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, 2012 and May 31, 2013. Our search yielded more than 6,500 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 2012*, unless progress in the field has been incremental. Emerging themes for this year's chapter include the role of somatic mutations in ATP1A1 and ATP2B3 in aldosterone-producing adenomas and secondary hypertension, structure-phenotype and genotype-phenotype correlations in congenital adrenal hyperplasia, the role of hair cortisol determination in clinical practice, and the potential therapeutic role of selective glucocorticoid receptor and estrogen receptor modulators.

Mechanism of the year Somatic mutations in ATP1A1 and ATP2B3 as causes of secondary hypertension

Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension

Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, Penton D, Schack VR, Amar L, Fischer E, Walther A, Tauber P, Schwarzmayer T, Diener S, Graf E, Allolio B, Samson-Couterie B, Benecke A, Quinkler M, Fallo F, Plouin PF, Mantero F, Meitinger T, Mulatero P, Jeunemaitre X, Warth R, Vilsen B, Zennaro MC, Strom TM, Reincke M

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Background: Primary aldosteronism is the most prevalent form of secondary hypertension. The aim of this study was to investigate the molecular mechanisms of autonomous aldosterone secretion.

Methods and Results: Using exome sequencing of aldosterone-producing adenomas (APAs), the authors identified somatic hotspot mutations in the ATP1A1 (encoding an Na⁺/K⁺ ATPase α subunit) and ATP2B3 (encoding a Ca²⁺ ATPase) genes in three and two of the nine APAs, respectively. Functional in vitro studies of the ATP1A1 mutants showed loss of pump activity and reduced affinity for potassium. Electrophysiological ex vivo studies on primary adrenal adenoma cells provided further evidence for inappropriate depolarization of cells with ATPase alterations. Mutation-positive cases showed male dominance, increased plasma aldosterone concentrations and lower potassium concentrations compared with mutation-negative cases.

Conclusions: Dominant somatic alterations in 2 members of the ATPase gene family result in autonomous aldosterone secretion.

Primary aldosteronism (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 blood pressure-matched patients with essential hypertension. Recent studies implicated mutations in the potassium channel gene *KCNJ5* as a cause of familial and sporadic forms of PA and estimated that 30–40% of aldosterone-producing adenomas (APAs) are caused by mutations in the *KCNJ5* gene [1, 2].

In the present study, Beuschlein and colleagues performed exome sequencing in tumor and matched control tissues from patients with PA who did not have somatic *KCNJ5* mutations. They identified multiple somatic variants in 2 members of the P-type ATPase gene family responsible for establishing and maintaining the electrochemical gradients across the plasma membrane, the ATP1A1, which encodes a Na⁺/K⁺ ATPase α subunit, and the ATP2B3, which encodes the plasma membrane Ca²⁺ ATPase, and proceeded to functional characterization of these variants. They demonstrated that somatic mutations in ATP1A1 and ATP2B3 were present in approximately 7% of APAs. In both instances, inactivation of the pump function, either indirectly (in the case of ATP1A1) or directly (in the case of ATP2B3), is predicted to increase intracellular calcium ion concentrations, which in turn leads to calcium-dependent signaling and increased aldosterone secretion. These findings expand the spectrum of somatic alterations leading to APAs to 2 members of the P-type ATPase pump family, and indicate new potential therapeutic targets for the most frequent secondary form of arterial hypertension.

New paradigms

Structure-phenotype correlations of human CYP21A2 mutations in congenital adrenal hyperplasia (CAH)

Structure-phenotype correlations of human CYP21A2 mutations in congenital adrenal hyperplasia

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Proc Natl Acad Sci USA 2013;110:2605–2610

Background: Mutations in the CYP21A2 gene, which encodes steroid 21-hydroxylase, account for 90–95% of cases of congenital adrenal hyperplasia (CAH). To date, more than 100 CYP21A2 mutations have been reported, which are associated with the salt-wasting, simple virilizing or non-classic form of CAH. The aim of this study was to investigate genotype-phenotype correlations in CAH using structural analysis.

Methods: A humanized model of CYP21A2 was constructed, using the crystal structure of its bovine homolog as a template to provide comprehensive structural explanations for the clinical manifestations caused by each of the known disease-causing missense mutations in CYP21A2.

Results: Mutations affecting membrane anchoring, disrupting heme and/or substrate binding, or impairing stability of CYP21A2, caused complete loss of function and salt-wasting disease. Mutations altering the transmembrane region or conserved hydrophobic patches caused up to a 98% reduction in enzyme activity and simple virilizing disease. Mutations interfering in oxidoreductase interactions, salt-bridge and hydrogen-bonding networks, and non-conserved hydrophobic clusters, caused mild non-classic CAH.

Conclusions: In silico evaluation of previously uncharacterized gene mutations may predict the often diverse phenotypes of CAH.

Although newborn screening for CAH is widely used and the reliability of steroid hormonal measurements is increasing, difficulties still remain regarding the prediction of the clinical phenotype. In the present study, the authors used a computational modeling to perform an exhaustive sequence-structure-clinical outcome analysis of known and previously unreported missense CYP21A2 mutations, and to investigate their relation to the phenotype and disease severity in patients with CAH. They show that this approach can predict the extent of loss of function of the steroidogenic CYP21A2 enzyme. With certain mutations, varying degrees of loss of function have been confirmed biochemically by overexpressing the mutated enzyme in vitro, whereas, in other cases, the extent to which the enzyme is disrupted remains unknown. This study adds new information on genotype-phenotype correlation for mutations in which enzyme activity was unknown. In addition, it shows high overall concordance between the extent and type of structural disruption of CYP21A2 and the clinical phenotype and severity of CAH. The prediction of clinical phenotype based on individual point mutations is important for clinical practice. However, clinicians still face difficulties in predicting the clinical phenotype within the group of simple virilizing CAH.

The assessment of cortisol in human hair: associations with sociodemographic variables and potential confounders

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Stress 2012;15:578–588

Background: The analysis of cortisol in human hair is a novel tool for the study of long-term cortisol exposure over extended periods. The validity of hair cortisol has been supported by several studies that showed considerable intraindividual stability. This study investigated the influence of potential confounding variables on hair cortisol.

