Congenital adrenal hyperplasia – current insights in pathophysiology, diagnostics and management

Claahsen-van der Grinten, Hedi; Speiser, Phyllis; Faisal Ahmed, S; Arlt, Wiebke; Auchus, Richard J; Falhammar, Henrik; Fluck, Christa; Guasti, Leonardo; Huebner, Angela; Kortmann, Barbara B; Krone, Nils; Merke, Deborah P; Miller, Walter L; Nordenstrom, Anna; Reisch, Nicole; Sandberg, David; Stikkelbroeck, Nike; Touraine, Philippe; Utari, Agustini; Wudy, Stefan A

DOI:
10.1210/endrev/bnab016

Document Version
Peer reviewed version

Citation for published version (Harvard):
Claahsen-van der Grinten, H, Speiser, P, Faisal Ahmed, S, Arlt, W, Auchus, RJ, Falhammar, H, Fluck, C, Guasti, L, Huebner, A, Kortmann, BB, Krone, N, Merke, DP, Miller, WL, Nordenstrom, A, Reisch, N, Sandberg, D, Stikkelbroeck, N, Touraine, P, Utari, A, Wudy, SA & White, P 2021, 'Congenital adrenal hyperplasia – current insights in pathophysiology, diagnostics and management', *Endocrine Reviews*.
https://doi.org/10.1210/endrev/bnab016

Congenital adrenal hyperplasia – current insights in pathophysiology, diagnostics and management

Hedi L. Claahsen – van der Grinten MD, PhD[1], Phyllis W. Speiser, MD[2], S. Faisal Ahmed FRCPCH[3], Wiebke Arlt MD, DSc[4], Richard J. Auchus MD, PhD[5], Henrik Falhammar, MD, PhD[6,7], Christa E. Flück MD[8], Leonardo Guasti PhD[9], Angela Huebner PhD[10], Barbara B.M. Kortmann, MD, PhD[11], Nils Krone MD[12,13], Deborah P. Merke MD[14], Walter L. Miller MD[15], Anna Nordenström MD PhD[16,17], Nicole Reisch MD[18], David E. Sandberg, PhD[19], Nike M.M.L. Stikkelbroeck MD, PhD [20], Philippe Touraine MD, PhD[21], Agustini Utari MD, PhD[22], Stefan A. Wudy MD[23], Perrin C. White MD[24]

[1]Radboud University Medical Centre, Amalia Childrens Hospital, Department of Pediatric Endocrinology, Nijmegen, The Netherlands

[2]Cohen Children's Medical Center of NY, Feinstein Institute, Northwell Health, Zucker School of Medicine, New Hyde Park, NY, USA

[3]Developmental Endocrinology Research Group, School of Medicine Dentistry & Nursing, University of Glasgow, Glasgow, Scotland, United Kingdom

[4]Institute of Metabolism and Systems Research (IMSR), College of Medical and Dental Sciences, University of Birmingham, United Kingdom, and Department of Endocrinology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

[5]Division of Metabolism, Endocrinology, and Diabetes, Departments of Internal Medicine and Pharmacology, University of Michigan, Ann Arbor, Michigan, USA

[6]Department of Molecular Medicine and Surgery, Karolinska Intitutet, Stockholm, Sweden

[7]Department of Endocrinology, Karolinska University Hospital, Stockholm, Sweden

[8]Pediatric Endocrinology, Diabetology and Metabolism, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland

[9]Centre for Endocrinology, William Harvey Research Institute, Bart's and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

[10]Division of Paediatric Endocrinology and Diabetology, Department of Paediatrics, Universitätsklinikum Dresden, Technische Universität Dresden, Dresden, Germany.

[11]Radboud University Medical Centre, Amalia Childrens Hospital, Department of Pediatric Urology, Nijmegen, The Netherlands

[12]Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

[13]Department of Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

[14]National Institutes of Health Clinical Center and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, Maryland, USA 20892

[15]Department of Pediatrics, Center for Reproductive Sciences, and Institute for Human Genetics, University of California, San Francisco, 94143

[16]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

[17]Pediatric Endocrinology, Karolinska University Hospital, Stockholm, Sweden

[18]Medizinische Klinik IV, Klinikum der Universität München, Munich, Germany

[19]Susan B. Meister Child Health Evaluation and Research Center, Department of Pediatrics, University of Michigan, Ann Arbor, Michigan, USA

[20]Radboud University Medical Centre, Department of Endocrinology, Nijmegen, Netherlands

[21]Department of Endocrinology and Reproductive Medicine, Center for Rare Endocrine Diseases of Growth and Development, Center for Rare Gynecological Diseases, Hôpital Pitié Salpêtrière, Sorbonne University Medicine, Paris, France

[22]Division of Pediatric Endocrinology, Department of Pediatrics, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

[23]Steroid Research & Mass Spectrometry Unit, Laboratory of Translational Hormone Analytics, Division of Paediatric Endocrinology & Diabetology, Justus Liebig University, Giessen, Germany

[24] Division of Pediatric Endocrinology, UT Southwestern Medical Center, Dallas TX, USA

Short title: Congenital adrenal hyperplasia

Key words: Steroid biosynthesis, 21-hydroxylase deficiency, *CYP21A2*, glucocorticoid, mineralocorticoid, cortisol, aldosterone

Address correspondence to: Perrin C White, MD, Division of Pediatric Endocrinology, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas TX 75390-9063, Perrin.white@utsouthwestern.edu, Telephone 214-648-3501, Fax 214-648-9772

Disclosures: SFA has received unrestricted educational grants from Diurnal and Neurocrine Biosciences. PWS has served as a consultant to Neurocrine Biosciences, Adrenas Therapeutics, OMASS Therapeutics, Certara and Gerson Lehman Group, and has received lecture fees from Sandoz/ Novartis. CEF has previously consulted for Adrenas Therapeutics, Inc. WA consults for

Diurnal Ltd. and Spruce Biosciences. RJA has previously consulted for Neurocrine Biosciences and Spruce Biosciences; consults for Adrenal Therapeutics, OMass Therapeutics, and Crinetics Pharmaceuticals; and receives contracted research support (2013-current) from Neurocrine Biosciences and Spruce Biosciences. HC has consulted for Neurocrine Bioscience and Spruce Bioscience. AN received lecture fees from Merck and has previously consulted for Diurnal. NPK has consulted for Neurocrine Biosciences, Inc., Diurnal Ltd., Novo Nordisk and Adrenas Therapeutics. NPK also reports speaker fees from Sandoz, Merck, and Novo Nordisk. NPK has received grant support from the Medical Research council, UK, International Fundraising CAH (IFCAH), and the Deutsche Forschungsgemeinschaft (DFG; KR3363/3–1). AH received grant support (IIT study) from Pfizer Pharma GmbH. WLM has previously consulted for Spruce Biosciences, Adrenas Therapeutics, EicOsis LLC, and BioMarin Pharmaceutical Inc. DM received unrelated research funds from Diurnal Limited through the National Institutes of Health Cooperative Research and Development Agreement. NR has consulted for Diurnal. HF has consulted for Neurocrine Biosciences, Inc., Diurnal Limited, Roche Diagnostics International Ltd and Adrenas Therapeutics. PCW previously obtained investigator-initiated research support from Janssen Pharmaceuticals; he consults for Neurocrine Biosciences and Crinetics Pharmaceuticals, and receives contracted research support from Neurocrine Biosciences.

Abbreviations

AAV	Adeno-Associated Virus
ACTH	Adrenocorticotropic hormone, corticotropin
BMD	Bone mineral density
BMI	Body Mass Index
CAH	Congenital Adrenal Hyperplasia
cIMT	Carotid intima media thickeness
COUP-TFII	Chicken Ovalbumin Upstream Promotor-Transcription Factor-2
CRF	Corticotropin-releasing factor
CRH	Corticotropin-releasing hormone
CYP21A2	21-hydroxylase
CYP19A1	Aromatase
Dex	Dexamethasone
DHEA	Dihydroeoiandrostenedione
DHEAS	Dehydroepiandrosterone sulfate
DELFIA	Dissociation-enhanced lanthanide fluoroimmunoassay
DHT	5-dihydrotestosterone
DOC	11 deoxycorticosterone
DSD	Differences in Sex development
ESC	Embryonic stem cell
FSH	Follicle stimulating hormone
GC	Gas Chromatography
HC	Hydrocortisone
HOMA-β	Homeostatic model assessment
HSD3B	3β-hydroxysteroid dehydrogenase
HSD17B	17β-hydroxyseteroid dehydrogenase
IPSC	inducible Pluripotent Stem Cell
LC	liquid chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LH	Luteinizing hormone
MC2R	Adrenocorticotropic hormone receptor
MLPA	Mulitplex ligation-dependent probe amplification
MR	Mineralocorticoid receptor
MS	Mass spectometry
NC	Non classic
OMM	Outer mitochondrial membrane
PGD	Preimplantation genetic diagnosis
POR	P450 oxidoreductase
RIA	Radioimmunoassay
TARTs	Testicular Adrenal Rest Tumors
SF-1	Steroidogenic factor-1
SRD5A1	5α-reductase type 1
StAR	Steroidogenic acute regulatory protein

SV	Simple virilizing
SW	Salt wasting
TNXB	Tenascin-X
11KT	11- ketotestosterone
11OHD	11-hydroxylase deficiency
17OH-Preg	17-hydroxypregnenolone
17OHD	17-hydroxylase deficiency
17OHP	17-hydroxyprogesterone
21OHD	21-hydroxylase deficiency

87

ABSTRACT

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders affecting cortisol biosynthesis. Reduced activity of an enzyme required for cortisol production leads to chronic overstimulation of the adrenal cortex and accumulation of precursors proximal to the blocked enzymatic step. The most common form of CAH is caused by steroid 21- hydroxylase deficiency due to mutations in CYP21A2. Since the last publication summarizing CAH in Endocrine Reviews in 2000 there have been numerous new developments. These include more detailed understanding of steroidogenic pathways, refinements in neonatal screening, improved diagnostic measurements utilizing chromatography and mass spectrometry coupled with steroid profiling, and improved genotyping methods. Clinical trials of alternative medications and modes of delivery have been recently completed or are under way. Genetic and cell-based treatments are being explored. A large body of data concerning long-term outcomes in patients affected by CAH, including psychosexual well-being, has been enhanced by the establishment of disease registries. This review provides the reader with current insights in congenital adrenal hyperplasia with special attention to these new developments.

I. INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an inherited inability to synthesize cortisol. Approximately 90-99% of cases of CAH are caused by 21-hydroxylase deficiency (21OHD) caused by mutations in the *CYP21A2* gene (1,2); the terms CAH and 21OHD will be used interchangeably in this article. The literature has historically described classic and nonclassic (NC) forms of this disorder, although current thinking views *CYP21A2* allelic variants and their phenotypic manifestations as a continuum. The classic form, occurring in 1 in 14,000 to 18,000 based on newborn screening (**Table 1**), is defined by severely reduced or absent enzyme activity with impaired cortisol production manifesting clinically in the neonatal period. In the most severe, salt-wasting (SW) form of classic CAH, there is little or no residual enzymatic activity, resulting in cortisol and aldosterone deficiency. Lack of negative feedback on the hypothalamic-pituitary-adrenal axis leads to excess adrenal androgen production as elevated precursor steroids are shifted to the non-affected androgen pathways. If not promptly treated, infants with this form of CAH quickly develop potentially fatal "salt-wasting crises" with hyponatremia, hyperkalemia, acidosis and shock. Those infants who produce slightly more aldosterone are less likely to suffer acute salt-wasting crisis, but such patients still have severe cortisol deficiency and markedly elevated adrenal androgen production. They are said to have "simple virilizing" (SV) CAH, associated with residual enzymatic activity of 1 – 5% of normal. All infants affected with classic CAH benefit from glucocorticoid plus adjunctive mineralocorticoid treatment at least within the first year of life, when there is relative renal tubular resistance to the salt-retaining effects of aldosterone in early infancy (28) and low sodium content of infant diets (29).

Whereas gonadal development is normal, severely increased prenatal adrenal androgen production leads to virilization of the female external genitalia (30), including variable degrees of

clitoral enlargement and labial fusion. The genital appearance of affected 46,XX infants is occasionally indistinguishable from that of male genitals with penis and scrotum but empty of gonads. Müllerian duct development is normal, except for the formation of a urogenital sinus with conjoined urethra and vagina. Thus, reproductive potential exists in females despite atypical external genitalia. Males have normal external genitalia. Wolffian duct development is normal in males but absent in females, who continue to produce COUP-TFII (Chicken Ovalbumin Upstream Promoter-Transcription Factor-2), which induces Wolffian duct involution (31).

Adverse sequelae in CAH patients occur both as a result of adrenal hormone imbalance, and from chronic glucocorticoid therapy (32). Androgen excess can cause inappropriately rapid somatic growth, accelerated skeletal maturation and reduced adult height. A systematic review and meta-analysis for >1000 classic CAH patients found shorter than average stature for mid-parental heights (-1.03 standard deviations, corresponding to ~7 cm) (33), but many of these children were diagnosed before the implementation of neonatal screening and did not receive the benefit of early initiation of treatment.

Elevated levels of adrenal androgens affect the hypothalamic-pituitary-gonadal axis. Central precocious puberty is a risk in patients experiencing prolonged periods of poor hormonal control. Young women with well-controlled CAH usually experience normal menarche (34), but poor control is associated with acne, female hirsutism, male pattern baldness, altered body habitus, irregular menses, and sub-normal fertility (35). Males with poor hormonal control may develop small testes and benign testicular adrenal rest tumors (TARTs) (see section VI.A.1) (36).

