4 deficit selectively affects the CD56dim subset of NK cells, which account for 90% of the mature circulating NK cells. The NK CD56dim subset originates from the NK CD56bright subset and the transition between these two subsets is associated with a decrease in the capacity of NK cells to proliferate. The concomitant observation of a lack of proliferation of NK CD56bright cells and of the loss of the NK CD56dim subset in the patients suggests that the final stage of NK differentiation requires the proliferation of NK CD56bright cells. Thus, the identification of this MCM4 deficiency sheds light not only on the genetic deficiency in these patients, but also on the mechanisms of NK cell differentiation, providing the first genetic evidence for the differentiation of CD56bright cells into CD56dim NK cells in humans.

BDNF and glucocorticoids regulate corticotrophin-releasing hormone homeostasis in the hypothalamus

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Proc Natl Acad Sci USA 2012;109:1305–1310

Background: Regulation of the hypothalamic-pituitary-adrenal (HPA) axis is critical for adaptation to environmental changes. Corticotrophin-releasing hormone (CRH), the principle regulator of the HPA axis, is an important target of negative feedback by glucocorticoids. Disruption of normal HPA axis activity is a major risk factor of neuropsychiatric disorders, in which decreased expression of the glucocorticoid receptor (GR) has been documented.

Methods and Results: Impairment of GR function in the paraventricular nucleus (PVN) resulted in an enhancement of CRH expression, an up-regulation of hypothalamic levels of BDNF and disinhibition of the HPA axis. BDNF is a stress and activity-dependent factor involved in many activities modulated by the HPA axis. Ectopic expression of BDNF in vivo increased CRH, whereas reduced expression of BDNF, or its receptor TrkB, decreased CRH expression and normal HPA function. The differential regulation of CRH relies upon the cAMP response-element binding protein coactivator CRTC2, which serves as a switch for BDNF and glucocorticoids to direct the expression of CRH.

Conclusions: These findings revealed a homeostatic mechanism by which hypothalamic BDNF and glucocorticoid signaling maintain CRH and glucocorticoid bioavailability.

The HPA axis is regulated by CRH and glucocorticoids. Stress activates the HPA axis via polysynaptic circuits that converge on the hypothalamic paraventricular nucleus (PVN) to activate CRH-producing neurons. Endocrine feedback control is characterized by the down-regulation of CRH and ACTH by glucocorticoids to prevent further increase in glucocorticoid concentrations. Therefore, maintenance of the HPA axis involves a homeostatic equilibrium between activation and inhibitory feedback. Both activation and feedback regulation are dependent upon the glucocorticoid receptor (GR), which is expressed in brain structures that control HPA axis reactivity. Neurotrophic factors, such as BDNF (brain-derived neurotrophic factor), are involved in regulating many functions of the HPA axis [2]. BDNF and its receptor, TrkB, are both expressed in the PVN and other brain regions, and play an important role in neuroprotection and synaptic plasticity. In the present study, the authors demonstrated that genetic disruption of GR in the PVN disinhibited both the HPA axis and the expression of hypothalamic BDNF. BDNF and glucocorticoid signaling intersect upon CRH. These findings provide a unique mechanism involving cAMP response-element binding protein (CREB) and its coactivator protein, CRTC2, which explains how BDNF can balance the ability of glucocorticoids to influence CRH expression specifically in the PVN. These findings revealed a homeostatic mechanism by which hypothalamic BDNF and glucocorticoid signaling maintain CRH and glucocorticoid bioavailability.

Reconciling the nutritional and glucocorticoid hypotheses of fetal programming

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FASEB J 2012;26:1866–1874

Background: Fetal growth restriction is associated with an increased risk of cardiometabolic and neuropsychiatric disorders in adulthood. Both maternal malnutrition (notably a low-protein (LP) diet) and stress/glucocorticoid exposure reduce fetal growth and cause persisting abnormalities in adult offspring. Deficiency of placental 11 β -hydroxysteroid dehydrogenase-2 (11 β -HSD2) is reduced by an LP diet and has been proposed as a unifying mechanism. The aim of the present study was to explore the role of glucocorticoids and placental 11 β -HSD2 in dietary programming.

Methods and Results: Pregnant mice were fed a control or isocaloric LP diet throughout gestation. The LP diet first elevated fetal glucocorticoid levels, then reduced placental growth and finally decreased fetal weight near term by 17%. Whereas the LP diet reduced placental 11 β -HSD2 activity near term by 25%, the activity was increased between 20 and 40% at earlier ages, implying that glucocorticoid overexposure in LP fetuses occurs via 11 β -HSD2-independent mechanisms. Heterozygous 11 β -HSD2^(+/-) crosses showed that although both LP and 11 β -HSD2 deficiency reduced fetal growth, LP indeed acted independently of 11 β -HSD2. Instead, the LP diet induced the fetal hypothalamic-pituitary-adrenal axis per se.

Conclusions: Maternal malnutrition and placental 11 β -HSD2 deficiency act via distinct processes to retard fetal growth, which both involve fetoplacental overexposure to glucocorticoids but from distinct sources.