Methods: The main study sample comprised 360 participants (172 women) covering a wide range of age (1–91 years, mean age 25.95). To examine the effect of natural hair color and young age on hair cortisol levels, two additional samples comprising 69 participants with natural blond or dark brown hair (hair color sample), 28 young children and 34 adults (young age sample) were also recruited. The influence of natural hair color, frequency of hair washes, age, sex, use of oral contraceptives (OC), and smoking status on hair cortisol levels was analyzed.

Results: No effect of natural hair color, OC use, and smoking status was found on hair cortisol levels (all p 's > 0.10). No influence of frequency of hair washes was seen for proximal hair segments ($p = 0.335$) but for the third hair segment, indicating lower cortisol content ($p = 0.008$). Hair cortisol levels in young children and older adults ($p < 0.001$) were elevated, while men showed higher hair cortisol levels than women ($p = 0.002$).

Conclusion: Hair cortisol measurement provides a useful tool in stress-related psychobiological research when applied with the consideration of possible confounders including age and sex.

A novel tool in the diagnosis and follow-up of (cyclic) Cushing's syndrome: measurement of long-term cortisol in scalp hair

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J Clin Endocrinol Metab 2012;97:E1836–1843

Background: Determination of cortisol concentrations in 24-hour urine collections and midnight saliva are standard screening tests for the diagnosis of Cushing's syndrome (CS). However, these tests do not provide historical information on cortisol exposure but rather reflect the cortisol concentrations of the preceding 24 h, at the most. Especially in the case of cyclic CS, the diagnosis can be missed if urine or saliva cortisol concentrations are used. Cyclic CS is a rare disorder that is characterized by alternating episodes of endogenous cortisol excess and normal cortisol secretion. The measurement of cortisol in scalp hair is a novel tool that might be helpful to establish the diagnosis of (cyclic) CS. The aim of this study was to investigate whether construction of retrospective timelines of hair cortisol correspond with symptomatic periods in patients with CS and suspected cyclic CS.

Methods: In 14 patients with confirmed CS and 6 patients with suspected cyclic CS, scalp hair cortisol was measured using ELISA after extraction with methanol. 96 non-obese, 43 overweight and 15 obese individuals were used as controls.

Results: Hair cortisol levels were significantly elevated in patients with CS compared with non-obese individuals ($p < 0.0001$). The sensitivity and specificity of hair cortisol measurements for CS were 86 and 98%, respectively. Hair cortisol timelines of patients with CS and cyclic CS corresponded with the clinical course of the disease.

Conclusion: Hair samples can provide a historical timeline that corresponds with the clinical course in patients with (cyclic) CS. This new diagnostic tool can contribute significantly to early recognition of patients suffering from cyclic CS.

The measurement of cortisol in scalp hair is a novel tool that obtains a retrospective index of cumulative cortisol exposure. Several studies have validated hair cortisol measurements and shown that hair

can be used to create a retrospective timeline of cortisol exposure, with every 1 cm of hair corresponding to a period of approximately 1 month. When using hair cortisol, one should take into consideration possible confounding influences. In the former study, Dettenborn and colleagues describe age, sex, and washout effect in the more distal hair samples as confounding factors, but other research groups did not find an effect of sex and washout in distal hair samples [3]. Factors including natural hair color, smoking status and the number of hair washes (in scalp-near hair segments) do not have a significant effect on hair cortisol levels. In addition, the use of oral contraceptives had no effect, indicating that hair cortisol levels reflect free cortisol concentrations. It should be noted, however, that the subjects who participated in the study of Dettenborn and colleagues were students, friends and family members of the wider research team, and that the sample of prepubertal children had a wide age range. Future studies should provide reference ranges for healthy children from the general population based on a larger sample size.

Hair cortisol measurement is also a promising novel tool in the diagnosis and follow-up of patients with Cushing's syndrome. Cushing's syndrome (CS) can be difficult to diagnose owing to the limitations of sensitivity and specificity of current diagnostic tests. Especially in cyclic CS, the diagnosis is difficult and can be easily missed when standard tests are performed during the periods of normal cortisol secretion. In the latter study, Manenschijs and colleagues show that hair cortisol can contribute significantly to early recognition of patients suffering from (cyclic) Cushing's syndrome. The method to measure cortisol in scalp hair has been used in only a few laboratories worldwide, and the normal values vary between institutions. To implement this method as a diagnostic tool in the clinical setting, further refinement and standardization of the technique is required. In addition, robust data on reference ranges and validation in pediatric adrenal diseases is needed before it can be used in the clinical setting for pediatric patients. Overall, determination of hair cortisol levels has major advantages over other currently used tests. The ability to reconstruct a retrospective timeline of long-term cortisol exposure and the non-invasiveness of hair sampling further underscore the usefulness of this method.

New concerns

Long-term effects of prenatal synthetic glucocorticoids

Impact of antenatal synthetic glucocorticoid exposure on endocrine stress reactivity in term-born children

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J Clin Endocrinol Metab 2012;97:3538–3544

Background: Antenatal glucocorticoid (GC) exposure has been discussed as a potent programming factor of hypothalamus-pituitary-adrenal (HPA) axis activity, producing sustained alterations in cortisol secretion throughout life. So far, the assessment of HPA axis activity in the offspring of mothers treated with synthetic GC has been limited to a time period shortly after birth, with prematurity being an important confounder in most prior studies. The aim of the present study was to investigate the long-term effects of antenatal GC therapy on the activity of the HPA axis in children born at term.

Methods: 209 full-term children aged 6–11 years were studied cross-sectionally and were divided into three groups: (i) children exposed to GC therapy prenatally; (ii) a control group of children not exposed to GCs, whose mothers had been hospitalized during pregnancy, and (iii) a control group of children not exposed to GCs, whose mothers had not been hospitalized during pregnancy. All subjects underwent a validated stress test (Trier Social Stress Test for Children) and cortisol concentrations in response to this test were determined in saliva.

Results: The increase in cortisol concentrations during the Trier test was greater in the exposed group ($n = 81$) than in the two non-exposed groups (respectively, $n = 43$ and $n = 85$).

Conclusion: Prenatal exposure to synthetic glucocorticoids is associated with increased activity of the HPA axis, and might predispose subjects to stress-related conditions in adult life.