Individuals affected with milder allelic variants (i.e., NC CAH) tend to present to medical attention after infancy, hence the former term, "late onset" CAH. The associated alleles encode enzymes with residual activity of 20-50%. Thus, these individuals typically have normal basal

cortisol and aldosterone production but mildly elevated levels of adrenal androgens; however, suboptimal cortisol levels after ACTH stimulation are reported in up to 30% of patients (37). Children may present with symptoms due to elevated adrenal androgens such as premature adrenarche, acne and accelerated skeletal maturation but many, especially males, are asymptomatic. Adolescent girls or adult women may present with hirsutism, oligomenorrhea, acne, and sub-normal fertility (37). Because NC CAH is not the primary target of neonatal screening and is rarely detected by that strategy, the true prevalence of this milder disorder is unclear. The estimated prevalence is ~1 in 200 in the Caucasian population (38).

Since the last publication summarizing CAH in Endocrine Reviews in 2000 (1), there have been numerous new developments. These include more detailed understanding of steroidogenic pathways, refinements in neonatal screening, improved diagnostic measurements utilizing high-throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled with steroid profiling, and improved genotyping methods. Clinical trials of alternative medications and modes of delivery have been recently completed or are under way, with the nearer prospect of genetic and cell-based treatments and a large body of data concerning long-term outcomes in patients affected by CAH, including psychosexual well-being, enhanced by the establishment of disease registries.

Much remains to be learned in several other domains spanning fetal life through adulthood. Both human and animal studies have illuminated risks of antenatal dexamethasone (Dex) treatment. Non-invasive prenatal diagnosis of CAH in families with known *CYP21A2* pathogenic genotypes has been accomplished by analysis of circulating free fetal DNA in maternal blood in proof-of-concept studies, but is not yet widely available. Genital reconstructive surgery in affected females is no longer viewed as an emergency procedure, and indeed the practice of genital surgery in infancy has been questioned. Shared decision making among parents, patients, surgeons,

endocrinologists, mental health providers, and support groups has been promoted as model for optimal care. Benefit-to-risk ratio for no surgery, early or late genital surgery for females with CAH remains to be determined. Unfortunately, even in advanced societies, medical care for CAH is neglected, increasing the risk for cardiovascular or metabolic morbidities due to suboptimal corticosteroid therapy. Methods to improve transition of care from pediatric to adult healthcare, as well as patient and provider education, are important goals.

This multi-authored review is the result of a planned European CAH Symposium, which was postponed due to the Covid-19 pandemic. The large international group of authors contributed innovative approaches to understanding and managing this condition.

II. BASIC PRINCIPLES OF STEROID SYNTHESIS AND ADRENAL ENZYMATIC DEFECTS

A. Physiology and pathophysiology of steroidogenesis

Steroidogenesis in the adrenal cortex takes place in three concentric zones: the outermost zona glomerulosa (mineralocorticoid biosynthesis), the zona fasciculata (glucocorticoid biosynthesis), and the innermost zona reticularis (sex steroid precursor biosynthesis). It entails conversion of cholesterol to active steroid hormones, and involves many enzymes, co-factors and accessory proteins (**Figure 1**). Most of these are expressed in the appropriate zones of the adrenal cortex, with others expressed in the gonads, placenta and some 'peripheral' tissues; these factors and the conditions caused by their mutations have been studied in detail (39). Mutations have been described in most of the genes encoding these proteins; those that disrupt cortisol synthesis with compensatory elevations in ACTH cause CAH, but in common parlance 'CAH' refers to 21OHD. This section describes all enzymatic conversions required to synthesize cortisol.

1. Cholesterol side-chain cleavage

Steroidogenesis is initiated by the conversion of cholesterol to pregnenolone, catalyzed by the cholesterol side-chain cleavage enzyme, CYP11A1 (P450scc). To initiate steroidogenesis, cholesterol from cytoplasmic storage depots must reach CYP11A1 on the inner mitochondrial membrane; this cholesterol influx requires the steroidogenic acute regulatory protein (StAR), acting on the outer mitochondrial membrane (OMM) (40). The action of StAR requires its phosphorylation and interaction with some other proteins, but the exact mechanism of StAR's action remains under investigation (41,42). Mutations in StAR cause another rare form of CAH, congenital lipoid adrenal hyperplasia, in which virtually no steroid hormones are made and 46,XY fetuses are phenotypically female due to impaired testicular steroidogenesis (43,44). CYP11A1 defects were once considered incompatible with term pregnancy; however, more than 30 cases of such defects have been reported (40). These two conditions are clinically and hormonally indistinguishable, but lipoid CAH is typically associated with very large adrenals, whereas CYP11A1 deficiency is not; gene sequencing is needed for definitive diagnosis. Milder 'non-classical' forms of these conditions have been reported with intermediate phenotypes (45-48). CYP11A1 is one of 7 human mitochondrial cytochrome P450 (CYP) enzymes, all of which require electron donation via ferredoxin and ferredoxin reductase (49). Mutations in ferredoxin have not been reported, but several patients have been described with ferredoxin reductase mutations that disrupt synthesis of iron/sulfur centers, causing neuropathic deafness, optic atrophy, encephalopathy and developmental delay (50-52); impaired steroidogenesis is to be expected but not yet reported.

2. 3β-hydroxysteroid dehydrogenase

Once pregnenolone is produced, it may be converted to progesterone by 3β-hydroxysteroid dehydrogenase (HSD3B, 3β-HSD). There are two human *HSD3B* genes: *HSD3B1* encodes an isozyme found in the placenta, brain, liver and elsewhere; *HSD3B2* encodes one found in the adrenals and gonads. Both of these isozymes can convert the Δ^5-steroids (pregnenolone, 17-hydroxypregnenolone (17OHPreg), dehydroepiandrosterone (DHEA) and androstenediol) to the corresponding Δ^4-steroids (progesterone, 17OH-progesterone (17OHP), androstenedione, testosterone) (53), but the placental/hepatic HSD3B1 has a low Michaelis-Menten constant (K_m), permitting it to act on low concentrations of steroids in the circulation(54), whereas the K_m for the adrenal/gonadal HSD3B2 is 10-fold higher (55), so it acts only on locally produced, intraglandular steroids. Mutations in *HSD3B2* cause a rare form of CAH, characterized by elevated ratios of Δ^5/Δ^4 steroids, notably 17OH-Preg/17OH-progesterone (17OHP), that are >8 SD above normal (56,57). The low K_m of hepatic HSD3B1 permits it to convert some of the elevated 17OH-Preg to 17OHP, engendering false positives in newborn screening programs for 21OHD (58). HSD3B2 deficiency causes DSD in both sexes: genetic females are mildly virilized because some fetal adrenal DHEA is converted to testosterone by HSD3B1; genetic males synthesize some androgens by peripheral conversion of DHEA, but these are insufficient for complete male genital development (59).

3. 17α-hydroxylase/17,20-lyase

Pregnenolone can also be converted to 17OH-Preg by 17α-hydroxylase (CYP17A1, P450c17). CYP17A1 catalyzes both 17α-hydroxylase and 17,20-lyase activities. The 17α-hydroxylase activity converts pregnenolone to 17OHPreg and progesterone to 17OHP. The 17,20-lyase activity can convert 17OH-Preg to DHEA, but very little 17OHP is converted to androstenedione because the human enzyme catalyzes this reaction poorly (60,61). The activities of CYP17A1 are expressed in

a zone-specific fashion: the enzyme is absent in the adrenal zona glomerulosa, hence pregnenolone yields mineralocorticoids; only the 17α-hydroxylase activity is found in the zona fasciculata, thus pregnenolone yields cortisol; both activities are present in the zona reticularis, hence pregnenolone yields 19-carbon (C19) precursors of sex steroids (Fig. 1). The principal factor regulating 17,20-lyase activity is electron transport from NADPH via cytochrome P450 oxidoreductase (POR) with the assistance of cytochrome b_5 (b5). *CYP17A1* mutations causing 17-hydroxylase deficiency (17OHD) are rare except in Brazil and China. Lack of CYP17A1 prevents sex steroid biosynthesis, yielding a female phenotype in 46,XY males and sexual infantilism in both sexes; overproduction of 11-deoxycorticosterone (DOC) in the zona fasciculata typically causes mineralocorticoid hypertension; cortisol is not produced, but corticosterone substitutes for glucocorticoid requirements (62). Rare cases of apparently isolated 17,20-lyase deficiency may be attributable to mutations in CYP17A1, b5 (*CYB*5 gene) or POR (63-65).

The enzymology of adrenal 21-hydroxylase (CYP21A2, P450c21, encoded by *CYP21A2* within the *HLA* locus), is discussed in section II.B.

4. P450 oxidoreductase

All microsomal cytochrome P450 (CYP) enzymes, including CYP17A1, CYP21A2, CYP19A1 (aromatase, P450aro), as well as the drug-metabolizing CYP enzymes of the liver, require the activity of POR, a flavoprotein that transfers electrons from NADPH to all microsomal CYP enzymes (49). Mutations in POR cause POR deficiency; patients have been described with highly variable clinical and hormonal findings depending on the underlying mutations (66-72). Most POR mutations impair CYP17A1, especially 17,20-lyase activity (including the G539R POR variant with a phenotype simulating isolated 17,20 lyase deficiency)(63,68,73), with CYP21A2 and CYP19A1 being affected variably, depending on the POR mutation. It is difficult to reach definitive conclusions

about phenotype-genotype correlations with such rare disorders, although there is a suggestion that compound heterozygotes carrying R457H in *trans* with null mutations tend to have a more severe phenotype (72). Findings range from severely affected infants with 46,XX and 46,XY disorders/differences of sex development (DSD), cortisol deficiency and the Antley-Bixler skeletal malformation syndrome to mildly affected women who appear to have polycystic ovary syndrome, or mildly affected men with gonadal insufficiency. The skeletal phenotype probably results from diminished activity of CYP26B1, a POR-dependent enzyme that degrades retinoic acid (74). POR mutations also result in clinically relevant disruption of hepatic CYP enzyme activity (75). Patients with POR deficiency typically have normal electrolytes and mineralocorticoid function, nearly-normal cortisol levels that respond poorly to ACTH stimulation, increased levels of 17OHP that respond variably to ACTH, and low levels of sex steroids. Impaired CYP21A2 activity may generate levels of 17OHP detected by newborn screening for 21OHD (66,76). Atypical genital development occurs in both sexes, with considerable variability. The 17,20-lyase activity of CYP17A1 is especially sensitive to disrupted electron transport (77), thus POR defects typically affect fetal testicular steroidogenesis. Virilization of 46,XX females has two causes. First, POR deficiency diverts steroids into the 'backdoor pathway' of dihydrotestosterone biosynthesis (Fig. 1), contributing to the prenatal female virilization (69,78-80). Second, as placental CYP19 (aromatase) requires POR, pregnant women carrying a fetus with the POR mutation R457H (but not POR A287P) may experience virilization during pregnancy (66-68), similarly to women carrying an aromatase-deficient fetus (81,82). The POR polymorphism A503V, which mildly affects many P450 activities, is found commonly - from 19% among African Americans to 37% of Chinese Americans (83), but does not affect the presentation of 21OHD (84).

5. 11β-Hydroxylase and aldosterone synthase

Steroid 11-hydroxylase (CYP11B1, P450c11β) and aldosterone synthase (CYP11B2, P450c11AS, P450aldo) are closely related enzymes that catalyze the final steps in the synthesis of glucocorticoids and mineralocorticoids, respectively; they are encoded by duplicated genes (39,85). Like CYP11A1, these are mitochondrial enzymes that require ferredoxin and ferredoxin reductase to receive electrons from NADPH. CYP11B1 is expressed abundantly in the zona fasciculata, where it converts 11-deoxycortisol to cortisol and DOC to corticosterone, and also in the zona reticularis, where it initiates the 11-oxo-pathway (see later) (86). CYP11B2 expression is less abundant and confined to the zona glomerulosa where it catalyzes the 11β-hydroxylase, 18-hydroxylase and 18-methyloxidase activities needed to convert DOC to aldosterone (87,88). Mutations in *CYP11B1* cause 11β-hydroxylase deficiency (11OHD), with deficient cortisol, increased adrenal sex steroids, female virilization, and increased DOC, causing mineralocorticoid hypertension; 17OHP may be elevated in the newborn, leading to misdiagnosis of 21OHD (89). Mutations in *CYP11B2* selectively impair aldosterone synthesis, causing hyponatremia and hyperkalemia with normal cortisol production (39,90). However, hyponatremia is typically less severe than in 21OHD because of continued DOC and cortisol secretion.

6. 17β-hydroxysteroid dehydrogenases

The synthesis of sex steroids requires the action of one of the 17β-hydroxysteroid dehydrogenases (17β–HSD, HSD17B). These enzymes differ in their structures, co-factor requirements, reactions catalyzed and tissue-specific expression (39). Several are important in steroidogenesis. HSD17B1 is required for the synthesis of ovarian estradiol and placental estrogens (91-93). No genetic deficiency syndrome for HSD17B1 has been described. HSD17B2 inactivates estradiol to estrone and testosterone to androstenedione in the placenta, liver, small intestine, prostate,

secretory endometrium and ovary. Whereas HSD17B1 is found in placental syncytiotrophoblast cells, HSD17B2 is expressed in endothelial cells of placental intravillous vessels, consistent with a role in defending the fetal circulation from transplacental passage of maternal estrogens and androgens. No deficiency state for 17βHSD2 has been reported. HSD17B3 is the testicular form of 17βHSD that completes the synthesis of testosterone from androstenedione; its mutations cause a form of 46,XY DSD (94,95). HSD17B5 (AKR1C3, an aldo-keto reductase enzyme), which is also a 3α-hydroxysteroid dehydrogenase, reduces androstenedione to testosterone (96) in the ovary and several non-steroidogenic tissues. AKR1C3 is expressed at low levels in the zona reticularis, accounting for the small amount of adrenally-produced testosterone (97). HSD17B6, also known as RoDH for its homology to retinol dehydrogenases (98), is expressed at low levels in the fetal testes, where it appears to catalyze oxidative 3αHSD activities in the alternative or "backdoor" pathway to 5α-dihydrotestosterone (DHT) synthesis (79,99)(see later).