Fetal growth restriction reflects an adverse intrauterine environment and is associated with a substantially increased risk of metabolic, cardiovascular, and behavioral disorders in adult life [3]. Maternal malnutrition or psychological stress and glucocorticoid exposure during pregnancy have been shown to constrain fetal growth and also to have long-term effects on adult health [4]; this phenomenon is called developmental programming. In both humans and rodents, circulating maternal glucocorticoid (cortisol in humans and corticosterone in rodents) concentrations during mid to late gestation are 5- to 10-fold higher than those in the fetus. The enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) catalyzes the inactivation of cortisol and corticosterone to inert cortisone and 11-dehydrocorticosterone (11-DHC), respectively, and is highly expressed in the placenta, where it protects the developing fetus from the deleterious effects of excess maternal glucocorticoids. Inhibition of 11 β -HSD2 or its bypass through administration of dexamethasone, which is poorly metabolized by 11 β -HSD2, reduces offspring birth weight and causes adult programming outcomes. Maternal malnutrition in humans reduces birth weight and placental size at term, which correlate with reduced placental 11 β -HSD2. In the present study, the authors directly tested the hypothesis that the maternal low-protein (LP) diet effects are mediated by increased fetal glucocorticoid exposure, specifically via a reduction in placental 11 β -HSD2. They showed that maternal protein malnutrition causes placental and then late fetal growth restriction. The growth restriction was preceded and accompanied by fetal glucocorticoid excess, which correlated closely and negatively with fetal growth by late gestation. Although 11 β -HSD2 deficiency also causes fetal growth restriction, placental insufficiency, and developmental programming, this was not the cause of LP-induced fetal growth restriction and, by implication, of programming by maternal malnutrition. Instead, the fetal HPA axis is prematurely activated in the LP fetus alongside fetoplacental IGF-II deficiency. It is clear that glucocorticoids are important in both models, yet the likely source and underlying processes differ.

Genotype-phenotype analysis in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency

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Background: P450 oxidoreductase deficiency (PORD) is a form of congenital adrenal hyperplasia that manifests with glucocorticoid deficiency, disordered sex development (DSD) and skeletal malformations. The aim of this study was to determine the genotype-phenotype correlation in a large cohort of subjects with PORD.

Methods: Thirty patients with PORD from 11 countries were recruited to participate in the study. Subjects underwent clinical, biochemical and genetic assessment, including multiplex ligation-dependent probe amplification (MLPA).

Results: Twenty-three P450 oxidoreductase (POR) mutations (14 novel) including an exonic deletion and a partial duplication were detected by MLPA. Only 22% of unrelated patients carried homozygous POR mutations. p.A287P was the most common mutation identified in 43% of unrelated alleles. Urinary steroid profiling showed characteristic PORD metabolomes with variable impairment of 17 α -hydroxylase and 21-hydroxylase. Adrenal insufficiency was diagnosed in 89% of patients using a short cosyntropin test. DSD was present in 15 of 18 46,XX and seven of 12 46,XY individuals. Homozygosity for p.A287P was invariably associated with 46,XX DSD but normal genitalia in 46,XY individuals. The majority of patients with mild to moderate skeletal malformations were compound heterozygous for missense mutations, whereas nearly all patients with severe malformations carried a major loss-of-function defect on one of the affected alleles.

Conclusions: MLPA is a useful addition to POR mutation analysis. Homozygosity for the most frequent mutation in Caucasians, p.A287P, allows for prediction of genital phenotype and moderate malformations. Adrenal insufficiency is frequent, easily overlooked, but readily detectable by cosyntropin testing.

P450 oxidoreductase deficiency (PORD) is a congenital adrenal hyperplasia variant owing to mutations affecting POR, which serves as mandatory electron donor enzyme to all microsomal cytochrome P450 (CYP) enzymes [5]. In PORD, deficient steroidogenesis is caused by indirect impairment of key enzymes involved in glucocorticoid and sex steroid synthesis, including 17 α -hydroxylase, 21-hydroxylase and P450 aromatase. In the present study, Krone and colleagues described in great detail the clinical, biochemical and genetic findings in a large Caucasian PORD cohort. They established the usefulness of MLPA as an addition to molecular genetic analysis of the POR gene, and provided a novel scoring system for standardized assessment of PORD-associated malformations. They showed that major loss-of-function mutations on one of the affected alleles are associated with severe malformations, whereas homozygosity or compound heterozygosity for missense mutations predicts a mild to moderate malformation phenotype. Importantly, homozygosity for the most common mutation in Caucasians, p.A287P, allows for prediction of the genital phenotype and is associated with mild to moderate malformations. Finally, adrenal insufficiency is present in the majority of patients and cannot be predicted by genotype.

18-Hydroxycorticosterone, 18-hydroxycortisol, and 18-oxocortisol in the diagnosis of primary aldosteronism and its subtypes

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J Clin Endocrinol Metab 2012;97:881–889

Background: The diagnosis of primary aldosteronism (PA) is made following a three-step procedure comprising of screening, confirmation testing, and subtype diagnosis. However, some tests are costly and unavailable in most hospitals. The aim of the study was to evaluate the role of serum 18-hydroxycorticosterone (s18OHB), urinary and serum 18-hydroxycortisol (u- and s18OHF), and urinary and serum 18-oxocortisol (u- and s18oxoF) in the diagnosis of PA and its subtypes, aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH).

Methods: 26 patients with low-renin essential hypertension (EH), 81 patients with PA (20 APA, 61 BAH), 24 patients with glucocorticoid-remediable aldosteronism, 16 patients with adrenal incidentaloma, and 30 normotensive subjects were recruited to participate in the study. s18OHB, s18OHF, and s18oxoF before and after saline load test (SLT) and 24-h u18OHF and u18oxoF were determined in all subjects.

Results: PA patients displayed significantly higher concentrations of s18OHB, u18OHF, and u18oxoF compared to EH and normal subjects. Patients with APA displayed significantly higher s18OHB, u18OHF, and u18oxoF concentrations than BAH patients. Similar results were obtained for s18OHF and s18oxoF. SLT significantly reduced s18OHB, s18OHF, and s18oxoF in all groups, but steroid reduction was much less for APA patients compared to BAH and EH. The s18OHB/aldosterone ratio after SLT more than doubled in EH but remained unchanged in APA patients.

Conclusions: u18OHF, u18oxoF, and s18OHB measurements in patients with a positive aldosterone/plasma renin activity ratio correlate with confirmatory tests and adrenal vein sampling in PA patients. If verified, these steroid assays would refine the diagnostic workup for PA.