Several studies have demonstrated interindividual variation in hypothalamus-pituitary-adrenal (HPA) axis regulation, which may lead to a wide variety of physical and psychological diseases. Adverse environmental conditions during prenatal development can result in marked changes of HPA axis function, which persist throughout life, a process commonly referred to as fetal programming for adult disease. Both maternal stress during pregnancy and fetal overexposure to glucocorticoids (GCs) are potent programming factors of the developing HPA axis across various species.

The study by Alexander and colleagues provides for the first time evidence for long-lasting effects of antenatal GC exposure on HPA axis reactivity, which may last a decade or more. These findings expand on previous studies focusing on HPA axis functioning shortly after birth by demonstrating that these effects persist until later in life. Although a limitation of this study is that it is cross-sectional, the results may have important implications with respect to stress-related physical and psychiatric disorders.

Dexamethasone induces germ cell apoptosis in the human fetal ovary

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Background: Pregnant women at risk for carrying a female fetus with classic congenital adrenal hyperplasia may be treated with synthetic glucocorticoids, such as dexamethasone (DEX), to prevent virilization of a female fetus. The present study assessed the potential deleterious effects of DEX exposure on fetal ovarian development.

Methods: Human fetal ovaries, available from legally induced abortions, were harvested 8–11 weeks after fertilization. The ovaries were cultured in the absence or presence of increasing concentrations of DEX for 14 days. Histological analyses were performed.

Results: The glucocorticoid receptor NR3C1 was present and the signaling pathway active in the fetal ovary, as demonstrated by the expression of NR3C1 target genes in response to DEX exposure. Exposure to DEX significantly increased the apoptotic rate, decreased germ cell density and the expression of the prosurvival gene KIT, but did not change oogonial proliferation.

Conclusion: In vitro exposure to high doses of DEX impairs human fetal oogenesis through increased apoptosis. Further studies are required to investigate the fertility of women exposed to DEX during fetal life.

Glucocorticoids (GCs) regulate a broad spectrum of physiologic functions essential for life and play an important role in basal and stress-related homeostasis. GCs are involved in the final maturation of fetal organ systems and modulate inflammatory and immune responses. Their actions are mediated by the nuclear glucocorticoid receptor (GR) [4]. Approximately 7% of pregnant women in Europe and North America are treated with dexamethasone (DEX) to promote lung maturation in fetuses at risk of preterm delivery and to prevent respiratory distress syndrome. In addition, maternal DEX administration is frequently used in classic congenital adrenal hyperplasia to prevent virilization of female fetuses.

This study investigated the effect of DEX on the ovary, as well as the long-term fertility of women exposed to DEX in utero. The findings indicate that DEX alone or in the presence of progesterone induced germ cell apoptosis in the human fetal ovary in vitro, however it is not known whether DEX has the same effect in vivo. Given that prenatal treatment with DEX was initiated in the early 1980s, and the first of these women are currently younger than 30 years of age, future studies are required to determine whether prenatal exposure of female fetuses to DEX compromises the oogonial stock and thus constitutes a risk factor for premature ovarian failure.

Effect of inhaled glucocorticoids in childhood on adult height

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N Engl J Med 2012;367:904–912

Background: Inhaled glucocorticoids are widely used in prepubertal children with asthma. It is known that they can cause a temporary reduction in growth velocity but are thought not to impair adult height.

Methods: Adult height was determined in 943 participants in the Childhood Asthma Management Program with a mean age of 25 years. The participants had been randomly assigned at the age of 5–13 years, to receive daily budesonide (400 µg), nedocromil (16 mg), or placebo for 4–6 years. Multiple linear regression was used to analyze the differences in adult height between the groups.

Results: Mean adult height was 1.2 cm lower (95% CI –1.9 to –0.5) in the budesonide group than in the placebo group ($p = 0.001$) and 0.2 cm lower (95% CI –0.9 to 0.5) in the nedocromil group than in the placebo group ($p = 0.61$). A larger daily dose of inhaled glucocorticoids in the first 2 years was associated with a lower adult height ($p = 0.007$). Decreased growth velocity in the budesonide group occurred primarily in prepubertal participants during the first 2 years of treatment.

Conclusion: The use of inhaled glucocorticoids in prepubertal children is associated with decreased adult height. The decrease in height induced in the first years of treatment is not progressive or cumulative.

The effect of inhaled glucocorticoids on height was thought to be growth retardation rather than growth suppression. Based on the results of retrospective studies with conflicting findings, it was presumed that children on inhaled glucocorticoids would grow for a longer period of time, eventually catching up for the decreased growth velocity seen in the first years of inhaled corticosteroid therapy in prepubertal children.

This study by Kelly and colleagues demonstrates that chronic inhaled glucocorticoid treatment administered to prepubertal children leads to impaired final height in a dose-dependent manner. The strengths of this study are the prospective randomized design, the long duration (from 1993) and the large sample size ($n > 1,000$ children with asthma). The negative effects of inhaled glucocorticoids on height were dependent on therapeutic index, which suggests that clinicians should be careful in the selection of the specific inhaled glucocorticoid medication and the delivery device. However, clinicians should also note that the potential reduction in adult height (mean –1.2 cm) is relatively small when balanced against the benefit of these drugs in controlling persistent asthma.

Important for clinical practice

Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency

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Proc Natl Acad Sci USA 2013;110:2611–2616

Background: Congenital adrenal hyperplasia (CAH) owing to 21-hydroxylase deficiency is characterized by impaired secretion of glucocorticoids and often mineralocorticoids, adrenal hyperandrogenism and impaired development and function of the adrenal medulla.

Methods: The CYP21A2 gene was studied in 1,507 families with at least 1 member affected with CAH due to 21-hydroxylase deficiency. The aim of this study was to report on the genotype-phenotype correlation, as well as the ethnic group and country of origin.

Results: Out of 45 genotypes, 21 yielded a phenotypic correlation in this patient cohort. Contrary to what is generally reported in the literature, certain mutations, such as the P30L, I2G, and I172N, yielded different CAH phenotypes. Although there was a strong genotype-phenotype correlation in salt

wasting and non-classic forms of the disease, in simple virilizing CAH a wide phenotypic variability was noted, particularly with the exon 4 I172N mutation. Finally, Middle Eastern and Ashkenazi Jewish populations showed a high frequency of the I2G and V281L mutations, respectively.