7. **Aromatase**

Aromatase (CYP19A1) converts 19-carbon androgens to 18-carbon estrogens (100). Aromatase is abundantly expressed in the ovary, placenta and is slightly expressed in fat, but is only expressed in the adrenal in certain malignancies. Nevertheless, it is central to the pathophysiology of fetal development in CAH. The fetus with CAH fetus is only virilized by its own adrenal androgens; even when maternal testosterone concentrations reach 300 ng/dl in a mother who herself has CAH, the female fetus is not virilized because placental aromatase inactivates the androgens from the maternal circulation (101).

B. Enzymology of CYP21A2

CYP21A2 (P450c21), like CYP17A1, is a microsomal or type II cytochrome P450, which catalyzes two essential reactions in adrenal steroidogenesis (39). The major substrate of CYP21A2

is 17OHP, which is converted to 11-deoxycortisol in the zona fasciculata during the biosynthesis of cortisol. In the zona glomerulosa, CYP21A2 21-hydroxylates progesterone to 11-deoxycorticosterone within the aldosterone pathway. Other hepatic cytochrome P450 enzymes have some 21-hydroxylase activity with progesterone as a substrate (102), but this activity does not rescue glucocorticoid deficiency in patients with classic CAH.

As with other microsomal P450s, CYP21A2 utilizes 2 electrons donated by POR to reduce molecular oxygen, producing a hydroxylated substrate and water. The enzymology of CYP21A2 is unusual for a cytochrome P450 in that the primary site of oxygenation is a methyl group, which is a kinetically disfavored site of hydrogen atom abstraction in the reaction cycle. The C-H bond breaking step is partially rate-limiting, and deuterium substitution at C-21 of progesterone shifts hydroxylation partially to the 16α-hydrogen (103). The x-ray crystal structures of bovine (104) and human CYP21A2 (105) with 17OHP bound to the active site explain this activity profile. The steroid substrate is held perpendicular to the heme ring with the A-ring 3-keto oxygen hydrogen bonded to arginine-234 (R234) furthest from the reactive iron-oxygen complex, with C-21 dangling just close enough for the reaction to occur. On the side of the active site, valine-359 (V359) holds the steroid substrate with hydrophobic interactions in the geometry required for 21-hydroxylation and limits access of other reaction sites, principally the C-16 protons; mutagenesis of V359 to the smaller amino acids alanine and glycine progressively shifts progesterone hydroxylation to the 16α-hydrogen (106). The crystal structures also contain a second molecule of steroid outside the active site where the F-G loop that forms the roof of the active site abuts the α-helical domain (104). Whether this second molecule reflects an intermediate state in substrate binding or simply a hydrophobic interaction that favors crystal formation is not known.

The common mutations that cause 21OHD have been compared to wild-type CYP21A2 as recombinant native enzymes in transfected mammalian cells (107), vaccinia-infected mammalian cells (108,109) and yeast (110) or as purified proteins modified for expression in *E. coli* and reconstitution in vitro (111). The catalytic activities of the mutants are reduced generally in proportion to the severity of the deficiency observed in patients with CAH. The studies of purified, reconstituted enzyme assays enable more detailed kinetic studies, which demonstrate that most mutations variably impair substrate binding, catalytic efficiency, and thermal stability in some combination. Extrapolation of these systems to the human adrenal in affected patients should be considered a good approximation but with limitations.

When using purified, reconstituted assay systems, investigators must add phospholipid and purified POR, in addition to the steroid substrate and NADPH. The phospholipid used does not exactly replicate the endoplasmic reticulum of adrenal cortex cells but does bring together CYP21A2 and POR in a proteoliposome to enable electron transfer and catalysis. The phospholipid composition is known to influence the reconstituted activity of CYP17A1 and other steroidogenic P450 enzymes (112), although CYP21A2 has not been studied well in this regard.

C. New Pathways; New Steroids

1. The alternative or "backdoor" pathway to dihydrotestosterone

In addition to the classic pathway via DHEA, androstenedione, and testosterone, the most potent endogenous androgen, 5-dihydrotestosterone (DHT), can also be synthesized via an alternative or "backdoor" pathway that bypasses the classical pathway intermediates (71,79,113-117). This alternative pathway is physiologically active during the major period of human sexual differentiation in the 6^{th} to 10^{th} week of human fetal development (79) and into the second trimester (118). To enter the alternative pathway to DHT, progesterone or 17OHP are 5α-reduced by steroid

5α-reductase type 1 (SRD5A1) to yield 5α-dihydroprogesterone and 17α-hydroxydihydroprogesterone, respectively (for clarity, only the alternative pathway from 17OHP is shown in Figure 1). These 3-ketosteroids are subsequently 3α-reduced to allopregnanolone and 17α-hydroxyallopregnanolone by isoforms of the AKR1C enzyme family. CYP17A1 converts allopregnanolone to 17α-hydroxyallopregnanolone and then to androsterone by its 17,20-lyase activity, serving as its preferred substrate. Androsterone, which is also an inactive metabolite of androstenedione and testosterone, can then be activated to DHT by sequential 17β-reduction and 3α-oxidase reactions (119) (**Figure 1**).

Because excessive 17OHP accumulation is a key characteristic of 21OHD, it is highly likely that the alternative pathway to DHT is a major contributor to fetal female virilization in 21OHD. Alternative pathway steroid metabolites can be detected in patients of all ages with 21OHD, most prominently in the neonate (120). These studies indicate that the high concentrations of 17OHP in individuals with 21OHD drive dihydrotestosterone production by the alternative pathway. The alternative pathway intermediate 17α-hydroxydihydroprogesterone (also termed 5α-17-hydroxypregnanolone) can be detected directly by urinary steroid profiling and indicates the activity of the alternative pathway (120,121).

2. The role of 11-oxo-androgens in CAH

After cleavage of the side chain by 17,20-lyase activity of CYP17A1 in the zona reticularis, the major 19-carbon product of the human adrenal cortex is dehydroepiandrosterone (DHEA) and its sulfate ester DHEAS. Whereas the latter is not a precursor to testosterone, DHEA is efficiently converted to androstenedione and also within the adrenal to lesser amounts of testosterone (122). Both androstenedione and testosterone are good substrates for CYP11B1. Precursor steroids accumulate in the adrenals of patients with 21OHD, and CYP17A1 and CYP11B1 activities are

high owing to chronic ACTH stimulation, hence the system can synthesize large quantities of 11OH-androstenedione, with concentrations exceeding that of androstenedione in both 21OHD patients and unaffected controls (123). 11-ketotestosterone (11KT) is primarily generated from circulating 11OH-androstenedione via the sequential action of 11β-HSD type 2 (which converts the 11β-hydroxyl to a keto group) and AKR1C3 (124). 11KT, which is in fact the major testicular androgen in teleost fishes (125), is nearly as potent as testosterone in transactivating the human androgen receptor (126). The intermediate 11-ketoandrostenedione – but not 11OH-androstenedione – is a much better substrate for AKR1C3 than androstenedione itself (127), which explains why 11KT is the second-most abundant circulating 11-oxo-androgen in both 21OHD patients and unaffected individuals. In addition, 11KT is a substrate for the 5α-reductases (124), yielding 11-ketoDHT (11KDHT), which appears to be a more potent androgen than 11KT (reviewed in (86)), but is not detected in relevant concentrations in circulation.

In women with 21OHD, 11KT rises roughly proportionately to testosterone (123), reflecting the adrenal rather than gonadal origin of these androgens. Furthermore, 11-oxo-androgens are poor substrates for aromatase; whereas 11-oxo-androgens can be converted to 11-oxygenated estrogens, the latter do not contribute substantially to the circulating estrogen pool (128). In contrast, 11KT is inversely proportional to testosterone in men (123) and in boys Tanner stage 3-5 (129) with 21OHD. This is because men with poor disease control produce more 11OH-androstenedione, which is preferentially metabolized to 11KT which then suppresses the hypothalamic-pituitary-testicular axis, thereby decreasing testicular secretion of testosterone. In men with good disease control, 11KT synthesis is low, whereas testicular testosterone synthesis is normal. Hence, a low 11KT/testosterone ratio in a man with 21OHD indicates both good disease control and good testicular function.

It is difficult to evaluate long-term disease control in adults with classic CAH. Assessing adrenal size, which might be the ultimate assessment, requires cross-sectional imaging with associated cost and radiation exposure. The 11-oxo-androgens (and 21-deoxycortisol), correlate better with adrenal size than traditional biomarkers of short-term disease control, such as androstenedione and 17OHP (129). Elevated 11-oxo-androgens are also predictive of menstrual irregularity in women and of TARTs in men with CAH (129). In contrast to DHEAS, androstenedione and testosterone, 11KT does not decline with age in women from 20 to 80-years old, and 11KT declines very gradually in men over the same age range (130). These data suggest that 11-oxo-androgens may be useful biomarkers of 21OHD control well into adulthood and in hypogonadal states. In patients with NC CAH, 11-oxo-androgens are elevated about 2-fold compared to women with clinical features of androgen excess, although 11-oxo-androgens alone cannot be used to establish the diagnosis of NC CAH (131). Finally, limited data suggest that 11-oxo-androgens are rather specific for 21OHD and are not elevated in other androgen-excess forms of CAH such as 11β-hydroxylase deficiency and 3βHSD2 deficiency, because either CYP11B1 activity or intra-adrenal androstenedione production are low, respectively (86,123).

In summary, androgens are generated in CAH patients via all three major pathways (132). First, classic pathway androgen synthesis is enhanced through increased conversion of accumulating 17OHP to androstenedione via the 17,20-lyase activity of CYP17A1, an ordinarily minor reaction compared to the preferred conversion of 17OH-Preg to DHEA (60). Second, the androstenedione so generated consequently drives increased substrate flow to the 11-oxoandrogen pathway, through conversion of androstenedione to 11β-hydroxyandrostenedione. Third, while the alternative pathway to DHT contributes to excess androgen generation in 21OHD, its relative

contribution appears to be more limited than that of classic and 11-oxo-pathways, as indicated by in vivo urinary steroid metabolite profiling in CAH patients during glucocorticoid therapy (133).

3. Biological activities of steroidal intermediates

Aside from defects in StAR and CYP11A1, in which essentially no steroids are secreted, a hallmark of inherited enzymatic defects in adrenal steroidogenesis is the accumulation of 'upstream' steroids, proximal to the affected enzymatic step, which provide useful diagnostic markers. In 21OHD, 17OHP, the steroid before 21-hydroxylase, accumulates and is traditionally used to diagnose 21OHD (1,134,135). Besides 17OHP, several other 'upstream' steroids such as pregnenolone, 17OH-Preg and progesterone, and may also accumulate but are not diagnostically specific. In the absence of 21-hydroxylase activity, a substantial portion of 17OHP is converted into 21-deoxycortisol by CYP11B1 (**Figure 1**). 21-deoxycortisol is a potentially useful marker for the diagnosis of 21OHD (136).

Some steroids that accumulate in 21OHD, including 21-deoxycortisol, progesterone and 17OHP, may also bind to glucocorticoid or mineralocorticoid receptors and act variously as either agonists or antagonists. In vitro, 21-deoxycortisol, corticosterone, 17OHP, and progesterone bind the glucocorticoid receptor with 24-43% of the affinity of cortisol. However, the transactivation activities of progesterone and 17OHP were only 0.2 to 0.8% of that for cortisol, whereas the transactivation activity of 21-deoxycortisol was 8.5% and 17% in two different assays (137,138). By contrast, 17OHP and progesterone inhibit aldosterone-mediated transactivation of the mineralocorticoid receptor in a dose-dependent fashion, explaining the strong anti-mineralocorticoid effect of 17OHP and progesterone in vitro (139,140). Androstenedione and testosterone had no effect on mineralocorticoid receptor transactivation (140).

The clinical implications of these findings are not yet completely understood. Some adult classic CAH patients stop glucocorticoid medication without developing symptoms and signs of adrenal insufficiency (137,141). Perhaps elevated levels of other steroids partially compensate for the low cortisol concentrations (142). Moreover, 21-hydroxylation of progesterone by hepatic cytochrome P450 enzymes other than CYP21A2 may permit some mineralocorticoid (11-deoxycorticosterone) synthesis (102). Clinical consequences of treatment lapses include androgen excess in women and TARTs in men, adrenal hyperplasia and/or tumors, as well as the theoretical risk of adrenal crisis in all patients.

III. GENETICS IN CAH

21OHD is caused by inactivating mutations in the gene coding for adrenal 21-hydroxylase (*CYP21A2,* older nomenclature *CYP21, CYP21B, P450c21B*; GeneID 1589).

A. *The CYP21 genes and the surrounding genetic region*

The *CYP21A2* gene encodes the microsomal P450 enzyme, 21-hydroxylase (CYP21A2, P450c21), a protein of 495 amino acids. *CYP21A2* is located in the HLA Class III region on the short arm of chromosome 6 (6p21.3), approximately 30-kilobases apart from the non-functional *CYP21A1P* pseudogene (**Figure 2**). *CYP21A2* and *CYP21A1P* both consist of 10 exons and share high nucleotide homology of about 98% and 96% in exons and introns respectively (143,144). *CYP21A1P* and *CYP21A2* are arranged in tandem with the *C4A* and *C4B* genes encoding the fourth complement factor (145). There are additional sense and antisense transcripts of unknown significance near or overlapping the CYP21 genes (146,147). The *C4/CYP21* unit is flanked by the serine-threonine kinase-19 (*STK19, RP1*) gene on the telomeric side and by the tenascin-X gene (*TNXB,* which encodes an extracellular matrix protein on the opposite DNA strand)(148) on the centromeric side, and their pseudogenes, *STK19B* and *TNXA,* forming a 30 kb tandem repeat

sometimes referred to as an RCCX module (*RP-C4-CYP21-TNX*) (149). The *STK19, C4* and *CYP21* genes are transcribed in the telomeric to centromeric direction, whereas *TNXB* is transcribed from the opposite strand. Most chromosomes have two copies of the module with a *CYP21A1P* pseudogene in the telomeric module and a *CYP21A2* gene in the centromeric module. However, this locus shows high structural variability with monomodular, trimodular or even quadrimodular haplotypes detected (150,151). The *TNXA* and *STK19B* pseudogenes were truncated during the duplication of the ancestral RCCX module. The last exons of *TNXA* and *TNXB* overlap the 3-prime untranslated regions of exon 10 of *CYP21A1P* and *CYP21A2*, respectively.