Primary aldosteronism (PA) is the most common form of secondary hypertension. The detection of PA is of particular importance because affected subjects are more prone to cardiovascular events and target organ damage than patients with essential hypertension. According to the Endocrine Society Guidelines, the diagnosis of PA is made following a three-step procedure comprising screening, confirmation/exclusion testing, and subtype diagnosis [6]. The most common screening test for PA is the measurement of the aldosterone/plasma renin activity (PRA) ratio (ARR); a positive ARR is followed by a confirmatory test, preferably using a saline load, to definitively confirm/exclude PA. However, there is no agreement on which of four accepted confirmatory tests should be performed because each test displays both advantages and potential pitfalls. Adrenal vein sampling (AVS) is the only reliable method to differentiate unilateral from bilateral PA; however, this test is expensive, requires a dedicated and expert radiologist, and is not available in most hospitals.

18-Hydroxycorticosterone (18OHB) is an intermediate precursor in aldosterone biosynthesis that originates from the conversion of corticosterone by the aldosterone synthase, although small amounts may be produced by the 11 β -hydroxylase. 18-Hydroxycortisol (18OHF) and 18-oxocortisol (18oxoF), known as “hybrid steroids” are produced by aldosterone synthase using 11-deoxycortisol as substrate, although 18OHF can also be produced by 11 β -hydroxylase. Given that aldosterone synthase expression is normally limited to the zona glomerulosa, and 17 α -hydroxylase and 11 β -hydroxylase necessary for cortisol synthesis occur in the zona fasciculata, production of 18OHF and 18oxoF is normally very low. Their synthesis is dramatically increased in glucocorticoid-remediable aldosteronism (GRA) due to the availability of substrate to the aldosterone synthase expressed in the zona fasciculata in GRA. Levels of 18OHF and 18oxoF were shown to be better than the dexamethasone suppression test for the diagnosis of GRA. In the present study, the authors evaluated the role of 18OHB, 18OHF, and 18oxoF in the diagnosis of PA and its subtypes. This is the largest study addressing the role of 18OHF, 18oxoF, and 18OHB in the diagnosis of PA and its subtypes. It demonstrated that none of the steroid assays were sensitive or specific enough to replace a confirmatory test and/or AVS in all patients. AVS is still the only reliable way to distinguish unilateral from bilateral forms of hyperaldosteronism.

Recovery of the hypothalamic-pituitary-adrenal axis in children and adolescents after surgical cure of Cushing's disease

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Background: Recovery of the hypothalamic-pituitary-adrenal (HPA) axis after transsphenoidal surgery (TSS) for Cushing's disease (CD) in children has not been adequately studied. The aim of this study was to assess the time to recovery of the HPA axis after TSS in children with CD.

Methods: 57 patients with CD (age range: 6–18 yr, mean: 13.0 ± 3.1 yr) given a standard regimen of glucocorticoid tapering after TSS were studied. ACTH (250 µg) stimulation tests were administered at 6-month intervals for up to 36 months. Age, sex, pubertal status, body mass index, length of disease, midnight cortisol, and urinary free cortisol at diagnosis were analyzed for effects on recovery. The main outcome measure was complete recovery of the HPA axis as defined by a cortisol concentration of at least 18 µg/dl in response to 250 µg ACTH.

Results: Full recovery of the HPA axis was documented in 43 (75.4%) of 57 patients, with 29 of the 43 (67.4%) and 41 of the 43 (95.3%) recovering by 12 and 18 months, respectively. The overall mean time to recovery was 12.6 ± 3.3 months. By receiver operating characteristic curve assessment, the cutoff of at least 10–11 µg/dl of cortisol as the peak of ACTH stimulation testing at 6 months after TSS yielded the highest sensitivity (70–80%) and specificity (64–73%) to predict full recovery of the HPA axis at 12 months. Two of the four patients that recovered fully within 6 months had recurrent CD.

Conclusions: This study presents a standardized tapering regimen for glucocorticoid replacement after TSS that led to recovery of the HPA axis in most patients within the first postoperative year. Although multiple factors may affect this process, an early recovery may indicate disease recurrence.

Recovery of normal pituitary function is essential for resumption of growth and development of children that undergo transsphenoidal surgery (TSS) for Cushing's disease (CD). Hypercortisolism suppresses hypothalamic and pituitary functions preoperatively. Unless TSS was complicated by multiple procedures and other untoward effects, hypercortisolism (and not surgery) appears to be mostly responsible for the pituitary hormone deficiencies seen postoperatively. This study proposes that an empirically made but standardized regimen for replacement and tapering of glucocorticoid replacement after TSS for CD leads to safe recovery of HPA axis function within 12 months after TSS in most patients. The standardized tapering regimen consisted of a starting dose of hydrocortisone of at 8–12 mg/m²/day in two divided doses with the larger dose (approximately two thirds of the total dose) administered in the morning. After 4 months, the hydrocortisone dose was reduced by 2.5 mg every 4–6 wk until the patients were on only 5 mg/d. This slow tapering method aimed at avoiding undertreatment with possible adrenal crises and overtreatment leading to HPA axis suppression. The data of this study also indicate that the severity of hypercortisolism before TSS is a factor that influences recovery, whereas early recovery is highly suspicious for failed surgical treatment.

Urine steroid metabolomics as a biomarker tool for detecting malignancy in adrenal tumors

Arlt W, Biehl M, Taylor AE, Hahner S, Libé R, Hughes BA, Schneider P, Smith DJ, Stiekema H, Krone N, Porfiri E, Opocher G, Bertherat J, Mantero F, Allolio B, Terzolo M, Nightingale P, Shackleton CH, Bertagna X, Fassnacht M, Stewart PM

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J Clin Endocrinol Metab 2011;96:3775–3784

Background: Adrenal tumors have a prevalence of approximately 2% in the general population. Adrenocortical carcinoma (ACC) is rare but accounts for 2–11% of incidentally discovered adrenal masses. The differentiation of ACC from adrenocortical adenoma (ACA) represents a diagnostic challenge in patients with adrenal incidentalomas. The aim of this study was to examine the diagnostic

value of a novel steroid metabolomic approach (mass spectrometry-based steroid profiling followed by machine learning analysis) for the detection of adrenal malignancy.