Conclusion: Knowledge of the genotype-phenotype correlation in CAH may help physicians in prenatal diagnosis and genetic counseling of parents who are at risk for having a child with CAH.

The structure of the human CYP21 gene was identified in the late 1980s and was followed by genetic screening in CAH families in the 1990s. Since then, many studies investigating the genotype-phenotype correlation in CAH have been published. Previous reports of genotype-phenotype correlations and recent *in silico* assignment of protein structure to a genotype suggest that the phenotype of a CAH fetus and the subsequent newborn can be predicted with reasonable certainty by performing prenatal genetic analysis. This study by New and colleagues explores this hypothesis. The Adrenal Steroid Disorders Program in New York has a large cohort of CAH patients. Over the last two decades, the authors have performed 8,290 DNA analyses of the CYP21A2 gene and report herein the results of the analysis, including data for 1,507 families who have at least 1 member affected with CAH. Their findings are important for several reasons. First, they analyzed a heterogeneous population as opposed to previous studies, which were drawn from specific countries. Second, their data provide a framework for physicians to ascertain the prevalent form of CAH for a given genotype. Third, a catalog of genotype-phenotype correlation is valuable for prenatal diagnosis of CAH, where the physician must rely on genetic data, as well as for the genetic counseling of parents who are at risk for having a child with CAH due to 21-hydroxylase deficiency.

Reduced cortisol metabolism during critical illness

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N Engl J Med 2013;368:1477–1488

Background: Critical illness is often associated with elevated cortisol concentrations, which are attributed to stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis. However, low ACTH concentrations have also been reported in critically ill patients, which may be due to reduced cortisol metabolism. The present study investigated whether critically ill patients had reduced cortisol metabolism, leading to hypercortisolism and subsequent reduction of ACTH by increased negative feedback on the HPA axis.

Methods: A total of 158 critically ill patients and 64 matched controls were tested in terms of several aspects of cortisol metabolism: (i) daily concentrations of ACTH and cortisol; (ii) plasma cortisol clearance, metabolism, and production during infusion of deuterium-labeled steroid hormones as tracers; (iii) plasma clearance of 100 mg of hydrocortisone; (iv) levels of urinary cortisol metabolites, and (v) levels of mRNA and protein in liver and adipose tissue, to assess major cortisol-metabolizing enzymes.

Results: Total and free circulating cortisol concentrations were consistently higher in critically ill patients than in controls, whereas ACTH concentrations were lower ($p < 0.001$ for both comparisons). Cortisol production was 83% higher in the patients ($p = 0.02$). There was a reduction of more than 50% in cortisol clearance during tracer infusion and after the administration of 100 mg of hydrocortisone in the patients ($p \leq 0.03$ for both comparisons). All these factors accounted for an increase by a factor of 3.5 in plasma cortisol levels in the patients, as compared with controls ($p < 0.001$). Impaired cortisol clearance also correlated with a lower cortisol response to ACTH stimulation. Reduced cortisol metabolism was associated with reduced inactivation of cortisol in the liver and kidney, as suggested by urinary steroid ratios, tracer kinetics, and assessment of liver biopsy samples ($p \leq 0.004$ for all comparisons).

Conclusion: During critical illness, reduced cortisol breakdown, related to suppressed expression and activity of cortisol-metabolizing enzymes, contributed to elevated cortisol concentrations and ACTH suppression.

Stress-induced activation of the HPA axis is well documented in critical illness. It was previously thought that this was the consequence of increased ACTH, however a paradoxical dissociation between cortisol and ACTH concentrations was often noted. This study demonstrates that a pituitary-

independent adrenal activation in association with reduced cortisol metabolism represent the main cause of the ACTH-independent hypercortisolemia in critically ill patients. Thus, the low cortisol response to ACTH stimulation does not necessarily reflect adrenal failure. The reduced cortisol metabolism is likely to be due to decreased activity of cortisol-metabolizing enzymes at the tissue level. These findings have several clinical implications. The contribution of reduced cortisol breakdown to hypercortisolemia during critical illness alters our understanding of the stress response. More specifically, these data suggest that 'stress doses' of hydrocortisone (200 mg/day), which are advocated to replace cortisol production in critically ill patients who are presumed to have adrenal failure, are at least three times too high. Finally, these findings suggest that a low cortisol response to ACTH stimulation does not necessarily reflect adrenal failure, since cortisol production in critically ill patients is not subnormal and the suppressed clearance maintains hypercortisolemia. Therefore, in critically ill patients in the ICU, reduced cortisol breakdown appeared to contribute to abnormal blood cortisol concentrations. These results have potential implications for the diagnosis of adrenal failure and its treatment in the ICU setting.

Clinical trials – new treatments

A potential therapeutic role of selective glucocorticoid receptor and estrogen receptor modulators

Differential targeting of brain stress circuits with a selective glucocorticoid receptor modulator

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Proc Natl Acad Sci USA 2013;110:7910–7915

Background: Glucocorticoid receptor antagonists may have several therapeutic indications for stress-related diseases, such as depression. However, blockade of all GR-dependent processes in the brain will lead to unnecessary and even counteractive effects, such as elevated endogenous cortisol concentrations. Selective glucocorticoid modulators are ligands that can act both as agonists and as antagonists and may be used to separate beneficial from harmful treatment effects. The aim of this research work was to study the high-affinity GR ligand C108297 as a selective modulator in the rat brain.

Methods and Results: C108297 induced a unique interaction profile between GR and its downstream effector molecules, the nuclear receptor co-regulators, compared with the full agonist dexamethasone and the antagonist RU486 (mifepristone). C108297 displayed partial agonistic activity for the suppression of hypothalamic corticotropin-releasing hormone (CRH) gene expression and potently enhanced GR-dependent memory consolidation of training on an inhibitory avoidance task. In contrast, it lacked agonistic effects on the expression of CRH in the central amygdala and antagonized GR-mediated reduction in hippocampal neurogenesis after chronic corticosterone exposure. Furthermore, the compound did not lead to disinhibition of the HPA axis.