CYP21A1P is transcribed but its mRNA cannot encode a functional protein owing to at least 10 deleterious mutations (143,144) including two frameshifts (8 bp deletion in exon 3, 1 bp insertion in exon 7, a nonsense mutation (p.Gln318stop; Q318X) (152), and a mutation in intron 2 that activates a cryptic splice site and causes an extra 19 nucleotides to be included in the mRNA (153). Missense mutations in the pseudogene include p.Pro30Leu (P30L) (108), p.Ile172Asn (I172N) (154), a cluster of missense mutations in exon 6, p.Ile236Asn, Val237Glu, Met239Lys (I236N, V237E, M239K), p.Val281Leu (V281L) (155) and p.Arg356Trp (R356W). Additionally, 4 single nucleotide differences in the 5' flanking region of *CYP21A1P* reduce its transcriptional activity to 20% of that of *CYP21A2* (see section III.B.3)(146). Note that there is a polymorphism of no functional significance in the hydrophobic leader sequence at the amino terminus of CYP21A2, consisting of a single amino acid insertion. Consequently, some publications and databases list mutations with positions incremented by 1; e.g. P31L instead of P30L.

B. *CYP21A2 gene expression*

1. Pattern of *CYP21A2* expression

By immunohistochemistry, *CYP21A2* expression is first detected robustly at 50–52 days postconception within the nascent inner fetal zone. Within the outer definitive zone, *CYP21A2*, is more weakly detected and persists up to 14 weeks postconception. All other enzymes required for cortisol biosynthesis are present as well, and cortisol concentrations within the fetal adrenal are high during the first trimester (156). From 14 to 22 weeks, *CYP21A2* is expressed only in the fetal and transitional zones, but not the definitive zone, and cortisol secretion is relatively low; definitive zone expression is detected starting at 23 weeks and continuing through the remainder of gestation, as cortisol secretion increases (157). Cortisol secretion in the first trimester suppresses DHEA production and thus minimizes fetal androgen secretion until placental aromatase expression increases in the second trimester, by which time differentiation of the external genitalia is complete and cannot be affected by DHEA levels. Cortisol is again required in the third trimester to support lung maturation, including surfactant production (158). Low expression of *CYP21A2* during the second trimester partially explains the high incidence of false-positives in newborn screens for CAH in premature infants (see section IV.A)(159).

In normal adult adrenal glands, *CYP21A2* immunoreactivity is detected in all three cortical layers, particularly the zonae glomerulosa and reticularis, with variegated expression in the zona fasciculata. The immunoreactivity is more intense in adrenals from patients with Cushing disease and at sites of regeneration in normal adrenal glands (160).

In addition to the adrenal cortex, *CYP21A2* is detected in other tissues by RT-PCR. These include lymphocytes, which also express an additional 21-hydroxylase activity that is not mediated by *CYP21A2* (161). *CYP21A2* is expressed throughout the human heart at levels approximately

0.1% those in the adrenal cortex. Expression patterns of other steroidogenic enzymes suggest autocrine or paracrine roles for corticosterone and deoxycorticosterone, but not cortisol or aldosterone, in the normal adult human heart (162).

2. Regulation of *CYP21A2* expression

Cortisol secretion is regulated mainly by ACTH, which acts via the Gα-protein coupled MC2R receptor to increase activity of adenylyl cyclase and thus increase intracellular levels of cAMP. This in turn increases activity of protein kinase A. The main effect of corticotropin releasing hormone (CRH) secreted by the hypothalamus is to increase ACTH secretion by the pituitary gland, but additionally, it may act directly on adrenocortical cells to increase cortisol secretion, and expression of *CYP21A2* and other steroidogenic enzymes (163). Infection, fever and pyrogens stimulate the release of interleukins IL-1 and IL-6, promoting secretion of CRH, and stimulate IL-2 and TNF promoting release of ACTH, increasing cortisol secretion during inflammation (164); IL-6 can also directly stimulate adrenal synthesis and release of cortisol (165).

In contrast, aldosterone secretion is regulated mainly by angiotensin II, which activates the Gq-protein coupled angiotensin 2 receptor (AT2R) , which acts primarily through the protein kinase C pathway but also through Ca^{2+} signaling (166). Additionally, high extracellular potassium levels trigger voltage sensitive calcium channels that also increase intracellular calcium levels. Calcium then increases activity of protein kinase C (167).

Regulation of *CYP21A2* expression is consistent with these tropic stimuli. In the H295R human adrenocortical cell line (168,169) and also in primary cultures of human adrenocortical cells (169,170), mRNA and/or protein expression of CYP21A2 are induced by increases in cAMP analogs and by angiotensin II or tetradecanoyl phorbol acetate, which stimulate protein kinase C. Insulin and IGF-I are additional trophic stimuli (170). Additional hormone and environmental

555 factors may regulate *CYP21A2* expression. These include orexins, which stimulate secretion of
556 cortisol (171), and potential endocrine disruptors including brominated flame retardants (172,173)
557 and organic freshwater contaminants (174).

558 ## 3. Transcriptional control of *CYP21A1P* and *CYP21A2*

559 The most important *CYP21A2* transcript begins 10–11 nucleotides before the initial AUG
560 codon (143). *CYP21A1P* is also transcribed specifically in the intact adrenal cortex at a level 10–
561 20% that of *CYP21A2* (146). However, the first 2 introns are inconsistently spliced out, and an
562 uncertain proportion of transcripts include additional exons in the region between the end of
563 *CYP21A1P* and the beginning of *C4B*. Some of these exons may overlap the truncated *TNXA* gene.
564 Adrenal transcripts in the same direction as *CYP21A2* have also been detected overlapping *TNXB*;
565 these are also of uncertain functional significance (175).

566 Similarly, *CYP21A1P* transcripts cannot be detected in primary cultures of human
567 adrenocortical cells, whereas *CYP21A2* is appropriately expressed under the same conditions
568 (170,176). In cultured mouse Y-1 or human H295 adrenocortical cells, the 5' flanking region of
569 human CYP21A2 drives basal expression of reporter constructs at levels 2.5–8 times higher than
570 the corresponding region of *CYP21A1P* (176-178). Sequences responsible for this difference have
571 been localized to the first 176 nucleotides (176) although sequences upstream of this region are
572 required for full expression. There are only 4 nucleotide differences (-126C>T, -113G>A, -
573 110T>C, and -103A>G) between *CYP21A1P* and *CYP21A2* in this region. The first two listed
574 affect binding of the Sp1 transcription factor. In patients with 21OHD, gene conversions involving
575 this region reduce but do not eliminate *CYP21A2* expression. In isolation with no additional
576 mutations present, they can be associated with NC CAH (179). When the gene conversion extends

to the P30L missense mutation (which is usually a Group C, i.e. nonclassic allele; see section III.D), it becomes a simple virilizing (Group B) allele (180).

The most important transcription factor for adrenal-specific expression of *CYP21A2* is steroidogenic factor-1 (SF-1, Ad4BP, NR5A1). This protein is also required for development of the adrenal gland and gonads (181,182). It interacts with specific DNA elements both within the proximal promoter and in intron 35 of the linked *C4B* gene (183).

Additional relevant transcription factors include nerve growth factor induced-B (NGFI-B, Nur77, NR4A1)(169,184), and Nur-related factor 1 (NURR1) (NR4A2); they may overlap in their functions (185). NGFI-B is phosphorylated under basal conditions and dephosphorylated in response to ACTH, which activates it. Thus it may help to mediate ACTH regulation of *CYP21A2* expression. These transcription factors may also be important for mediating gene regulation by angiotensin II (167). A third closely related transcription factor, neuron-derived orphan receptor 1 (NOR1, NR4A3), is also expressed in the adrenal cortex and may function similarly (186).

C. *Molecular genetics of CAH*

Over 90% of mutations causing 21OHD are the consequence of intergenic recombinations within the 30 kb tandem repeat (**Figure 2**), promoted by the high recombination rate in the HLA region along with the nucleotide identity shared across the 30 kb repeat. These include both deletions generated by unequal meiotic crossing-over during gametogenesis, and gene conversions between *CYP21A2* and the *CYP21A1P*, generated by either meiotic or mitotic events (187). Unequal crossovers, owing to misalignment of the 30 kb tandem duplication, can occur with break points anywhere along the duplicated region. Breakpoints originating in *STK19* or *C4A* lead to a net deletion of one of the *C4* genes and *CYP21A1P* but leave *CYP21A2* unaffected. Such a

configuration occurs on at least 5% of normal chromosomes (145). Breakpoints originating in *CYP21A1P* yield a deletion of *C4B* and a single chimeric *CYP21* gene that has a 5' end corresponding to *CYP21A1P* and a 3' end corresponding to *CYP21A2*. This chimeric gene usually (but not always (188)) includes *CYP21A1P* mutations that prevent synthesis of a functional protein, so it represents a null allele and thus is usually referred to as a *CYP21A2* deletion. Occasionally a breakpoint occurs in the *TNX* genes, leading to complete deletion of *C4B* and *CYP21A2*, and a *TNXB/TNXA* chimeric gene (149,150,189). Homozygosity for such a chimera leads to a contiguous gene syndrome consisting of CAH and Ehlers-Danlos syndrome (190), which is rarely clinically reported in patients with severe CAH. However, 7-14% of patients with CAH have heterozygous *TNXB* mutations (191,192). This extended phenotype has been termed the CAH-X syndrome. CAH-X is associated with joint hypermobility, chronic arthralgia, joint subluxations, hernias, and cardiac defects (193,194). Deletions account for approximately 20% of mutant alleles in 21OHD (189,195-213). *CYP21A2* gene duplications are relatively common in some populations (214,215). Many of these alleles carrying a *CYP21A2* gene duplication have a p.Gln318X (Q318X) mutation in the duplicated *CYP21A2* gene next to the *TNXB* gene, and a wild-type *CYP21A2* gene next to the *TNXA* pseudogene. Importantly, such alleles are non-disease causing, but can be easily misinterpreted (214).

Approximately 70-75% of disease-causing *CYP21A2* mutations arise from the transfer of deleterious mutations from *CYP21A1P*, i.e, gene conversion (**Figure 3**). In addition, over 200 pseudogene-independent mutations are listed in the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk) and the Pharmacogene Variation Consortium (https://www.pharmvar.org/gene/CYP21A2). Most of these rare mutations are sporadic. However, due to founder effects increased frequencies of some pseudogene-independent mutations are

observed in some populations. Deletions, the splice site mutation in intron 2 (c.293-13A/C>G) and p.Ile172Asn (I172N) are the most common mutations in most populations (189,195-213). The p.Val281Leu (V281L) mutation is by far the most common allele detected in patients with NC CAH and is highly prevalent in Ashkenazi Jews (155). Novel or rare mutations account for about 3-5% of detected mutations in large cohorts. The vast majority of these rare mutations have been identified in single families or small populations. Approximately 1-2% of *CYP21A2* disease causing mutations arise *de novo*.

D. *Genotype-phenotype correlation*

In descending order of compromised 21-hydroxylase activity, 4 general groups of *CYP21A2* mutations have been established to predict the phenotype (**Figure 3**) (195,196,201,203,208,213,216). Deletions, large gene conversions, nonsense mutations, frameshifts, and missense mutations that totally abolish enzymatic activity are SW classic alleles, comprising mutation Group 0 ("null"). A single nucleotide mutation that alters splicing of intron 2 (c.293-13A/C>G, "intron 2 G" mutation) (153) is particularly common, comprising 20-25% of mutant alleles in most populations (**Table 2**). It has been seen in both SW and SV patients, suggesting that there is a small amount of normally-spliced mRNA; it is generally considered its own separate Group A (in the first analysis of this sort (195), Groups 0 and A were referred to as Groups A1 and A2, respectively). A nonconservative amino-acid substitution, p.Ile172Asn (I172N)(154) reduces enzymatic activity to <5% of normal and is associated with the SV form of the disorder (mutation Group B)(107,109,221). Finally, missense mutations such as p.Val281Leu (V281L) and p.Pro30Leu (P30L) (108) reduce enzyme activity to ~20-50% of normal (mutation Group C), and are associated with NC CAH. Although enzyme function in vitro appears to be

similar (111), clinical observations suggest that patients carrying the P30L allele are somewhat more symptomatic, straddling the border between SV and NC forms of CAH (213,222). As noted in section III.B.3, in many cases this may be a consequence of gene conversions extending into the 5' flanking transcriptional regulatory region, thus impairing gene expression (180).

CAH due to 21OHD is an autosomal recessive condition. About 65–75% of 21OHD patients are compound heterozygotes; i.e., they carry different mutations on each allele. In cohort studies, the clinical phenotype of CAH strongly correlates with the less severely impaired *CYP21A2* allele (**Figure 3**); 96% of individuals carrying two Group 0 alleles have SW CAH, whereas 97% of those with at least one Group C allele have NC CAH. The correlation is somewhat less strong for the groups with enzymatic impairment of intermediate severity (Groups A and B, and the P30L mutation). To some extent, this reflects the fact that the distinctions between SW and SV CAH, or SV and NC CAH, are a continuum, and not absolute. For example, many SV CAH patients nevertheless require mineralocorticoid supplementation early in life and might be classified as SW, whereas the distinction between the SV and NC forms can be particularly challenging in males. Without exhaustive sequencing, it is difficult to rule out the existence of additional mutations in introns or flanking regions that might affect mRNA processing or gene expression; as noted in section III.B.3, one transcriptional control region is several kilobases away from *CYP21A2*, in the *C4B* gene (183). Data correlating genotype with intermediate phenotypes are limited and often are not presented in a way that permits meta-analysis. In both American (195,223) and German (201) data, median (interquartile range) Prader virilization scores (**Figure 4**) for females in Groups 0, A, B and C are 4 (3-5), 4 (3-5), 3 (2-4) and 0 (0-2) respectively. (201). A similar correlation of severity with genotype is seen when evaluating genital appearance in adult women (224). No factors modulating androgen effects have been demonstrated to influence the degree of virilization

associated with each genotype group. Basal levels of 17OHP are also correlated with genotype (195,200,201,225), with different studies reporting mean levels (in ng/dl) in Groups 0/A, B and C of 23-41,000, 10-18,000, and 3-8000, respectively. However, there is substantial overlap in values between genotype groups. Adult height and mean hydrocortisone doses are also influenced by genotype (226). There are limited data directly correlating psychosexual functioning with genotype (224,225), but gender dysphoria does tend to be most severe in women with SW CAH, which in turn is highly correlated with group 0 and A genotypes (227). Long-term health outcomes in adults do not correlate well with the genotype (212). However, girls and women with more severe *CYP21A2* genotypes appear to have an increased risk for psychiatric conditions (228) and variations of the complement component C4 may influence the risk of psychopathology (229). In summary, genotype-phenotype correlations are strong but not absolute, and clinical management should be based on clinical and hormonal data.