Methods: Quantification of 32 distinct adrenal derived steroids was carried out by gas chromatography/mass spectrometry in 24-h urine samples from 102 ACA patients (age range 19–84 yr) and 45 ACC patients (20–80 yr). The underlying diagnosis was ascertained by histology and metastasis in ACC and by clinical follow-up (median duration 52 (range 26–201) months) without evidence of metastasis in ACA. Steroid excretion data were subjected to generalized matrix learning vector quantization (GMLVQ) to identify the most discriminative steroids.

Results: Steroid profiling revealed a pattern of predominantly immature, early-stage steroidogenesis in ACC. GMLVQ analysis identified a subset of nine steroids that performed best in differentiating ACA from ACC. Receiver-operating characteristics analysis of GMLVQ results demonstrated sensitivity = specificity = 90% employing all 32 steroids and sensitivity = specificity = 88% when using only the nine most differentiating markers.

Conclusions: Urine steroid metabolomics is a novel, highly sensitive, and specific biomarker tool for discriminating benign from malignant adrenal tumors, with obvious promise for the diagnostic work-up of patients with adrenal incidentalomas.

Adrenocortical carcinoma (ACC) is a rare tumor, however, it accounts for 2–11% of incidentally discovered adrenal masses. Even when basing the histopathological assessment on the entire tumor specimen, the differentiation between benign and malignant lesions represents a major diagnostic challenge. In this study, Arlt and colleagues undertook steroid metabolite excretion analysis by mass spectrometry followed by computational analysis, and investigated the performance of this novel biomarker tool in detecting malignancy and hormone excess in a large cohort of patients with adrenal tumors. They demonstrated that this method has a sensitivity and specificity of 90% for discriminating ACCs from ACAs. They also identified the 11-deoxycortisol metabolite THS to be the most discriminative steroid in differentiating ACC from ACA. THS was significantly increased in both ACA and ACC patients compared with controls, but excretion levels were significantly higher in ACC than in ACA, suggesting inhibition or lack of expression of 11 β -hydroxylase, the enzyme that converts the glucocorticoid precursor 11-deoxycortisol to active cortisol. These data clearly indicate that urine steroid metabolomics represents a novel and highly promising biomarker approach to the differential diagnosis of adrenal tumors. Before implementation of this novel approach as a diagnostic test in routine clinical practice, prospective validation in large cohorts of patients with adrenal tumors is recommended.

Clinical trials – new treatments

A dual-release formulation of hydrocortisone for the treatment of adrenal insufficiency

Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation

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J Clin Endocrinol Metab 2012;97:473–481

Background: Patients with adrenal insufficiency (AI) have increased morbidity and mortality rate on standard replacement therapy. The aim of this study was to compare the pharmacokinetics and metabolic outcome between a once-daily (OD) oral hydrocortisone dual-release tablet and the same daily dose of thrice-daily (TID) dose of conventional hydrocortisone tablets.

Methods: An open, randomized, two-period, 12-wk crossover multicenter trial with a 24-wk extension was conducted at five university hospital centers. The trial enrolled 64 adults with primary AI; 11 had

concomitant diabetes mellitus (DM). The same daily dose of hydrocortisone was administered as OD dual-release or TID.

Results: Compared with conventional TID, OD provided a sustained serum cortisol profile 0–4 h after the morning intake and reduced the late afternoon and the 24-h cortisol exposure. The mean weight (difference: –0.7 kg, $p = 0.005$), systolic blood pressure (difference: –5.5 mm Hg, $p = 0.0001$), diastolic blood pressure (difference: –2.3 mm Hg; $p = 0.03$), and glycated hemoglobin (absolute difference: –0.1%, $p = 0.0006$) were all decreased after OD compared with TID at 12 wk. Compared with TID, a reduction in glycated hemoglobin by 0.6% was observed in patients with concomitant DM during OD ($p = 0.004$).

Conclusions: The OD dual-release tablet provided a more circadian-based serum cortisol profile and was associated with reduced body weight, reduced blood pressure and improved glucose metabolism. Furthermore, glucose metabolism improved in patients with concomitant DM.

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 AD have shown a more than double the standardized mortality rate despite contemporary optimal glucocorticoid replacement therapy [7]. 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 [8]. 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. A novel once-daily (OD) dual-release hydrocortisone tablet, based on an immediate-release coating together with an extended-release core, was developed to obtain a more physiological circadian-based serum cortisol exposure-time profile. The cortisol time-exposure profile achieved using the OD dual-release hydrocortisone treatment improved cardiovascular risk factors, glucose metabolism and QoL in comparison with conventional treatment. The OD dosing achieved a high and reliable bio-availability and consistent-exposure serum cortisol profile more resembling normal physiology, avoiding the last two peaks during TID, all of which may be of importance for improving outcome in AI patients.

New Mechanisms

Novel mechanisms that control adrenal steroidogenesis

Transcriptional control of adrenal steroidogenesis: novel connection between Janus kinase 2 protein and protein kinase A through stabilization of cAMP response element-binding protein transcription factor

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J Biol Chem 2011;286:32976–32985

Background: In the adrenal gland, adrenocorticotropin (ACTH) acts through the cAMP protein kinase (PKA) transduction pathway and represents the main regulator of genes involved in glucocorticoid synthesis. The present study investigated the influence of JAK/STAT signaling on the steroidogenic activity of adrenocortical cell cultures.