Conclusion: C108297 represents a class of ligands that has the potential to more selectively abrogate pathogenic GR-dependent processes in the brain, while retaining beneficial aspects of GR signaling.

Glucocorticoids are essential for adaptation to stressors, but prolonged or excessive exposure to these hormones has been consistently implicated in the development of stress-related psychopathologies, such as depression. Their actions are mediated by the glucocorticoid receptor (GR). The GR is widely distributed in the brain, where it affects many different processes, including learning and memory, adult neurogenesis, and neuroendocrine negative feedback regulation. Although GR antagonism of particular processes may be of therapeutic benefit, blocking other GR-mediated effects may actually counteract the potential therapeutic efficacy.

In this study, Zalachoras and colleagues provide proof of principle for selective GR modulation in the brain with relevance for stress regulation, cognition and psychopathology. They demonstrated that a

selective high-affinity GR ligand, C108297, showed GR antagonism in relation to GR-dependent CRH mRNA regulation in the amygdala and corticosterone-induced reduction in hippocampal neurogenesis. The agonistic effects of C108297 included enhanced memory consolidation of emotionally arousing training and a suppression of hypothalamic CRH expression. The compound did not lead to net inhibition of glucocorticoid negative feedback, as evidenced by the unaltered circulating corticosterone concentrations. Future studies are required to investigate the effects of C108297 on peripheral tissues, as well as in clinical trials in human subjects.

Targeting estrogen receptor- α reduces adrenocortical cancer (ACC) cell growth in vitro and in vivo: potential therapeutic role of selective estrogen receptor modulators (SERMs) for ACC treatment

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J Clin Endocrinol Metab 2012;97:E2238–2250

Background: Adrenocortical carcinoma (ACC) is a rare tumor characterized by an increased production of IGF-II and upregulation of estrogen receptor (ER)- α . This study examined the role of ER α in 17 β -estradiol (E₂)- and IGF-II-dependent ACC growth, and whether selective ER modulators are effective in controlling ACC growth in vivo.

Methods: The human adrenocortical cell line H295R was used as an in vitro model to generate xenograft tumors in athymic nude mice.

Results: In H295R cells, IGF-II controlled expression of steroidogenic factor-1, which in turn, increased aromatase transcription and, consequently, estrogen production, thereby inducing cell proliferation. ER α silencing significantly blocked E₂- and IGF-II-dependent cell proliferation. Furthermore, ER α mediated E₂-induced non-genomic signaling that stimulated IGF-I receptor (IGF1R), ERK1/2, and AKT phosphorylation, resulting in a ligand-independent activation of the IGF1R-induced pathway. In addition, E₂ potentiated this pathway by upregulating IGF1R expression as a consequence of increased cAMP-responsive element-binding protein activation and binding to IGF1R promoter. The estrogen antagonist, hydroxyltamoxifen, the active metabolite of tamoxifen, reduced IGF1R protein levels and both E₂- and IGF-II-induced cell proliferation. Moreover, H295R xenograft growth was strongly reduced by tamoxifen.

Conclusions: These findings establish a critical role for ER α in E₂- and IGF-II-dependent ACC proliferation and provide a rationale for targeting ER α to control the proliferation of ACC.

Adrenocortical carcinoma (ACC) is a rare tumor with poor prognosis. At present, in localized ACC, only surgery provides a chance for long-term cure. However, the recurrence rates following radical resection may be as high as 60–80%, indicating the need for adjuvant treatment. To date, treatment with mitotane remains the common therapy, mostly because of its ability to impair adrenal steroidogenesis. Research studies indicate that genetic mutations in the adrenal gland lead to the initiation of a malignant tumor. The most consistent and dominant genetic change in ACC is the perturbation of the imprinted IGF2 locus (11p15). IGF2 is overexpressed in 90% of ACCs together with the IGF-I receptor gene (IGF1R). In vitro studies using the adrenal cancer cell line H295R confirmed a direct involvement of the IGF-II/IGF1R system in adrenocortical tumor cell proliferation, and showed that upon ligand binding, the intrinsic tyrosine kinase of the IGF1R caused activation of phosphatidylinositol 3-kinase (PI3K)/AKT and the Raf-1/MAPK kinase/ERK pathways, thereby stimulating cellular proliferation. In addition, receptors for growth factors, like IGF-II, are able to also activate protein kinase C (PKC).

In the present study, Sirianni and colleagues demonstrate the important role that ER α plays in mediating the mitogenic activity of E₂ and IGF-II in ACC. Both E₂ and IGF-II induced many comparable responses in H295R cells, including activation of IGF1R/AKT signaling. An anti-IGF1R antibody inhibited basal and IGF-II-induced cell proliferation but not E₂-dependent cell growth. Furthermore, targeting ER α was effective in controlling E₂- and IGF-II-dependent cell proliferation, indicating a central role for ER α in the mechanisms controlling ACC cell proliferation. These findings support the possibility of using anti-estrogens for the purpose of controlling ACC cell proliferation.

The dorsal aorta initiates a molecular cascade that instructs sympathoadrenal specification

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Background: The autonomic nervous system, which includes the sympathetic neurons and adrenal medulla, originates from the neural crest. The aim of this study was to investigate how neural crest cells (NCCs) generate sympathetic and medullary lineages during embryogenesis.

Methods and Results: Combining avian blood vessel-specific gene manipulation and mouse genetics, the authors demonstrated that the dorsal aorta acts as a morphogenetic signaling center that coordinates NCC migration and cell lineage segregation. Bone morphogenetic proteins (BMPs) produced by the dorsal aorta are critical for the production of the chemokine stromal cell-derived factor-1 (SDF-1) and neuregulin-1 in the para-aortic region, which act as chemoattractants for early migration. Later, BMP signaling is directly involved in the sympathomedullary segregation.

Conclusions: These data provide insights into the complex developmental signaling cascade that instructs one of the earliest events of neurovascular interactions guiding embryonic development.