By analyzing the CYP21A2 crystal structure, novel insights into the underlying molecular pathology have been gained (105,230). Null and other severely deleterious mutations commonly disrupt heme and/or substrate binding domains, the anchoring of the protein to the membrane, or impair protein stability. Mutations categorized as group B partially impair membrane anchoring or affect conserved hydrophobic clusters within the protein. Milder mutations (group C) result in less severe alterations, often interfering with electron transfer from POR, salt-bridge and hydrogen-bonding networks, and non-conserved hydrophobic clusters (105). However, other factors potentially influence enzymatic activity including mRNA expression, splicing and stability, and protein stability.

IV. DIAGNOSTICS

A. Neonatal screening

1. Benefits

Neonatal screening for classic CAH was introduced to prevent morbidity and mortality due to adrenal crisis. Currently, all 50 states in the US, 35 other countries, and portions of 17 additional countries screen for CAH (231,232). Results of these screens indicate that the incidence of classic CAH in most populations is approximately 1:14,000 to 1:18,000. **Table 1** summarizes data since 2008; data reviewed 1997-2004 are similar (233-235). Although newborn screening for CAH is now performed in an increasing number of countries, protocols and reported outcomes vary widely (236).

Screening markedly reduces the time to diagnosis of infants with classic CAH (89,237-239), consequently reducing morbidity and mortality. Diagnosis is more likely to be delayed in males owing to the lack of genital ambiguity. Thus, a relative paucity of males in a patient population may be taken as indirect evidence of unreported deaths from salt-wasting crises. Females do outnumber males in some (12,240,241) although not all (242) retrospective studies in which CAH was clinically diagnosed without neonatal screening. Moreover, there is a greater preponderance of severe genotypes in screened infants than in those ascertained prior to screening, again suggesting extra deaths of severely affected infants prior to screening (2,243). Nevertheless, infant deaths from CAH are now rare (0-4%) in advanced economies even without screening (244,245).

Infants ascertained through screening have less severe hyponatremia and shorter hospitalizations (238,242,246,247). The delay before correct sex assignment of severely virilized females is also markedly reduced (233). Moreover, males with SV CAH, and (mildly) virilized females, may otherwise not be diagnosed until later in childhood, at which time height may already

713 be compromised. Although not an aim of screening, children with NC CAH are occasionally
714 diagnosed. In some cases, the consequent close monitoring and, if necessary, treatment may
715 improve adult height.

716 ## 2. Initial screening methodology

717 First-tier screens for CAH due to 21OHD employ immunoassays to measure 17OHP in dried
718 blood spots on the same filter paper ("Guthrie") cards used for other newborn screening tests
719 (238,248,249). Radioimmunoassay (RIA) was the first method developed (250), but automated
720 time-resolved dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) has almost
721 completely supplanted other immunoassays (251).

722 The main drawback to screening is that false positive rates are high, leading to substantial costs
723 for evaluation and increasing parental concern. Several factors limit accuracy of these tests. First,
724 premature, sick, or stressed infants tend to have higher levels of 17OHP than term infants; as
725 studied by high-performance liquid chromatography, preterm infants have a functional deficiency
726 of several adrenal steroidogenic enzymes with a nadir in function at 29 weeks of gestation (159).
727 This "adrenal prematurity" can generate many false positives unless screening programs use higher
728 screening cut-offs for preterm infants. For example, in 26 years of operation of the Swedish
729 screening program, the positive predictive value for full-term infants was 25%, whereas it was
730 only 1.4% for preterm infants, and it correlated very strongly with gestational age (252). There
731 are no universally accepted standards for stratifying infants. Most laboratories use a series of birth
732 weight-adjusted cut-offs (253,254) but actual gestational age, or both, might be preferable, because
733 gestational age correlates much better with 17OHP levels (27,255).

734 Second, 17OHP levels are normally high after birth, decreasing rapidly during the first few
735 postnatal days in healthy infants. In contrast, 17OHP levels increase with time in infants with

21OHD. Thus, diagnostic accuracy is poor in the first 2 days, and screening a second sample several days later improves both sensitivity and positive predictive value with the risk of delaying treatment (89,249,256). Moreover, a comparison of one-screen versus two-screen state programs found a higher incidence of 21OHD when a second screen was employed (257). It has been suggested that preterm infants should have additional samples rescreened at 2 and 4 weeks of age, most practical in a hospitalized population where potential salt-wasting can be monitored (254).

Multiple courses of antenatal corticosteroids might reduce 17OHP levels and thus potentially increase the likelihood of false negative screens. Studies have reported inconsistent effects of antenatal corticosteroid administration in practice (258,259). Testing of later samples should minimize this problem, but the delay may increase the risk of salt-wasting crisis.

Female infants have lower mean circulating 17OHP levels than males, slightly reducing screening sensitivity (260). Because almost all females with SW CAH are virilized, most of them are diagnosed based on clinical symptoms, and therefore the reduced sensitivity is not usually problematic. However, even severely virilized girls can be missed as virilization is not always noticed at physical examination (2,22,261,262). Finally, immunoassays may lack specificity; this is discussed in Section IV.B.

3. **Second-Tier Screening**

Because 21OHD is a rare disease, the positive predictive value of neonatal screening is low, even though the specificity and sensitivity of the tests are very high (232). The positive predictive value might be improved with a second-tier screen.

a) Biochemical Second Screens

Direct biochemical analysis of steroids in blood samples using LC-MS/MS can obviate the specificity problems of immunoassays (263-265) and both heel stick blood samples (266) and urine

samples (267,268) can also be analyzed by mass spectrometric methods. Measuring 21-deoxycortisol instead of 17OHP may improve diagnostic accuracy (136). Measuring steroid ratios further improves the screening specificity of LC-MS/MS. Such ratios have included (17OHP+androstenedione)/cortisol (263,269,270), 17OHP/11-deoxycortisol (271), and (17OHP+21-deoxycortisol)/cortisol(265). Some (272,273) but not all (254) laboratories have reported markedly superior results with these approaches, with one recent report claiming a positive predictive value of 71% (270). A recent statistical approach using principal component analysis of six steroid levels (17OHP, both first and second-tier, 11-deoxycortisol, androstenedione, 21-deoxycortisol and cortisol) achieved a positive predictive value of 67% (274). Consistency of results might be improved by mandating participation in national proficiency testing programs (275). However, caution should be exercised in developing reference ranges for assays using dried blood spots that have been stored for prolonged periods at room temperature, because cortisol and 11-deoxycortisol are not stable past 4 weeks of such storage (276).

b) Molecular Genetic Second Screens.

CYP21A2 mutations can be detected in DNA extracted from the same dried blood spots used for hormonal screening (see Section IV.A.2). Because >90% of mutant alleles carry one or more of a discrete number of mutations, we can assume with >99% confidence that samples that carry none of these mutations are unaffected. Several studies of genotyping of samples from screening programs have suggested that this is a potentially useful adjunct to hormonal measurements (277-282), but there has been no large-scale study of its efficacy as a second-tier screen in actual use.

B. Biochemical evaluation

Determining levels of steroid hormones and their precursors is a mainstay of diagnosis and management of CAH. Currently, the determination of steroid hormones rests on analytical techniques either based on the principle of immunoassay or on chromatographic methods coupled to mass spectrometry (283).

The specificity of the antibody is crucial for the reliability of an immunoassay. Inefficient discrimination between the analyte and structurally closely related substances will lead to cross reactivity with consequent overestimation of the amount of analyte. The overestimation of 17OHP in serum or plasma of premature infants, neonates or young infants by immunoassay techniques used in screening procedures or clinical routine with the consecutive risk of over-diagnosing 21OHD, presents a typical and important example of this phenomenon (284). Cross-reactivity has been documented with 17-OH pregnenolone sulfate, a steroid originating in extremely high amounts from the fetal zone of the fetal adrenal (285), and 15β-hydroxylated compounds apparently generated by gut bacteria and resorbed through the entero-hepatic circulation (286). There may be additional substances in dried blood spots that interfere with immunoassays (matrix effect)(287). The DELFIA was reformulated in late 2009 to make it less sensitive to cross-reacting compounds in premature infants (288). This modification improved the positive predictive value from 0.4% to 3.7% for the first screen (249). The specificity of immunoassays may be further improved with organic extraction to remove cross-reacting substances, such as steroid sulfates. Additional preparative steps such as extraction, chromatographic pre-purification or dilution can help to circumvent matrix effects.

Currently, mass spectrometry represents the most versatile and exact of all analytical techniques for steroids. Initial separation by liquid chromatography (LC) or gas chromatography

(GC) can consistently improve specificity, and it also permits multi-component analysis, i.e. the simultaneous determination of multiple analytes in a single run. This development laid the foundation for the field of metabolomics, which presents the unbiased and systematic study of small molecules present in a biological sample. If mass spectrometry records all ions of a particular mass range, this is called an "untargeted" mode. If operated in "targeted" mode, mass spectrometry only records preselected ions (283).

Of all separation techniques, GC provides the best resolution of steroids. In combination with MS as the detection method, GC-MS presents a robust analytical tool, unsurpassed in determining simultaneously a multitude of steroids including precursors or metabolites of progestins, glucocorticoids, mineralocorticoids (all C_{21}-steroids), androgens (C_{19}-steroids) and estrogens (C_{18}-steroids) (289,290). GC-MS is particularly useful for urinary steroid metabolome analysis, but it can also be applied to the analysis of blood or tissues (291) or be used as a gold standard in quality assurance (292). As over two-thirds of steroid hormones and their metabolites are excreted into urine, the measurement of these urinary steroids provides an integrated picture of a patient's steroid hormonal status (steroidal fingerprint) and has enormous diagnostic power. Adrenal enzyme defects show unique metabolic patterns (disease signatures, metabotypes) (293). Usually, a spot urine sample is sufficient for diagnosing an adrenal enzyme defect (268,294,295). Timed samples (e.g. 24-hr urine) provide additional information on hormonal production rates via determination of steroid excretion rates (296,297). This information aids the diagnosis of hormonal overproduction syndromes, e.g. Cushing syndrome or tumors, as well as in assessing compliance with hormonal therapy in CAH (298,299). Moreover, this approach has been used to dissect the contribution of the three androgen biosynthesis pathways discussed in Section II.C (133,300,301). Unbiased systems biology approaches allow for clustering and describing various metabotypes

(302), reclassifying hitherto uncharacterized conditions (303) or improving metabolic monitoring of 21OHD (304,305).

LC-MS is a more recent technique than GC-MS (303). Tandem MS (MS/MS) provides an extra level of filtering, thus improving the relatively poor separation properties of LC. Simple work up procedures and short run times permit much greater throughput than with GC-MS (283). Currently, determination of most clinically relevant steroid hormones in plasma or serum can be carried out by LC-MS/MS. It is the technique of choice for determining conjugated steroids (306). However, factors such as relatively low chromatographic resolution and lower ionization fraction, compared with electron impact in GC-MS, can impair the specificity of LC-MS/MS. Thus, GC-MS and LC-MS/MS should be considered complementary techniques.

Whatever analytical method is used, thorough method validation is a *sine qua non*. Important aspects of method validation comprise assessment of sensitivity, precision, reproducibility, accuracy, limits of quantification and detection, recovery, stability, carryover and matrix effects. Recommendations have been published for the hormonal diagnosis of steroid related disorders (307).

C. Molecular genetic testing for CYP21A2 gene mutations

Southern blot analysis was originally the gold standard for the detection of *CYP21A2* gene deletions but is no longer used clinically because it requires relatively large amounts of high-quality DNA, is labor intensive, and time consuming. Moreover, *CYP21A1P* duplications and certain other rearrangements at this locus may impede the detection of *CYP21A2* gene deletions or duplications (308). The most widely used current approach for gene dosage determination is multiplex ligation-dependent probe amplification (MLPA). MLPA requires only small amounts of

DNA for detection of gene deletions, rearrangements and fusion genes (212,309-311). However, complex rearrangements can lead to challenges interpreting the correct genotype. The design of *CYP21A2*-specific primers for PCR-based amplification is crucial to avoid amplification of the pseudogene and allele dropout by non-amplifying PCR fragments. This represents a challenge due to the high number of polymorphisms within *CYP21A2* and the high sequence identity with its *CYP21AP1* pseudogene. A variety of targeted molecular genetic strategies for detecting the common mutations have been published and are established in diagnostic laboratories. However, direct sequencing of the amplified PCR products combined with a method for the detection of gene deletions and chimeric genes are the only available strategies that allow for the detection of close to 100% of *CYP21A2* mutated alleles. If possible, carrier testing should be performed in the parents to set phase (i.e., confirm the parental origin of each mutation), which is required to determine compound heterozygosity, distinguish hemizygosity from homozygosity in the index case, and estimate the recurrence risk.