Methods and Results: Pharmacological or siRNA-mediated inhibition of Janus kinase 2 (JAK2) reveals its essential role in both basal and ACTH/cAMP-induced steroidogenesis. In addition, nuclear JAK2 regulates the amount of active transcription factor CREB (cAMP response element-binding protein) through

tyrosine phosphorylation and prevention of proteasomal degradation, which in turn leads to transcriptional activation of the rate-limiting steroidogenic Star gene.

Conclusions: These findings demonstrate a novel link between PKA and JAK2, by which nuclear JAK2 signaling controls adrenal steroidogenesis by increasing the stability of CREB.

Steroidogenesis is regulated mainly through the adrenocorticotropin (ACTH) activation of the cAMP-dependent protein kinase A (PKA) signaling pathway. Chronic response to ACTH results in increased transcription of genes encoding steroidogenic enzymes and the proteins responsible for cholesterol mobilization and transport, such as the steroidogenic acute regulatory protein (STAR). cAMP-induced PKA activation results in the phosphorylation of the transcription factors GATA-4-binding protein, steroidogenic factor 1 (SF1), CAAT enhancer-binding protein (C/EBP) and cAMP response element-binding protein (CREB), which in turn stimulate the transcription of genes involved in steroidogenesis. Although cAMP is an essential inducer of steroidogenesis, other mechanisms triggered by paracrine and endocrine signals have been involved in the regulation of steroid synthesis in basal or stress-related conditions. They can either act independently or in synergy with the PKA signaling. In the present study, the authors demonstrated that Janus kinase 2 (JAK2), which is well known to be involved in GH, prolactin and leptin signaling, was also involved in basal and ACTH-induced steroidogenesis. In adrenocortical cells, activated JAK2 levels were not sensitive to ACTH. They exerted an essential control on basal and ACTH-induced steroidogenesis by interacting with the PKA through the modulation of CREB protein accumulation. CREB-induced Star transcription was dependent on JAK2 but was independent of signal transducer and activation of transcription 5 (STAT5). In adrenocortical cells, JAK2 acted in the nucleus to stabilize the Ser(P)133-CREB by preventing its early proteasomal degradation. This, in turn, allowed stimulation of Star gene transcription. These data demonstrate for the first time that STAT5-independent nuclear JAK2 signaling plays an essential role in adrenal steroidogenesis by increasing the stability of the transcriptionally active CREB protein, thereby allowing the expression of CREB steroidogenic target genes.

Inner mitochondrial translocase Tim50 interacts with β 3-hydroxysteroid dehydrogenase type 2 to regulate adrenal and gonadal steroidogenesis

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Background: In the adrenal glands, testes and ovaries, β 3-hydroxysteroid dehydrogenase type 2 (β 3HSD2) catalyzes the conversion of pregnenolone to progesterone and dehydroepiandrosterone (DHEA) to androstenedione. β 3HSD2 is synthesized in the cytosol and is imported into the inner mitochondrial membrane (IMM) by translocases. Steroidogenesis requires that β 3HSD2 acts as both a dehydrogenase and isomerase. To achieve this dual functionality, β 3HSD2 must undergo a conformational change; however, the underlying mechanisms remain unknown.

Methods and Results: Fractionation assays demonstrated that β 3HSD2 is associated with the IMM but did not integrate into the membrane. Mass spectrometry and Western blotting of mitochondrial complexes and density gradient ultracentrifugation showed that β 3HSD2 formed a transient association with the translocases Tim50 and Tom22 with Tim23. Tim50 knockdown inhibited catalysis of DHEA to androstenedione and pregnenolone to progesterone. Although Tim50 knockdown decreased β 3HSD2 expression, restoration of expression via proteasome and protease inhibition did not rescue activity. In addition, protein fingerprinting and CD spectroscopy revealed the flexibility of β 3HSD2, a necessary characteristic for forming multiple associations.

Conclusions: Tim50 regulates the expression and activity of β 3HSD2, which represents a new role for translocases in steroidogenesis.

Most mitochondrial proteins are synthesized as precursors on cytosolic polysomes and imported across the outer (OMM) and inner mitochondrial membranes (IMM). Translocases are loosely associated protein complexes at the mitochondrial membranes, which mediate protein import and sorting. Translocase complexes found at the OMM are called Tom (translocase, outer membrane), while those at the IMM are called Tim (translocase, inner membrane).

The IMM-associated 3 β -hydroxysteroid dehydrogenase (3 β HSD2) catalyzes the conversion of pregnenolone to progesterone, 17 α -hydroxy-pregnenolone to 17 α -hydroxy-progesterone, and dehydroepiandrosterone (DHEA) to androstenedione in conjunction with NAD⁺ as a cofactor. 3 β HSD2 has both dehydrogenase and isomerase activities. In this study, Pawlak and colleagues tested the hypothesis that the bifunctional activity of 3 β HSD2 requires the protein to undergo a conformational change. They showed that 3 β HSD2 associated with the IMM but did not integrate into it. This likely was the result of the interaction between 3 β HSD2 and Tim50, resulting in a transient complex formation of 3 β HSD2, Tom22, Tim23, and Tim50. Moreover, restoration of 3 β HSD2 expression in the Tim50 knockdown cells via proteasome and protease inhibition did not rescue the steroidogenic activity. Therefore, the association of 3 β HSD2 with the translocase appears to play a key role in the regulation of enzymatic activity. The absence of Tim50 inhibited the catalysis of DHEA to androstenedione and pregnenolone to progesterone but did not affect the expression of endoplasmic reticulum resident cytochrome P450c17.

New genes

Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma

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Background: Hereditary pheochromocytoma (PCC) is often caused by germline mutations in one of nine susceptibility genes described to date, but there are familial cases without mutations in these known genes.

Methods and Results: Sequencing of the exomes of three unrelated individuals with hereditary PCC (cases) identified mutations in MAX, the MYC-associated factor X gene. Absence of MAX protein in the tumors and loss of heterozygosity caused by uniparental disomy supported the involvement of MAX alterations in the disease. A follow-up study of a selected series of 59 cases with PCC identified five additional MAX mutations and suggested an association with malignant outcome and preferential paternal transmission of MAX mutations.