The autonomic nervous system (ANS) plays a pivotal role in a wide range of physiological functions, including homeostasis and stress defense, and dysfunction of the ANS leads to a variety of diseases. However, it is not clear how the ANS is established during development at the cellular and molecular levels. The ANS consists of sympathetic and parasympathetic neurons, as well as stress defense tissues, including the adrenal gland. In the trunk of the early developing embryo, progenitors of sympathetic ganglion (Sg) emerge as a derivative of neural crest cells (NCCs), an embryonic progenitor cell population emigrating from the dorsal aspect of the neural tube. In a defined region along the anteroposterior body axis, Sg-progenitor cells also give rise to adrenal medulla (Am) cells, which later become associated with the adrenal cortex, a tissue of different origin. Thus, Sg cells and Am cells are produced from common progenitors (SA progenitors). The SA progenitors first migrate ventrally toward the dorsal aorta, the first-formed embryonic blood vessel, and subsequently these cells segregate into the Sg and Am lineages in the para-aortic region. Therefore, the dorsal aorta has long been anticipated to play a role in the regulation of sympathoadrenal lineages.

In the present study, Saito and colleagues unveiled the molecular and cellular mechanisms through which the sympathetic nervous system and stress defense organ are established. This occurs in the vicinity of the dorsal aorta, where multiple factors are repeatedly used in a manner regulated spatiotemporally by the dorsal aorta. The authors demonstrated that the dorsal aorta acts as a morphogenetic center for SA migration and Sg-Am segregation, and that bone morphogenetic proteins (BMPs) play a critical role for these activities. Therefore, the dorsal aorta serves as a critical relay point to coordinate the diversification of sympathetic nervous system.

Disrupted cortical function underlies behavior dysfunction due to social isolation

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Background: Stressful events during early childhood may have a profound influence on emotional and cognitive behavior throughout life. This study explored the mechanisms through which stress affects neonatal brain circuit formation.

Methods and Results: Neonatal isolation prevented long-term potentiation and experience-dependent synaptic trafficking of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors normally occurring during circuit formation in the rodent barrel cortex. This inhibition of AMPA receptor trafficking was mediated by an increase of the stress glucocorticoid hormone and was associated with

reduced calcium/calmodulin-dependent protein kinase type II (CaMKII) signaling, resulting in attenuated whisker sensitivity at the cortex.

Conclusions: Neonatal social isolation alters neuronal plasticity and disrupts the molecular, cellular, and circuit developmental processes, leading to behavioral dysfunction.

Mental stress may have different effects on the nervous system. Although brief, mild stress can evoke emotional arousal and enhance learning, chronic elevated stress can induce deficits in neuronal function [5, 6]. In particular, social isolation early in life caused by neglect can induce mental stress and lead to various mental illnesses, such as depression, drug addiction and anxiety disorders. Although numerous studies have revealed profound effects of stress on neural function, few have comprehensively related molecular, synaptic, and circuit deficits to behavioral aberrations.

In this study, Miyazaki and colleagues found that the stress produced by neonatal social isolation disrupts the synaptic delivery of AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors, circuit formation in the cortex, and behaviors related to somatosensory function. The authors focused on the plasticity in the barrel cortex, since it is likely to play an important role in the integration of social information. They found that brief periods of neonatal social isolation alter the formation of proper neuronal circuitry by a glucocorticoid signaling-mediated blockade of experience-dependent synaptic AMPA receptor insertion, and leads to long-lasting behavioral perturbations. Therefore, neonatal social isolation alters neuronal plasticity and disrupts the molecular, cellular, and circuit developmental processes, leading to behavioral dysfunction.

New genes

Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency

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Background: Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterized by an inability of the adrenal cortex to produce cortisol in response to ACTH. The NNT gene is a highly conserved, antioxidant defense gene that encodes nicotinamide nucleotide transhydrogenase.

Methods and Results: Using targeted exome sequencing, the authors identified mutations in the NNT gene in subjects with FGD. In mice with *Nnt* loss, higher levels of adrenocortical cell apoptosis and impaired glucocorticoid production were observed. NNT knockdown in a human adrenocortical cell line resulted in impaired redox potential and increased reactive oxygen species (ROS) levels.

Conclusions: These findings indicate that NNT may have a role in ROS detoxification in human adrenal glands.

Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterized by an inability of the adrenal cortex to produce cortisol in response to ACTH. Affected subjects usually present within the first few months of life with symptoms related to cortisol deficiency. Fifty percent of FGD cases are caused by mutations in *MC2R*, *MRAP* or *STAR*, all encoding components of the ACTH signaling-steroidogenic pathway [7]. To determine other genetic causes in patients with FGD, the authors used targeted exome sequencing in probands from consanguineous families with FGD of unknown etiology. They identified one variant (c.1598C>T; p.Ala533Val) in *NNT* (encoding nicotinamide nucleotide transhydrogenase) that was homozygous in affected individuals, heterozygous in the parents and absent in controls. *NNT* is a highly conserved gene encoding an integral protein of the inner mitochondrial membrane. Under most physiological conditions, this enzyme uses energy from the mitochondrial proton gradient to produce high concentrations of nicotinamide adenine dinucleotide phosphate (NADPH). Detoxification in mitochondria of reactive oxygen species (ROS) by

glutathione peroxidases depends on this NADPH for regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) to maintain a high GSH/GSSG ratio. These findings suggest that FGD is due to defective oxidative stress responses. In addition, they suggest that, at least in humans, NNT is of primary importance for ROS detoxification in adrenocortical cells, highlighting the susceptibility of the adrenal cortex to this type of pathological damage. Over time, affected individuals may develop other organ pathologies related to impaired antioxidant defense and will therefore need careful monitoring.

Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia

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Background: Hypoxia-inducible factors are transcription factors controlling energy, iron metabolism, erythropoiesis and development. When these proteins are dysregulated, they contribute to tumorigenesis and cancer progression. However, mutations in genes encoding α subunits of hypoxia-inducible factors (HIF- α) have not previously been identified in any cancer.

Methods and Results: Herein the authors report two novel somatic gain-of-function mutations in the gene encoding hypoxia-inducible factor 2 α (HIF2A) in 2 patients, 1 presenting with paraganglioma and the other with paraganglioma and somatostatinoma, both of whom had polycythemia. The two mutations were associated with increased HIF-2 α activity and increased protein half-life.