D. Prenatal diagnosis

Prenatal diagnosis can be performed when both parents are carriers of *CYP21A2* mutations; most often this situation arises when they have a previous child with 21OHD. The possible methods for prenatal diagnosis have increased over the past decades. However, methods involving invasive sampling should only be performed if the results will lead to changes in approach or treatment (312).

Analysis of fetal hormones in amniotic fluid was the initial method available for prenatal diagnosis (313-315). Fetal cells obtained this way were originally used for HLA typing to determine the inheritance of maternal and paternal haplotypes (*CYP21A2* is located in the *HLA*

complex)(316) but can also be used for genetic analysis, although cells must first be cultured, a time-consuming process.

Chorionic villus sampling to obtain fetal DNA can be performed as early as gestational week 10-11, as compared to week 12-14 for amniocentesis (317). This method is available in many countries and centers today. Both amniocentesis and chorionic villus sampling are associated with a small but increased risk of fetal loss (318).

1. Non-invasive methods

Cell-free fetal DNA can be isolated from maternal plasma (319). Unlike fetal cells, it disappears from the maternal circulation shortly after delivery (319,320) and therefore does not confound prenatal genetic investigations in subsequent pregnancies (321,322). Prenatal sex typing (SRY detection) can be performed using PCR amplification of cell-free fetal DNA as early as week 6–9 (323) and may be useful in decisions regarding prenatal treatment with dexamethasone to minimize treatment of male fetuses (see section V.C) (324). Next generation sequencing of cell-free fetal DNA can ascertain mutations, but it is challenging to detect *CYP21A2* mutations in this manner because the vast majority of such mutations are already present in the *CYP21A1P* pseudogene. Instead, targeted massive parallel sequencing of cell-free fetal DNA in maternal plasma can identify SNPs flanking *CYP21A2* that are specific for the mother, father, and proband (previous child), thus constructing haplotype blocks to determine the maternal and paternal alleles inherited by the fetus (325). The technique is promising but costly; it requires specific resources and personnel and is not yet available as part of routine clinical care.

2. Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is available in many countries for families at risk of having a child with a severe genetic condition including 21OHD. PGD requires an in vitro

fertilization approach and enables implantation only of embryos without the specific genetic disorder. It may present ethical challenges beyond the scope of this review (326). The preferred approach to obtain DNA for PGD is a biopsy at day 5-6 from the trophectoderm of the blastocyst when it comprises about 120 cells. The inner cell mass that will develop into the fetus, from which 5-10 cells are required, can then be separated from the trophectoderm (312). If the first polar body of the oocyte is used in PGD, the procedure is performed before fertilization occurs, and the analysis offers the unique possibility of pre-conceptional diagnosis. The disadvantage for autosomal recessive disorders is that only the oocyte is assessed and the paternal allele is not included in the assessment. The rate of fetal anomalies is not increased with PGD, rather it is thought that if damage occurs during the procedure it is lethal to the embryo (327).

V. MANAGEMENT

A. Hormonal treatment of classic CAH

Treatment of classic CAH is intended to replace both glucocorticoid and if necessary, mineralocorticoid hormones to prevent adrenal and salt-wasting crisis and to reduce excessive corticotropin driving adrenal androgen secretion (134). Clinical goals are normal growth and development and pubertal maturation from birth to adolescence, and prevention of adrenal crisis, virilization, and other long-term complications discussed below (134,328,329). The levels of evidence for management guidelines are detailed in The Endocrine Society's Clinical Practice Guideline published in 2018 (134) and are not repeated here. There are no large-scale prospective randomized trials for any therapies discussed here. As such the evidence is generally of low or moderate quality and rests to some extent on expert opinion, values and preferences. Glucocorticoid replacement in CAH faces three particular challenges. First, it aims to replace

cortisol, but current treatment strategies cannot completely mimic the circadian rhythm of cortisol with a typical early morning rise of cortisol leading to a peak concentration at 6-8 AM and a nadir at midnight (330). Second, it aims to mimic the adaptation to stress (331). These distinctive features of physiological cortisol biosynthesis could only be closely mimicked by an infusion pump (332,333) which, however, is neither practical nor cost-effective and thus not routinely available. Third, it aims to restore negative feedback on pituitary ACTH drive thereby controlling adrenal androgen excess (334). Normalizing ACTH levels in CAH patients requires supraphysiological glucocorticoid doses compared to the mere replacement doses of cortisol required in other forms of adrenal insufficiency. Treatment in classic CAH therefore constantly struggles to prevent overtreatment with multiple adverse side effects on growth and on metabolic, cardiovascular and bone health, or undertreatment, which carries risks life-threatening adrenal crises and virilization or, in children, accelerated skeletal maturation with consequently reduced adult height. Both over- and undertreatment also affect reproductive function in both sexes.

1. Treatment in the neonatal period and early infancy

In growing children with classic CAH, the preferred glucocorticoid is the synthetic form of cortisol, hydrocortisone (HC), because its shorter half-life minimizes the adverse side effects typical of longer-acting, more potent glucocorticoids, especially growth suppression (134). As cortisone must be converted to cortisol for bioactivity (335,336) and differences in conversion rates may influence drug effectiveness, cortisone acetate is not recommended (337,338). HC should be given in 3 to 4 divided doses totaling 10-15 mg/m^2 daily (**Table 3**), a supraphysiological dose under which many patients show satisfactory control of adrenal androgen production. Although there are data to suggest that 4 daily doses are preferable (339), this may not be practical for many patients or their families. Data remain inconclusive regarding morning versus evening

dose-weighting (340,341). The total dosage should be individualized based on adequate monitoring (see below) and may need to be increased for short periods in certain circumstances; such increased needs are described below. Therefore, all children with CAH should be under the care of a pediatric endocrinologist (342).

In the neonatal period, some clinicians exceed the recommended glucocorticoid dose in order to reduce elevated androgen levels as quickly as possible. If this treatment strategy is adopted, more frequent monitoring is necessary to rapidly reduce the dose when target levels of monitored steroids are achieved, to avoid adverse effects of high doses of glucocorticoids (343). After a few months, maintenance daily totals of about 3-4 mg HC divided in 3 doses (i.e., 1-2 mg three times daily) are usually sufficient. Infants have low sensitivity to androgens, and completely suppressed adrenal androgens should not be the main goal in the first year of life. Commercial HC tablets, which may be extemporaneously crushed and mixed into food or suspended, are preferred as there have been reports of variable dose accuracies in compounded preparations (344,345). A suspension would be preferable for small children, but the commercial suspension was withdrawn 20 years ago owing to unreliable therapeutic effects, although in some countries reliable solutions are now available (346). An immediate-release granule formulation of hydrocortisone (Infacort®/ Alkindi®, Diurnal Ltd.) available in 0.5, 1, 2 and 5 mg preparations has been approved for use in the EU and USA (347,348).

Mineralocorticoid replacement is achieved with fludrocortisone. Monitoring is discussed in section V.A.5. Subclinical or overt aldosterone deficiency is present in all forms of classic CAH (349,350). Therefore, fludrocortisone is given to all newborns with classic CAH detected in neonatal screening programs even before hyponatremia develops. Due to relative mineralocorticoid resistance and the anti-mineralocorticoid effects of elevated 17OHP in this

965 period, neonates and young infants require higher fludrocortisone doses than older children,
966 typically 100-200 µg/day but occasionally more, divided in 1-2 oral doses. However, this treatment
967 requires frequent monitoring of electrolytes, plasma renin and blood pressure and tapering the
968 fludrocortisone dose in order to avoid iatrogenic hypertension. Because of a lower glomerular
969 filtration rate, immature renal tubules and the low sodium concentration in breast milk and infant
970 formula, infants often require additional supplemental sodium chloride to maintain sodium balance.
971 The recommended dosage is approximately 1-2 g (4-8 mEq/kg/d) NaCl given in divided doses
972 ideally using a standard saline solution (351) or crushed, aliquoted sodium chloride tablets. In
973 patients receiving high doses of fludrocortisone, NaCl supplementation may not be needed (352).
974 Moreover, 0.1 mg of fludrocortisone has the glucocorticoid potency of ~1 mg of hydrocortisone,
975 so high fludrocortisone doses may permit (or require) a reduction of the hydrocortisone dose in
976 young children.

977 2. **Treatment during childhood**

978 Children younger than 18 months should be monitored at least every 3 months, while older
979 children should be monitored every 4-6 months or more frequently after a change in dosing. The
980 suggested target 17OHP range is 100-1200 ng/dl (3-36 nmol/l) when measured in the early
981 morning before medication (337,338), and/or age-appropriate androstenedione levels. Attempts to
982 normalize 17OHP levels should be avoided because of the risk of HC overdosing causing
983 iatrogenic Cushing syndrome. Because prepubertal children normally have low circulating sex
984 steroid levels, adequate androgen suppression is important to achieve normal growth and puberty.
985 **Table 3** provides suggested dosing guidelines (134). HC dosing requirements may vary and
986 depend on differences in HC absorption and half-life (353). Long-acting glucocorticoids should
987 be avoided in growing children except for short intervals when necessary to restore hormonal

control, or if HC is unavailable (**Table 4)**. If used, care must be taken to avoid overdosing, which will suppress growth (354-356), and the dose should be decreased as quickly as possible once hormonal control is achieved.

Aldosterone deficiency is described in up to 75- 90% of all classic CAH patients, now viewed as a continuum of phenotypes rather than strict divisions between SV and SW disease. In classic CAH after infancy, fludrocortisone is usually given in doses ranging between 50-200 µg. Fludrocortisone has a biological half-life of approximately 18 – 36 hours. Therefore, low doses can be administered once a day, although doses above 0.1 mg may still be divided to be given twice daily. In hot and humid weather conditions, some endocrinologists suggest a seasonal increase in fludrocortisone, although increased salt intake may suffice. In contrast to glucocorticoid treatment fludrocortisone does not need to be increased during illness (Section V.A.6)

3. Treatment during puberty and adolescence

Puberty is often associated with difficult hormonal control, even if the replacement dose seems adequate and there is good adherence to the medication regimen. During puberty, the pharmacokinetics of HC may be altered by increased clearance due to decreased activity of 11β-HSD1. Therefore, higher glucocorticoid doses are necessary during puberty (357). However, as adult height of patients with CAH correlates negatively with the glucocorticoid dose administered in early puberty (356), HC doses exceeding 17 mg/m^2 per day should be used with care. Treatment should be continued with the lowest effective dose to achieve treatment goals, prioritizing height attainment over arbitrary steroid measurements. At or near the completion of growth, long-acting glucocorticoids might be considered but are not preferred.

Management of CAH during adolescence and the transition from pediatric to adult health care is challenging (358). CAH patients may have poorer health, beginning in adolescence and persisting into adulthood, highlighting the importance of this period in patient care (359). Multi-disciplinary transition clinics involving pediatric and adult endocrinologists, gynecologists, urologists and psychologists may promote good medical adherence among adults with CAH (134). Uninterrupted glucocorticoid and mineralocorticoid administration at the transition from adolescence to adulthood is required to prevent increased morbidity and mortality, particularly from adrenal crises. Treatment regimens should be reassessed and adapted to the recommendations for adult CAH patients. Importantly, mineralocorticoid requirements, which change from birth to adolescence, should be reassessed in adolescence/young adulthood to avoid mineralocorticoid over- and under-replacement (360). Transition, however, is more than just prescribing steroid replacement for primary adrenal insufficiency and must address sex- and gender-specific issues (361,362). In females, obesity and hyperandrogenism are common problems leading to menstrual irregularities and hirsutism (359). Gynecologic evaluation should be offered to all adolescents with CAH at transition, particularly in cases of blocked menstrual flow, planned penetrative vaginal intercourse or desired pregnancy (134,363). Boys should have a testicular ultrasound upon completion of puberty and regular examination for TARTs (359.). All patients should be aware of the risk of reduced fertility with poor medical adherence (134). Psychosexual and genetic counseling of the adolescent patient are strongly recommended during transition (364).

4. Treatment of adults

Treatment of adults with classic CAH aims to replace the missing cortisol and aldosterone, and ameliorate adrenal androgen excess (334). Optimal hormone replacement theoretically should enable normal quality of life and life expectancy. However, this aim is not always achieved, and

adults with classic CAH suffer from multiple disease-associated comorbidities (365-367), reduced health-related quality of life (360) and increased mortality (368).

The decision of which preparation to use for glucocorticoid treatment in adults with classic CAH is based on the clinical experience of the individual physician and on the needs of each patient (**Table 4**). In general, the lowest possible doses should be prescribed that minimize risk of adrenal crises and control androgen excess. HC is associated with better bone mineral density and better metabolic and cardiovascular outcome than dexamethasone in both sexes; prednisolone and prednisone have adverse effect profiles intermediate between HC and dexamethasone (366,369). Therefore, immediate-release HC remains the preferred option for glucocorticoid treatment in adulthood. Due to its short half-life of 4-6 hours, however, it needs to be taken 3-4 times per day and requires reliable adherence.

When patient adherence to a three times daily regimen is difficult, a twice daily regimen with prednisolone or prednisone (e.g. 1-5 mg per dose, for a total daily dose of 20-25% of the previous hydrocortisone dose) might be used instead (360,370,371). Prednisolone/prednisone also has been used for fertility induction when it might be more effective and can be continued throughout pregnancy (372). Dexamethasone (Dex) also effectively helps to establish regular menstrual cycles, but it is long-acting and associated with more adverse metabolic side effects (373). In contrast to prednisolone, prednisone or HC, dexamethasone traverses the placenta and therefore should be avoided during pregnancy (the use of Dex for prenatal treatment of a possibly affected fetus is discussed in Section V.C)(134,374-377). Due to its strong adrenal-suppressive effect, Dex is preferred in the treatment of TARTs (378,379). For this purpose, it needs to be given in supraphysiological doses, and should be given for short duration to avoid adverse metabolic effects such as weight gain, striae, edema and glucose intolerance.