Conclusions: These findings elucidate the mechanisms underlying the development of hereditary PCC, as well as those of metastatic potential.

Pheochromocytoma (PCC) is a rare neural crest cell tumor mainly localized within the adrenal medulla. Approximately 30–40% of individuals with PCC are familial cases, who show dominant autosomal inheritance caused by germline mutations affecting one of nine susceptibility genes: RET, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, NF1 or TMEM127. A subset of tumors from familial cases with PCC without germline alterations in the susceptibility genes identified to date showed a homogeneous expression profile, suggesting that mutations in the same as yet unidentified gene could be responsible for these cases. Next-generation sequencing is an emerging new tool for discovering genes responsible for genetically heterogeneous diseases. In the present study, next-generation sequencing allowed the identification of MAX as a new PCC tumor suppressor gene. MAX is the most conserved dimerization component of the MYC-MAX-MXD1 network of basic helix-loop-helix leucine zipper (bHLHZip) transcription factors that regulate cell proliferation, differentiation and apoptosis. In this network, MAX plays an essential role, as it is the common interaction partner for both MYC and MXD1 proteins. The discovery of MAX germline mutations in individuals with a hereditary adrenal tumor suggests a role for the MYC-MAX-MXD1 network in the development and progression of neural crest tumors. Moreover, the malignant behavior of MAX-related PCCs supports the idea that MAX loss of function is correlated to metastatic potential. The results of this study elucidate the mechanisms

underlying the development of hereditary PCCs, as well as those of their metastatic potential. In addition, they have clinical implications for genetic counseling and genetic testing algorithms. Further studies are required to determine the prevalence of MAX mutations in PCC and the phenotype associated with MAX mutations.

Reviews

Development and function of the human fetal adrenal cortex: a key component in the fetoplacental unit

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The human fetal adrenal cortex plays a pivotal role in the regulation of intrauterine homeostasis and in fetal development and maturation. The steroidogenic activity is characterized by early transient cortisol biosynthesis, followed by its suppressed synthesis until late gestation, as well as extensive production of dehydroepiandrosterone and its sulfate, precursors of placental estrogen, during most of gestation. Recent studies revealed that the development and/or function of the fetal adrenal cortex may be regulated by a number of molecules, including transcription factors, extracellular matrix components, locally produced growth factors, and placenta-derived CRH, in addition to the primary regulator, fetal pituitary ACTH. The role of the fetal adrenal cortex in human pregnancy and parturition appears highly complex. The actions of hormones operating in the human fetoplacental unit are most likely mediated by mechanisms including target tissue responsiveness, local metabolism and bioavailability, as well as changes in circulating concentrations. The comprehensive study of such molecular mechanisms and the newly identified factors implicated in adrenal development should enhance our understanding of the development and function of the human fetal adrenal cortex.

The human fetal adrenal (HFA) cortex shares its unique structural and functional organization with higher primates, such as the rhesus monkey and baboon. The HFA cortex is an active endocrine organ in which most steroidogenic activity is exerted in a specialized cortical compartment known as the fetal zone (FZ), a unique feature of fetal adrenals in humans and some higher primates, but not in other species, such as rodents and sheep. Accumulating evidence suggests that growth of the HFA appears to involve cellular proliferation, hypertrophy, apoptosis, and migration. Appropriate development and function of the fetal adrenal cortex are critical for fetal maturation and perinatal survival. Moreover, the fetal adrenal cortex must itself undergo maturation in preparation for its essential role postnatally, i.e., production of glucocorticoids, androgens, and mineralocorticoids for fetal intrauterine homeostasis, and to insure adrenal cortical autonomy once the placenta has separated. This article presents an overview of recent key observations that serve to gain a better understanding of the developmental processes and functional aspects of the HFA.

The syndrome of 17,20lyase deficiency

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Disorders of steroidogenesis have been instrumental in delineating human steroidogenic pathways. Since 1972, several patients have been reported as having ‘17,20lyase deficiency,’ but there have been inconsistent genetic findings. A single enzyme, cytochrome P450c17, catalyzes both 17 α -hydroxylase activity and 17,20lyase activity. The 17,20lyase activity is especially sensitive to the activities of the accessory proteins P450 oxidoreductase and cytochrome b₅. The first cases of genetically and biochemically proven 17,20lyase deficiency were reported in 1997, in which specific P450c17 mutations were

identified that lost 17,20lyase activity but not 17 α -hydroxylase activity. Subsequent work identified other P450c17 mutations and mutations in the genes encoding P450 oxidoreductase and cytochrome b₅. Recently, the initially reported cases from 1972 were found to carry mutations in two aldo-keto reductases, AKR1C2 and AKR1C4. These AKR1C isozymes catalyze 3 α -hydroxysteroid dehydrogenase activity in the so-called 'backdoor pathway' by which the fetal testis produces dihydrotestosterone without the intermediacy of testosterone. 17,20Lyase deficiency should be considered a syndrome with multiple causes, and not a single disease. Study of this very rare disorder has substantially advanced our understanding of the pathways, mechanisms and control of androgen synthesis. Mutations in other as yet unidentified genes may also cause this phenotype.

Apparent 17,20lyase deficiency is a complex syndrome that can be caused by mutations in several genes. Although this disorder is rare, its study provides an excellent example of how the detailed study of a rare disorder has substantially improved our understanding of a basic physiological process: in this case, illustrating the key roles of POR and cytochrome b₅ in the synthesis of adrenal androgens, and leading to the discovery of the crucial role of AKR1C2 in fetal testicular synthesis of DHT and the role of fetal testicular DHT in normal male genital development. 17,20Lyase deficiency is an extremely difficult diagnosis to establish, so that endocrinologists must be skeptical about clinical case reports of this disorder that do not include a genetic diagnosis and proof that the identified mutation indeed reduces 17,20lyase activity while preserving 17 α -hydroxylase activity. In addition, one might consider other genes, which, if disordered, might cause a clinically similar syndrome. This study reviews the biochemistry, genetics and clinical disorders of 17,20lyase activity, which converts 21-carbon precursors of glucocorticoids to 19-carbon precursors of sex steroids.