Conclusions: These findings indicate that patients presenting with paragangliomas or somatostatinomas might benefit from screening for HIF2A mutations.

Hypoxia-inducible factors are transcription factors that respond to changes in tissue oxygen concentration. These highly conserved proteins are composed of α and β subunits. The HIF- β subunit is constitutively expressed, whereas the α subunits are inducible by hypoxia and are associated with aggressive, treatment-refractory tumors. Under normoxic conditions, HIF-1 α , HIF-2 α , and HIF-3 α are hydroxylated on specific prolyl residues, allowing for recognition by the von Hippel-Lindau (VHL) tumor-suppressor protein, ubiquitination and rapid degradation through the proteasome. However, under hypoxic conditions, prolyl hydroxylation of HIF- α proteins is reduced, resulting in their stabilization and, in turn, transcription of genes involved in the hypoxia response, including angiogenesis, glycolysis, apoptosis, proliferation and growth.

In this study, Zhuang and colleagues report two novel somatic gain-of-function HIF2A mutations in paragangliomas and somatostatinomas that were also associated with polycythemia. These mutations affect prolyl hydroxylation and VHL protein binding, resulting in reduced HIF-2 α degradation but intact transcriptional activity and activation of genes downstream of HIF-2. They found increased erythropoietin mRNA and protein levels in both tumors, which may result in a high serum erythropoietin level and polycythemia. These findings indicate that patients presenting with paragangliomas or somatostatinomas might benefit from screening for HIF2A mutations.

Reviews

Role of KCNJ5 in familial and sporadic primary aldosteronism

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Primary aldosteronism (PA) is characterized by increased aldosterone production and comprises both sporadic forms, caused by an aldosterone-producing adenoma or bilateral adrenal hyperplasia, and familial forms. Angiotensin II and serum K⁺ are the two principal physiological regulators of aldoste

rone synthesis that reverse the high resting K⁺ conductance and hyperpolarized membrane potential of adrenal glomerulosa cells. The resulting membrane depolarization results in opening of the voltage-gated Ca²⁺ channels and an increase in intracellular Ca²⁺ that stimulates aldosterone biosynthesis. Point mutations in the KCNJ5 gene, which encodes the G-protein-activated inward rectifier K⁺ channel 4 (GIRK4), have been implicated in the pathogenesis of both sporadic and familial forms of PA. These mutations interfere with the selectivity filter of GIRK4 causing Na⁺ entry, cell depolarization and Ca²⁺ channel opening, resulting in constitutive aldosterone production. KCNJ5 mutations have been reported in approximately 40% of sporadic aldosterone-producing adenomas. This review summarizes the role of GIRK4 in adrenal pathophysiology and provides an overview of the clinical and biochemical phenotypes resulting from KCNJ5 mutations in patients with sporadic and familial 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. 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 [8]. The clinical spectrum of the condition is 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. Therefore, there is striking variation in the phenotype and clinical outcome resulting from different mutations in *KCNJ5* in subjects with PA with and without adrenal hyperplasia. This review discusses the clinical and biochemical phenotypes resulting from *KCNJ5* mutations in patients with sporadic and familial primary aldosteronism.

Hair cortisol, stress exposure, and mental health in humans: A systematic review

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Chronic stress has deleterious effects on health and contributes to the development of mental illness. An important development in the last few years has been the employment of hair cortisol analysis, which provides the unique possibility to assess the long-term systematic levels of cortisol retrospectively. This review synthesizes the body of published research on hair cortisol, chronic stress and mental health. The studies on hair cortisol and chronic stress show increased hair cortisol levels in a wide range of situations. With respect to mental illnesses, the results differed between diagnoses. In major depression, the hair cortisol concentrations were increased, whereas for bipolar disorder, cortisol concentrations were only increased in patients with a late age-of-onset. In patients with anxiety, hair cortisol levels were decreased. For exposure to chronic stressors, the effect sizes on hair cortisol levels were medium to large, whereas for psychopathology, the effect sizes were small to medium. This is a first implication that the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in the development and/or maintenance of psychopathology may be more subtle than it is in healthy but chronically stressed populations.

The activity of the HPA axis in psychoneuroendocrine research is usually assessed by determining cortisol concentrations. Until a few years ago, cortisol has solely been analyzed from blood serum, saliva, or urine. These analyses offer the possibility to explore the dynamics and the concentration of acutely (serum, saliva) or short-term (urine) circulating cortisol. Unfortunately, the cortisol concentrations obtained using these methods show considerable intra- and interindividual differences, which are due to cortisol's circadian rhythm, as well as its pulsatile secretion, daily variation and reactivity to acute transient stress. In addition, the use of oral contraceptives has been reported to increase the adrenal cortisol production. These techniques further call for invasive or frequent sampling and they are especially prone to measurement error, given that the samples are often collected by the participants. Furthermore, these techniques do not cover the long-term effect of stress exposure very well.

In the last few years, a new and very different method to measure cortisol exposure in humans has been developed, which involves the extraction of cortisol from human hair [9]. This is a promising technique with numerous advantages, such as the non-invasiveness, the standardized sampling and the possibility to use hair as a retrospective biomarker of cortisol exposure. As hair grows approximately 1 cm per month, hair analysis offers the possibility to show the average long-term activity of the HPA axis, and to compare several hair segments/months with each other, including segments before the presence of a stressful event. As hair cortisol most probably reflects the amount of free, unbound cortisol, it is also unaffected by oral contraceptives. For the analysis, a strand of hair is usually cut from the scalp and minced, and the cortisol in this hair is then extracted by methanol and further analyzed by either immunoassays or liquid chromatography-mass spectrometry. This review systematically synthesizes and discusses the published research studies on hair cortisol, chronic stress and mental health.

Of marsupials and men: 'backdoor' dihydrotestosterone synthesis in male sexual differentiation

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Following the development of the fetal bipotential gonad into a testis, male genital differentiation requires testicular androgens. Testosterone produced by the fetal Leydig cells is converted to dihydrotestosterone in the genital skin and leads to labioscrotal fusion. An alternative 'backdoor' pathway of dihydrotestosterone (DHT) synthesis that bypasses testosterone has been described in marsupials, but its relevance to human biology has been uncertain. The classic and backdoor pathways share many enzymes, however 3 α -reductase, AKR1C2, is unique to the backdoor pathway. Human AKR1C2 mutations result in disordered sexual differentiation, indicating that both pathways are required for normal human male genital development. Therefore, fetal DHT acts both as a hormone and as a paracrine factor, substantially revising the classic paradigm for fetal male sexual development.