Sustained-release HC preparations have been developed as an alternative to longer-acting synthetic corticosteroids such as prednisone/prednisolone or dexamethasone. A modified-release HC formulation Plenadren® (Shire Services BVBA, Belgium), is approved in Europe for treatment of adrenal insufficiency in adults. When given once daily to patients with primary adrenal insufficiency, it significantly improves metabolic variables such as body weight, body mass index (BMI) and HbA1c, compared to conventional hydrocortisone replacement of the same daily dose (380,381). However, data on its use in CAH patients are lacking. Clinical experience shows that once daily hydrocortisone therapy fails to control early morning rise of ACTH with subsequent excess of adrenal androgens in CAH, requiring an additional glucocorticoid dose in the evening. Excessive nighttime glucocorticoid administration has potential adverse metabolic consequences (371,382). Another modified-release preparation (Chronocort®, Diurnal, UK) addressing this CAH-specific challenge is currently under regulatory review for the treatment of CAH. It exerts a delayed (4 hours following intake) and sustained action (383). If taken at 2300 (11 PM), the delayed release mimics the overnight rise and following morning peak of cortisol (383,384). A second dose is given in the morning (7 AM) ensuring cortisol supply during the day. A phase III trial including 122 patients with classic CAH revealed superior hormonal control during the early morning and early afternoon compared to patients receiving standard glucocorticoid therapy (338,385).

5. **Monitoring**

Regular follow-up should include measurement of height, weight, blood pressure and physical examination. In children, special attention should be paid to accelerated or reduced height velocity, rapid weight gain, skin and mucosal hyperpigmentation, signs of virilization, pubic hair onset, development of apocrine odor and signs of central precocious puberty such as breast development

or testicular enlargement. Medical history concerning symptoms of salt craving, phases of unusual fatigue during the day, irregular menstrual cycles in girls and skin hyperpigmentation point to the need for medication titration **(Table 5).**

Laboratory monitoring traditionally relies on consistently timed serum 17OHP, androstenedione and plasma renin levels, whereas ACTH measurements are superfluous (**Table 6**). Plasma renin activity and direct renin levels are extremely variable and should be used along with standing blood pressure and electrolytes to titrate mineralocorticoid dosing (134,386). Other hormonal monitoring approaches have been suggested but are not yet used routinely. Adrenal-specific metabolites such as 21-deoxycortisol (387) and 11-oxygenated androgens (86) may provide more direct evidence for adrenal androgen production in CAH. Steroids can be measured in blood, urine (268,305), saliva (388) and dried filter-paper blood samples (389,390) and fluctuate with both the circadian rhythm and the timing of glucocorticoid intake (298,391-393).

Regular bone age X-rays in growing children beyond 2 years of age are helpful to detect unwanted bone age advancement as a result of cumulative exposure to excess adrenal androgens. The clinician should be alert to signs of central precocious puberty (e.g., testicular enlargement in boys, breast development in girls) because elevated adrenal androgens may activate the hypothalamic-pituitary-gonadal axis (134). The decision to adjust HC and fludrocortisone doses should consider clinical symptomatology and should not solely rely on laboratory data. Monitoring for reproductive complications are discussed below including reduced fertility in females (section 0), and TARTs in males (see section VI.A.1).

6. Management of adrenal emergency in CAH

The overwhelming majority of patients with CAH survive into adulthood, but with shortened life expectancy. Adrenal crises were responsible for 42% of deaths in 588 patients with CAH in a

Swedish population-based study; those with the SW form were especially at risk, as they had the lowest cortisol and aldosterone reserves (368). In a retrospective matched-cohort study in the UK, all-cause mortality rates were higher in patients with CAH, with a mean age at death of 54.8 years versus 72.8 years in controls (394). The incidence of adrenal crisis in adults with adrenal insufficiency is estimated to be 5–10 crises/100 patient years with a mortality rate of 0.5/100 patient years (395). Studies of children report similar findings. Two German studies estimated the incidence of adrenal crisis after the neonatal period to be 4.9-6.5 adrenal crises/100 patient years (396,397). In an American series, 55/155 children with SW CAH were hospitalized a total of 105 times over a 14-year period (398). In an Australian population-based study of children and adolescents with CAH, both hospital admission and the risk of adrenal crisis decreased with age (399). A large multicenter international study of 518 children from low-middle income as well as high income countries reported an adrenal crisis rate of 2.6/100 patient years (400).

Adrenal crisis is most often triggered by infectious illness (397,401,402). A population-based retrospective cohort study (drug prescriptions and clinical diagnoses) in the UK reported increased rates of infectious illnesses in patients with CAH (401). Gastrointestinal illnesses and upper respiratory tract illnesses are the most common precipitants of adrenal crises in both children and adults (397,398,401,402). Socioeconomic factors influence risk; in the USA, patients with government insurance (reflecting low family income) were twice as likely to be hospitalized as patients with commercial insurance (398). Pre-school children, adolescents, males and those with SW CAH, were more likely to experience sick days requiring stress dosing. Patients treated with higher glucocorticoid doses were less likely to suffer illness requiring stress dosing. The frequency of adrenal crises has decreased over time, perhaps due to greater awareness of this risk during sick days. None of the adrenal crises reported in a multicenter study were fatal (400).

Hypoglycemia can occur unexpectedly (396), may be associated with seizures, and can occasionally result in permanent neurologic sequelae, especially in children (402,403). Patients with SW CAH have adrenomedullary dysfunction with epinephrine deficiency (404) and this contributes to the risk of hypoglycemia especially in young children.

Protocols for the prevention and treatment of adrenal crisis are based on expert opinion and clinical experience (134,405,406). "Sick day rules" aim to prevent acute deterioration and a life-threatening adrenal crisis. However, the definition and reporting of sick days is more variable than that of adrenal crises, with evidence of systematic variation between centers (400). Adverse outcomes in children are related more to hypoglycemia than to electrolyte disturbances (396,402,407); thus, frequent intake of carbohydrates is important (402). Oral stress doses (2-3 times usual doses) of glucocorticoid cannot always prevent the progression to adrenal crisis and the occurrence of hypoglycemia (407,408). Increased HC doses are suggested with infectious illnesses (**Table 7**). Hydrocortisone sodium succinate for intramuscular injection should be prescribed with instructions for home use if oral medication is not tolerated during episodes of major stress (e.g., febrile illness with vomiting), especially for patients residing far from medical facilities. Once brought to emergency care, intravenous HC and isotonic fluids should be given. Continuous intravenous infusion of hydrocortisone sodium succinate might have a theoretical advantage over intermittent bolus administration because of lower variability and avoidance of regular troughs in plasma cortisol levels (385,410), but these two approaches have not been compared directly and clinical outcomes are likely similar. Stress dosing is indicated for pregnant women in active labor, similar to that used in major surgical stress (405). Stress dosing is not recommended for everyday mental and emotional stress, minor illness or before routine exercise (134,405,406). Serum cortisol did not exceed 10μg/dL (276 nmol/L) in healthy children

undergoing minor surgical procedures; therefore, stress dosing for minor procedures (e.g. brief medical or dental procedures performed under local anesthetic with or without light sedation) should be individualized (249,411).

Approximately one-third of patients with NC CAH have mild but clinically silent cortisol impairment (412,413) and the risk of adrenal crisis is unknown. Adrenal crisis has only been reported in NC CAH patients receiving glucocorticoid therapy in the setting of iatrogenic tertiary hypothalamic-pituitary-adrenal axis suppression (134,402). Thus, stress dosing for the prevention of adrenal crisis is recommended for glucocorticoid-treated patients with NC CAH. The Endocrine Society Clinical Practice Guideline (134) suggests HC stress dosing in the case of severe illness, major surgery, major trauma or childbirth for untreated individuals with a suboptimal ACTH test (in adults, cortisol below 14-18 µg/dL, <400–500 nmol/l).

In general, prevention of adrenal crisis in patients with known adrenal insufficiency is best accomplished through repeated structured patient education regarding "sick day rules" (414,415). All patients should wear medical alert identification tags or have an emergency card (and/or emergency information on their mobile phones) indicating adrenal insufficiency. A medical card developed by the European Society of Endocrinology is downloadable and includes guidance for healthcare providers as well (https://adrenals.eu/emergency-card/). A UK version including a QR code rapidly linking emergency personnel to instructions on adrenal crisis treatment is available (https://www.endocrinology.org/media/3652/steroid-nhs-card.jpg).

B. Treatment of NC CAH

In NC CAH the estimated residual enzymatic activity of CYP21A2 is about 20 – 50% based on in vitro or in silico studies, resulting in a generally mild but highly variable phenotype

(212,217,416). In contrast to classic CAH, no general guidelines exist for the management and follow-up of these patients and the overall evidence of recommendations for NC CAH is low (134,342,417-419). Decisions about starting treatment should be individualized and based mainly on clinical symptoms; the Endocrine Society guidelines do not recommend routine treatment with glucocorticoid in asymptomatic individuals (134). The general treatment goals in children are to maintain normal growth and pubertal development and to minimize risk of therapies; children should be regularly monitored clinically for height, weight, signs of androgen excess, puberty and bone age advancement (420).

When glucocorticoid treatment is required, HC is preferred, as with classic CAH. Patients receiving glucocorticoid therapy require stress-dosing per guidelines (see Section V.A.6). Mineralocorticoid supplementation with fludrocortisone is not required.

1. Growth

In contrast to untreated children with SV CAH, children with NC CAH may not have increased growth velocity although bone age maturation can be accelerated, potentially leading to reduced adult height (421,422). However, most studies describe nearly normal adult height in NC CAH patients (417,423-425). Glucocorticoid treatment should be reserved for patients who suffer from androgen excess, although criteria for deciding when symptomatology warrants treatment are not well defined. Supraphysiological dosages of glucocorticoids similar to those used to treat classic CAH patients may be necessary to suppress adrenal androgen production (426). Treatment will suppress the hypothalamic-pituitary-adrenal axis requiring stress dosing in case of illness. In many cases glucocorticoid treatment may be discontinued after reaching adult height, if the individual is otherwise asymptomatic (134). Adverse effects such as excess weight gain may make continued glucocorticoid treatment less desirable.

2. Puberty

Children with NC CAH can present with signs of increased adrenal androgen production such as premature pubarche, acne, mild hirsutism and menstrual disturbances that can progress over time (427), but in contrast to classic CAH, central precocious puberty is infrequently observed (37). Glucocorticoids can lower adrenal androgen concentrations ameliorating signs of hyperandrogenism, but prolonged glucocorticoid treatment may have long term adverse effects. Alternative treatment options in adolescent and young adult females to induce menstrual cycles and improve acne and hirsutism include oral contraceptives containing progestins with low androgenic activity such as desogestrel (428). Antiandrogens can be considered as an add-on for patient-important hirsutism that persists despite oral contraceptives (see the next section).

3. NC CAH in adult women

Most patients diagnosed with NC CAH are females suffering from mild adrenal androgen excess without clinically relevant deficiencies of gluco- and mineralocorticoids (134,429). Typical symptoms in affected women are hirsutism, oligo- and amenorrhea, acne, alopecia and sub- or infertility (427). Sometimes the diagnosis is made within the course of evaluation for adrenal incidentalomas (430-432). The main treatment goal is to reduce adrenal androgens and symptoms of androgen excess. Clinical studies comparing different treatment approaches in adults with NC CAH are lacking; treatment should only be started in symptomatic patients desiring treatment (134). The risks, benefits and effectiveness of various treatment options should be discussed. Fertility and childbearing in women with NC CAH are discussed in section VI.B).

4. Additional treatments for signs of androgen excess

Hirsutism is the most prevalent symptom in women with NC CAH, but also the most difficult to treat (433). Clinical experience suggests that a combination of oral contraceptives, topical eflornithine and cosmetic treatment (shaving, chemical depilatories, plucking, tweezing, threading, waxing or epilation therapy, electrolysis and intense pulsed light) might be the most effective treatment approach (434). For the treatment of acne and androgenic alopecia a dermatologist should be consulted.

Oral contraceptives act on the production, transport (increase of sex hormone binding globulin) and action of androgens. Anti-androgenic oral contraceptives containing cyproterone acetate, chlormadinone acetate, dienogest, or drospirenone effectively reduce androgenic symptoms. If hirsutism is the leading symptom, oral contraceptives are the preferred treatment (134). One randomized study in 30 women with NC CAH found cyproterone acetate to be more effective than hydrocortisone for isolated hirsutism (435).

Spironolactone, flutamide and finasteride can be used to treat hirsutism (433), acne and androgenic alopecia (436) but are teratogenic and not approved for this use. Eflornithine hydrochloride cream is used as topical therapy for facial hirsutism (433). It prevents hair growth by inhibiting the anagen phase of hair production. Eflornithine irreversibly binds to ornithine decarboxylase and thus prevents the natural substrate, ornithine, from accessing the active site. It is most effective when combined with physical means of hair removal, such as topical lasers.

5. Treatment of adult men with NC CAH

As androgen production in the testis far outweighs adrenal androgen production, men generally do not experience symptoms of androgen excess requiring treatment, and therefore remain undiagnosed. In rare cases, severe acne, reduced fertility or adrenal incidentaloma lead to the

diagnosis of NC CAH in men (429). TARTs are rare in men with NC CAH (437-439). Therefore, routine scrotal ultrasound is not recommended in NC CAH males.

C. Prenatal treatment

1. **Purpose**

Since the mid-1980's, prenatal treatment with high doses of Dex has been proposed for to pregnant women with a fetus at risk for classic CAH using a treatment protocol of 20 μg/kg/day, maximum 1.5 mg/day, with the aim of preventing prenatal virilization of the external genitalia in affected girls (313,317,440). The treatment is effective in ameliorating virilization of the external genitalia if started by gestational week 6-7 (441); in most centers this is before a fetal diagnosis can be made (see section IV.D). If prenatal diagnosis, most often by chorionic villus biopsy obtained in week 10-11, shows that the fetus is a girl with classic CAH, the treatment is continued until term, but otherwise stopped. The treatment is controversial due to safety concerns (442). Risk-benefit assessments must consider that, on average, 8 pregnancies at risk for CAH must be treated for every affected female who might benefit from the treatment (443-446). Endocrine societies and others have stated that the treatment is experimental and should only be performed in centers taking part in long-term research studies of these treated pregnancies (134,328,375,377,447).