Autoimmune Addison disease: pathophysiology and genetic complexity

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Autoimmune Addison disease is a rare autoimmune disorder with symptoms that typically develop over months or years. Following the development of serum autoantibodies to the key steroidogenic enzyme, 21-hydroxylase, patients have a period of compensated or preclinical disease, characterized by elevations in adrenocorticotrophic hormone and renin, before overt, symptomatic adrenal failure develops. Local failure of steroidogenesis, causing breakdown of tolerance to adrenal antigens, might be a key factor in disease progression. The etiology of autoimmune Addison disease has a strong genetic component in man. Allelic variants of genes encoding molecules of both the adaptive and innate immune systems have now been implicated, with a focus on the immunological synapse and downstream participants in T lymphocyte antigen-receptor signaling. With the exception of MHC alleles, no major or highly penetrant disease alleles have been found to date. Future research into autoimmune Addison disease, using genome-wide association studies and next-generation sequencing technology, will elucidate our understanding of the etiology of this disease.

Autoimmune Addison disease (AAD) is a rare endocrine condition with a prevalence in white European populations of 110–140 cases per million [9], making it 30-fold less prevalent than type 1 diabetes (T1DM) and 200-fold rarer than autoimmune thyroid diseases. Like other autoimmune diseases, AAD is more frequent in women (female:male ratio 1.5–3.5:1) and often presents in individuals between the ages of 30 and 50 years, although it can affect individuals at any age. AAD is a condition that results from the interaction of currently unknown environmental factors with incompletely defined genetic factors in a susceptible individual. The number of susceptibility loci known to contribute to this disease is gradually increasing, but those discovered so far make only a modest contribution to disease susceptibility. Studies of larger cohorts of patients with AAD are required. In addition to the candidate-gene approach, genome-wide association studies and whole-genome sequencing could offer unique insights into the genetic etiology of AAD. With an increased understanding of the etiology of AAD and the biological pathways involved, new therapeutic targets and diagnostic tools could be developed that will offer an improved outcome in patients with the condition.

Familial longevity is marked by lower diurnal salivary cortisol levels: the Leiden Longevity Study

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Background: Research findings are inconsistent whether hypothalamic-pituitary-adrenal (HPA) signaling becomes hyperactive with increasing age, resulting in increasing cortisol concentrations. Recent evidence suggests that offspring from long-lived families are biologically younger. The aim of this study was to assess whether these offspring have a lower HPA axis activity, as indicated by lower concentrations of cortisol and higher cortisol feedback sensitivity.

Methods: In a cohort of subjects from the Leiden Longevity Study comprising 149 offspring and 154 partners, salivary cortisol concentrations were determined at four time points within the first hour upon awakening and at two time points in the evening. A dexamethasone suppression test was performed as a measure of cortisol feedback sensitivity. Age, gender and body mass index, smoking and disease history (type 2 diabetes and hypertension) were considered as possible confounding factors.

Results: Salivary cortisol secretion was lower in offspring compared to partners in the morning (area under the curve: 15.6 versus 17.1 nmol/l, respectively; $p = 0.048$) and in the evening (area under the curve: 3.32 versus 3.82 nmol/l, respectively; $p = 0.024$). Salivary cortisol concentrations were not different after dexamethasone (0.5 mg) suppression between offspring and partners (4.82 versus 5.26 nmol/l, respectively; $p = 0.28$).

Conclusions: Offspring of long-lived families display a lower HPA axis activity, but not a difference in cortisol feedback sensitivity; however, further studies are required to characterize the HPA axis in offspring and partners are needed.

In this study, Noordam and colleagues investigated the relation between cortisol concentrations and cortisol feedback sensitivity with familial longevity. They showed that salivary cortisol concentrations were lower in the offspring of long-lived families compared with their partners both in the morning and in the evening. The authors also showed that cortisol feedback sensitivity, as estimated by a dexamethasone suppression test, was similar between offspring and partners, suggesting that the difference between offspring and partners in salivary cortisol concentration is most likely not caused by a difference in cortisol feedback sensitivity. However, the dose of dexamethasone used in this study (0.5 mg) might have been too high for studying cortisol sensitivity in a general population, given that it leads to a near-complete suppression in most individuals. The range of cortisol concentrations following a lower dose of dexamethasone (e.g. 0.25 mg) is much wider, and may be more appropriate to identify differences in cortisol sensitivity among individuals.

Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults

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PLoS One 2012;7:e30148

Background: A history of early adverse experiences is an important risk factor for adult psychopathology. Alterations in stress sensitivity and functioning of the hypothalamic-pituitary-adrenal (HPA) axis may underlie the association between stress and risk for psychiatric disorders. The aim of this study was to examine whether early-life stress leads to epigenetic modifications of the glucocorticoid receptor (GR) gene in humans.

Methods: The degree of methylation of a region of the promoter of the human GR gene (NR3C1) was examined in leukocyte DNA from 99 healthy adults. Participants reported on their childhood experiences of parental behavior, parental death or desertion, and childhood maltreatment. On a separate day, participants completed the dexamethasone/corticotropin-releasing hormone (Dex/CRH) test.

Results: Disruption or lack of adequate nurturing, as measured by parental loss, childhood maltreatment, and parental care, was associated with increased NR3C1 promoter methylation ($p < 0.05$). In addition, NR3C1 promoter methylation was linked to attenuated cortisol responses to the Dex/CRH test ($p < 0.05$).