Following embryonic sex determination, in which the presence of a Y chromosome drives the differentiation of the primitive, bipotential gonad into a testis, androgens produced by the fetal testis mediate both the stabilization of structures derived from the wolffian ducts and the closure of the urogenital sinus, resulting in the male phenotype. Two androgens, testosterone and dihydrotestosterone (DHT), are needed to produce the human male phenotype. Testosterone produced in the fetal testis reaches the genital skin of the fetal labioscrotal folds, where it is converted to DHT by 5 α -reductase. DHT induces labioscrotal fusion, the development of the phallic urethra and some phallic enlargement. In the 'classic' pathway of androgen synthesis, DHEA and androstenedione or androstenediol are key intermediates in the synthesis of testosterone, and fetal DHT is produced in situ in its target organ, the genital skin, where it acts as a paracrine factor. An alternative, or 'backdoor', biosynthetic pathway leading to the production of DHT without going through testosterone has been described in marsupials and rodents [10], but the potential relevance of this pathway to human genital masculinization has been unclear. Recent studies have indicated that this pathway plays an important role in human male sexual development. Patients with genetic lesions in AKR1C2, a 3 α -hydroxysteroid dehydrogenase (3 α HSD) that participates in the backdoor pathway but not in the classic pathway of testosterone biosynthesis, had a disorder of sexual development (DSD). This unique, newly described form of DSD supports the idea that the backdoor pathway is essential for normal male sexual development, thereby revising former views of the mechanisms of normal and pathological masculinization.

Familial glucocorticoid deficiency: new genes and mechanisms

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Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterized by an inability of the adrenal cortex to produce cortisol in response to ACTH. Affected subjects usually present within the first few months of life with symptoms related to cortisol deficiency. Fifty percent of FGD cases are caused by mutations in the ACTH receptor (MC2R), its accessory protein (MRAP) or in the steroidogenic acute regulatory protein (STAR), all encoding components of the ACTH signaling-steroidogenic pathway. Recent studies have demonstrated that the mini-chromosome maintenance-deficient-4 homologue (MCM4) and nicotinamide nucleotide transhydrogenase (NNT) genes, which are involved in DNA replication and antioxidant defense, respectively, also play a role in the development of FGD. These findings expand the spectrum of pathogenetic mechanisms causing adrenal disease and indicate that the adrenal may be hypersensitive to replicative and oxidative stress.

Familial glucocorticoid deficiency (FGD) is a rare autosomal disorder characterized by unresponsiveness of the cells of the zona fasciculata within the adrenal cortex to stimulation by ACTH. It is characterized by isolated glucocorticoid deficiency and elevated plasma ACTH concentrations. Glucocorticoid deficiency may lead to hypoglycemic seizures and/or failure to thrive within the neonatal period or very early childhood, while older children may present with recurrent infections. The condition is potentially fatal if left untreated and in the long-term recurrent hypoglycemia can lead to learning difficulties in addition to other neurological sequelae. Excessive plasma ACTH often results in hyperpigmentation due to overstimulation of melanocortin-1 receptors (MC1R). Defects in the ACTH receptor pathway, MC2R and its accessory protein MRAP, as well as STAR are implicated in the pathogenesis of FGD. More recently the discovery of defects in DNA replication and antioxidant genes, MCM4 and NNT, expands the spectrum of pathways that can be pathogenic in FGD. Over time, patients with MCM4 or NNT mutations may develop other organ pathologies related to their impaired gene functions. This review summarizes the genetic defects and underlying mechanisms in patients with FGD.

References

1. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, Lolis E, Wisgerhof MV, Geller DS, Mane S, Hellman P, Westin G, Åkerström G, Wang W, Carling T, Lifton RP: K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* 2011;331:768–772.
2. Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, Mulatero P, Samson-Couterie B, Hahner S, Quinkler M, Fallo F, Letizia C, Allolio B, Ceolotto G, Cicala MV, Lang K, Lefebvre H, Lenzini L, Maniero C, Monticone S, Perrocheau M, Pilon C, Plouin PF, Rayes N, Seccia TM, Veglio F, Williams TA, Zinnamosca L, Mantero F, Benecke A, Jeunemaitre X, Reincke M, Zennaro MC: Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension* 2012;59:592–598.
3. Manenschijn L, Koper JW, Lamberts SW, van Rossum EF: Evaluation of a method to measure long-term cortisol levels. *Steroids* 2011;76:1032–1036.
4. Nicolaides NC, Galata Z, Kino T, Chrousos GP, Charmandari E: The human glucocorticoid receptor: molecular basis of biologic function. *Steroids* 2010;75:1–12.
5. Hu H, Real E, Takamiya K, Kang MG, Ledoux J, Haganir RL, Malinow R: Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* 2007;131:160–173.
6. Enthoven L, Oitzl MS, Koning N, van der Mark M, de Kloet ER: Hypothalamic-pituitary-adrenal axis activity of newborn mice rapidly desensitizes to repeated maternal absence but becomes highly responsive to novelty. *Endocrinology* 2008;149:6366–6377.
7. Meimaridou E, Hughes CR, Kowalczyk J, Guasti L, Chapple JP, King PJ, Chan LF, Clark AJ, Metherell LA: Familial glucocorticoid deficiency: new genes and mechanisms. *Mol Cell Endocrinol* 2013;371:195–200.
8. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, Lolis E, Wisgerhof MV, Geller DS, Mane S, Hellman P, Westin G, Åkerström G, Wang W, Carling T, Lifton RP: K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* 2011;331:768–772.
9. Raul JS, Cirimele V, Ludes B, Kintz P: Detection of physiological concentrations of cortisol and cortisone in human hair. *Clin Biochem* 2004;37:1105–1111.
10. Wilson JD, Shaw G, Leihy ML, Renfree MB: The marsupial model for male phenotypic development. *Trends Endocrinol Metab* 2002;13:78–83.