Conclusions: Childhood maltreatment or adversity may lead to epigenetic modifications of the human GR gene. Alterations in methylation of this gene could underlie the associations between childhood adversity, alterations in stress reactivity, and risk for psychopathology.

Research studies in rodents, non-human primates and humans have documented the impact of early life experiences on the neurobiological mechanisms regulating stress responses and mood and anxiety disorders. Young children are dependent on caregivers for their basic physical, social and emotional needs. When this is poorly provided, the child develops a strategy adaptive to stressful life. Such children undergo substantial developmental changes in neural pathways involved in regulating emotion and behavior. As a result, disruption of early care-giving can produce profound and long-lasting changes in these neurobiological and behavioral systems. Early-life stress is a risk factor for major depression, post-traumatic stress disorder, and drug abuse, among other conditions. In this study, Tyrka and colleagues demonstrated that early-life stress, in the form of loss of a parent during childhood, maltreatment, and low parental care, was associated with epigenetic changes to the promoter region of the glucocorticoid receptor gene. Furthermore, methylation of the promoter region of this gene was linked to alterations in HPA axis function. These findings provide preliminary support for the hypothesis that altered expression of the GR due to cytosine methylation of the gene promoter could be a mechanism of the neuroendocrine effects of early-life stress, and could predispose to the development of major depression and post-traumatic stress disorder. Further work is needed to determine whether these findings are specific to lymphocytes and whether this reflects changes in central regulation of the glucocorticoid receptor in brain regions involved in stress responses and mood and anxiety disorders.

Follow-up on Yearbook 2011

K⁺ channel mutations are a cause of early-onset familial hypertension

Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5

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Background: Mutations in the KCNJ5 (potassium inwardly rectifying channel, subfamily J, member 5) gene are implicated in primary hyperaldosteronism and in autonomous glomerulosa cell proliferation in humans. These mutations alter the channel selectivity filter, producing increased Na⁺ conductance and membrane depolarization, the signal for aldosterone production and proliferation of adrenal glomerulosa cells.

Methods and Results: The members of four kindreds with early onset primary aldosteronism of unknown cause were studied. Sequencing of KCNJ5 gene revealed that affected members of two kindreds had KCNJ5(G151R) mutations. These individuals had severe progressive aldosteronism and hyperplasia requiring bilateral adrenalectomy in childhood to control arterial blood pressure. Affected members of the other two kindreds had KCNJ5(G151E) mutations. These subjects had easily controlled hypertension and no evidence of hyperplasia. Electrophysiological studies of channels expressed in 293T cells demonstrated that KCNJ5(G151E) was the more extreme mutation, producing a much larger Na⁺ conductance than KCNJ5(G151R), resulting in rapid Na⁺-dependent cell lethality.

Conclusions: These findings demonstrate striking variations in the phenotype and clinical outcome resulting from different mutations of the same amino acid in KCNJ5 and have implications for the diagnosis and pathogenesis of primary aldosteronism with and without adrenal hyperplasia.

Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism

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Background: Mutations in the KCNJ5 gene have been described recently in aldosterone-producing adenomas (APAs). The aim of this study was to investigate the prevalence of KCNJ5 mutations in patients with primary aldosteronism and their correlation with clinical, biological and molecular findings.

Methods and Results: KCNJ5 sequencing was performed on somatic (APA, n=380) and peripheral (APA, n=344; bilateral adrenal hyperplasia, n=174) DNA of patients with primary aldosteronism, collected through the European Network for the Study of Adrenal Tumors. Transcriptome analysis was performed in 102 tumors. Somatic KCNJ5 mutations (p.Gly151Arg or p.Leu168Arg) were found in 34% of APA. They were significantly more prevalent in females (49%) than males (19%; $p < 0.001$) and in younger patients (42.1 ± 1.0 vs. 47.6 ± 0.7 years; $p < 0.001$) and were associated with higher preoperative aldosterone levels (455 ± 26 vs. 376 ± 17 ng/l; $p = 0.012$) but not with therapeutic outcome after surgery. Germline KCNJ5 mutations were found neither in patients with APA nor those with bilateral adrenal hyperplasia. Somatic KCNJ5 mutations were specific for APA, since they were not identified in 25 peritumoral adrenal tissues or 16 cortisol-producing adenomas.

Conclusions: Although a large proportion of sporadic APAs harbors somatic KCNJ5 mutations, germline mutations are not similarly causative for bilateral adrenal hyperplasia. KCNJ5 mutation carriers are more likely to be females; younger age and higher aldosterone concentrations at diagnosis suggest that KCNJ5 mutations may be associated with a more florid phenotype of primary aldosteronism.

Primary hyperaldosteronism (PA) is the most common cause of secondary hypertension. Its prevalence is estimated to be approximately 10% within the hypertensive population and higher among patients with hypertension refractory to medical treatment. Early diagnosis and treatment of patients with PA is of fundamental importance because they have significantly higher incidence of stroke, myocardial infarction, and atrial fibrillation compared with age-, sex-, and BP-matched patients with essential HTN. Familial hyperaldosteronism (FH)-III is an autosomal dominant condition characterized by early-onset hypertension, non glucocorticoid-remediable hyperaldosteronism and hypokalemia. The genetic cause of FH-III is attributed to mutations in the KCNJ5 gene encoding the potassium channel Kir-3.4 [10]. The clinical spectrum of the condition in the limited number of families reported to date appears to be broad, ranging from mild or moderate hypertension, which is responsive to medical therapy, to more severe and refractory to medical treatment hypertension that requires bilateral adrenalectomy. The above studies demonstrate the striking variations in the phenotype and clinical outcome resulting from different mutations in KCNJ5 in subjects with PA with and without adrenal hyperplasia. They also showed that the absence of adrenal hyperplasia in subjects with genetically confirmed FH-III is thought to arise as a result of mutations in the KCNJ5 gene that produce a much larger Na^+ conductance, leading to rapid Na^+ -dependent cell lethality.

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