2. **Fetal safety**

There have not been randomized studies of prenatal dexamethasone (Dex) treatment, and so all discussions of adverse effects are based on animal or retrospective data. Dex is a pluripotent gene regulator and its introduction at a critical stage of embryonic development may impact much more than the developing hypothalamic-pituitary-adrenal axis. Numerous studies (**Table 8**) have delineated adverse outcomes affecting brain, cardiovascular, renal, reproductive, thyroid and

metabolic functions in non-human mammalian species exposed to glucocorticoids in utero (reviewed in (375)).

With respect to human teratogenicity, systematic review and meta-analysis found an odds ratio of 1.41 (95% confidence interval 1.14-1.74) for cleft lip and palate in case-control series of infants whose mothers were treated with glucocorticoids in the first trimester (468). Even when exposed later in gestation, multiple doses of antenatal steroids for preterm labor increased the number of infants with birth weight < 10^{th} percentile and the risk for cerebral palsy (469). Among pregnancies at risk for CAH, prenatally Dex-treated newborns have lower, but nominally normal, birth weights compared to untreated controls (470); the decrease averages ~400 g (471). Other adverse events including failure to thrive, stroke-like events and midline defects have been observed in both short-term and full-term treated cases at risk for CAH (472-474).

Prenatal Dex treatment has shown inconsistent long-term effects on cognition and behavior (**Table 9**). One study showed no cognitive differences but increased shyness and emotionality in treated children (475). A larger follow-up study from the same group of 126 non-CAH and 48 with CAH short-term exposed children and 8 girls with CAH treated until term did not show any effects on motor, cognitive, and social development or scholastic competence using parental questionnaires (476). In a later report including two different age groups (5-12 and 11-24 years) using neuropsychological testing, there were no significant findings in children without CAH (480). Swedish studies of healthy non-CAH children exposed to Dex only during the first trimester have shown negative effects on cognition, especially verbal working memory (477); a sexually dimorphic effect with a more pronounced negative effect on working memory and executive function was observed in the girls (483). A follow-up with a second neuropsychological testing in

a subgroup of the cohort as adults showed less pronounced effects indicating a possibility for compensatory mechanisms over time (485).

In two cohorts of girls with CAH treated throughout gestation, neurocognitive outcomes were negatively affected for mental processing and spatial memory (480) and broad deficits were found in most measures of cognition (488). In contrast, a Polish study reported better cognitive results in general in 9 girls with CAH who were treated throughout pregnancy, but 8 unaffected girls who had been treated with Dex had worse results than controls (481).

Possible imprinting effects of prenatal exposure to Dex have only begun to be explored. Differences in DNA methylation in peripheral CD4+ T-cells seemed to be related to sex (485). Of particular interest were methylation effects of the genes *BDNF* and *FKBP5*, relevant for the development of the central nervous system, and *NR3C1* encoding the glucocorticoid receptor. There were also associations between DNA methylation and performance on cognitive tasks.

Moreover, first trimester Dex exposure of non-CAH fetuses is associated with differences in brain morphology (489). MRI studies in adults showed enlargement of the amygdala, increased left superior frontal gyrus, and widespread white matter changes. The pathophysiology behind the observed neuropsychological effects of early Dex exposure are largely unknown. Infants prenatally exposed to betamethasone have altered responses of the HPA axis and a higher incidence of mental and behavioral disorders (490,491).

Negative effects on glucose and lipid metabolism in childhood and in young adulthood have been reported in individuals without CAH but exposed to Dex during the first trimester. Lower insulin secretion, followed by lower glucagon secretion was reported in a French study (486). A lower HOMA-β was reported in the Swedish cohort, significant in girls but not in boys. Plasma glucose levels were higher in the younger treated group with no sex difference. In older adolescents

and young adults, total cholesterol and LDL cholesterol were higher in the treated individuals (487). It is unknown if this implies an increased risk of developing metabolic syndrome later in life.

In order to minimize the exposure to Dex, efforts have been made to develop diagnostic techniques using cell free fetal DNA in maternal blood samples, but these are not yet routinely available (see section IV.D)(325). Dose adjustments, with lower doses during the later phases of pregnancy, have been discussed (158) but such studies have not been reported.

D. New medical strategies (Figure 5)

The treatment goals for classic CAH include both hormonal replacement and reducing adrenal androgen production. Glucocorticoid therapy is used to achieve both goals, but normalizing adrenal androgen production requires supraphysiologic doses that are higher than required to replace the cortisol deficiency, contributing to comorbidities. Modified and delayed-release HC formulations were discussed in Section V.A.4 (492). Continuous subcutaneous delivery of HC is also suitable for mimicking physiologic cortisol secretion patterns and is useful in patients with rapid cortisol metabolism or impaired gut absorption (493), but this approach is less practical than oral drugs for widespread chronic use.

Alternatively, medications that lower androgen production and/or action can be added to physiologic glucocorticoid therapy, similar to doses used to treat primary adrenal insufficiency. The combination of testolactone (an aromatase inhibitor) and flutamide (an androgen receptor antagonist) with 8 mg/m^2/d HC normalized growth and bone maturation in a 2-year randomized trial of 28 children (494). A long-term study of this combination to determine the efficacy of this regimen on improving adult height will soon be completed (NCT00001521). Abiraterone acetate is a potent CYP17A1 inhibitor used to treat prostate cancer (495) When added to HC 20 mg/d, 6

days of treatment with 100-250 mg/d of abiraterone acetate normalized androstenedione in 6 adult women (496) with parallel reductions in testosterone, androgen metabolites, and 11-oxo-androgens (497). Abiraterone acetate therapy can cause DOC accumulation and consequent hypertension and/or hypokalemia in patients with prostate cancer via CYP21A2-mediated 21-hydroxylation of intra-adrenal progesterone (497), however, this conversion cannot occur in patients with classic CAH (497). Abiraterone acetate is likely to be most useful in prepubertal children with classic CAH to suppress androgens and estrogens until the anticipated age of puberty, and a phase I trial testing this approach is underway (NCT02574910). Abiraterone acetate monotherapy might cause DOC accumulation in patients with NC CAH if not combined with glucocorticoid therapy or a mineralocorticoid receptor antagonist. Moreover, its use in pubertal girls would require concomitant estrogen treatment, for example with oral contraceptive pills. Third-generation anti-androgens such as enzalutamide, apalutamide, and darolutamide have not been tested in CAH patients but also might be useful treatments in women of reproductive age willing to use contraception.

Agents that reduce the ACTH-mediated drive for androgen production are possible approaches. The binding of corticotropin-releasing hormone to its type 1 receptor (CRHR1) is a major input to corticotropes, raising intracellular cyclic AMP and stimulating ACTH secretion. A single-dose, fixed-sequence study of 8 women given 300 or 600 mg of the CRHR1 antagonist NBI77860 at 2200 (10 PM) showed significant reductions in ACTH and 17OHP over the ensuing 16 hours relative to a control period during which glucocorticoid treatment was withheld (498). The CRHR1 antagonists tildacerfont and crinecerfont were tested in 14-day continuous-dosing trials (NCT03257462 and NCT04045145, respectively), and tildacerfont therapy was extended in a 3-month trial (NCT03687242). Peer-reviewed results are unavailable as yet. Additional trials are

required to further assess the long-term benefits of these treatments. Theoretically, an anti-ACTH antibody (499) or an antagonist of the melanocortin type 2 receptor (MC2R, the ACTH receptor) (500) might also reduce adrenal androgen synthesis in patients with classic CAH, but these approaches have only been tested in preclinical models (501). It should be kept in mind that most of these approaches do not eliminate the need to treat with, and monitor adequacy of, glucocorticoid replacement, albeit perhaps in lower doses.

Unilateral or bilateral adrenalectomy has been suggested as an approach to long-term management of classic CAH to limit adrenal androgens (502). A recent meta-analysis of 48 CAH cases, 34 (71%) described symptomatic improvement after bilateral adrenalectomy but with 34 cases (10%) reporting short-term and 13 cases (27%) long-term adverse outcomes, including an increased risk of adrenal crisis (503). The subsequent development of adrenal rest tumors due to elevated ACTH levels even in women has been reported (504,505), which defeats the purpose and allows the recrudescence of androgen excess. Consequently, this approach has fallen out of favor (134). Adrenolytic therapy with mitotane has been reported in men with TARTs as an approach to restore fertility (506), but long-term outcomes have not been published. The adrenolytic drug nevanimibe was testing in a dose-escalation study of 14-day treatment periods interrupted with 14-day placebo periods, up to 1000 mg twice daily (507). The median 17OHP was consistently lower in treatment periods and rose during placebo periods, consistent with a reversible effect on steroidogenesis, but only 20% met the primary endpoint (17OHP ≤2x upper limit of normal). A study using longer treatment periods in order to achieve greater and more sustained reductions in adrenal-derived androgens was initiated (NCT03669549) but terminated after an interim analysis (https://clinicaltrials.gov/ct2/show/NCT03669549, accessed 22 Dec 2020). Thus current data do not support the approach of "medical adrenalectomy".

Growth hormone has been used to improve height in children with CAH (508-510). Growth hormone treatment for a mean duration of 5.6 years achieved nearly-adult height in 34 children with CAH (12 NC-CAH patients). In some of the patients, GnRH analogue was also used to delay puberty (509). Controlled studies in larger groups of patients are lacking. Therefore, growth hormone, with or without GnRH analogue therapy, cannot be generally recommended as adjunctive therapy.

E. Novel cell- and gene- based therapies

1. Potential cell-based therapies for CAH

Cellular reprogramming is the process whereby a fully differentiated, specialized cell type is forced to acquire a different phenotype that it would not reach under normal physiological conditions. Somatic cells can be induced to de-differentiate to an Embryonic Stem Cell (ESC)-like phenotype by forcing the expression of specific transcription factors; these cells, termed Inducible Pluripotent Stem Cells (iPSCs), are donor-specific and phenotypically highly similar to ESCs (511). An example of cell therapy used ESCs- and iPSCs-derived pancreatic beta cells for potential treatment for type 1 diabetes (512).

An alternative strategy for reprogramming somatic cells without an intermediate state (iPSCs) is through lineage-conversion (also known as direct reprogramming or transdifferentiation), which entails the forced expression of lineage-determining transcription factors (513). Various human and mouse cell types have been used for lineage conversion to an adrenocortical phenotype (514). Adrenocortical-like cells have also been established from cells derived from human skin, blood and urine cells in humans using a combination of steroidogenic factor-1 (SF-1, NR5A1) expression (through lentiviral delivery) and activation of Protein Kinase-A (PKA) and GnRH

pathways (515). These reprogrammed cells displayed ultrastructural features resembling steroid-secreting cells (such as larger mitochondria with a densely packed inner mitochondrial membrane), *de novo* expressed steroidogenic enzymes and secreted steroid hormones in response to physiologic (such as ACTH) and pharmacologic (such as non-degradable cyclic AMP-dependent PKA activators) stimuli. They are also viable when transplanted into the mouse kidney capsule or intra-adrenally. Importantly, the hypocortisolism observed in cells reprogrammed from epithelial cells recovered from the urine of patients with CAH (due to mutations in *CYP21A2*, *STAR*, *HSD3B2* and *CYP11A1*) was rescued by expressing the wild-type version of the defective enzyme. These studies model CAH in a dish to test personalized interventions. In the future, one could attempt to apply gene-editing on cells reprogrammed from patients to achieve normal steroidogenesis. The same approach could be employed *in vivo*, through delivery of gene-editing tools to the adrenal with viral vectors. Other, as yet untested, methodologies include establishment of adrenocortical-like cells from iPSCs and adrenocortical organoids capable of self-renewal.

2. Potential gene-based therapies for CAH

Gene therapy using Adeno-Associated Viruses (AAVs) is an alternative option, tested in an animal model of 21OHD. The active murine gene is named *Cyp21a1*, while the duplicated pseudogene is *Cyp21a2-p*. Mice bearing a deletion of approximately 80 kilobases of chromosome 17 (516), including the *Cyp21* locus showed perinatal lethality, elevated ACTH, cortical hyperplasia with lack of proper zonation, accumulation of steroid precursors and both glucocorticoid and mineralocorticoid deficiency (517). Intra-adrenal injection of AAVs carrying human *CYP21A2* reverted the CAH-like phenotype for 40 days. A drawback of AAVs is their induction of an inflammatory response, but adrenals of mice treated with gene therapy did not show active inflammation, possibly due to high intra-adrenal levels of glucocorticoids. (517).

Restoration of adrenocortical function in *Cyp21a1*-null mice was also achieved through AAV-mediated delivery of murine *Cyp21a1* to the thigh muscles, suggesting that functional 21-hydroxylase enzymatic activity does not have to be confined in the adrenal (518). Intravenous injections of AAVrh10-CAG-human CYP21A2-HA vector endowed with adrenocortical tropism efficiently restored near-normal adrenal function; cells in the zona fasciculata, but not in the zona glomerulosa or capsule, were efficiently transduced two weeks after a single AAV injection, and this was concomitant with a reduction of progesterone and ACTH levels (519). However, the restoration of proper steroidogenesis was only transient. A likely explanation of such phenomenon lies within the biology of the gland; through the use of specific transgenic mouse model (lineage tracing) we now know that the adrenal cortex undergoes a self-renewal process, and key paracrine effectors supporting a dynamic centripetal streaming of adrenocortical cells have been identified (520). Adrenocortical self-renewal relies on the differentiation of at least two cell populations of progenitor cells, located in capsular (expressing the transcription factor Gli1) and subcapsular compartments (secreting the morphogen Sonic Hedgehog, Shh). These two cell populations are able to differentiate and become fully mature steroidogenic cells forming the distinct histological and functional layers of the zona glomerulosa and zona fasciculata. If *Cyp21*--AAVs are not able to transduce adrenocortical stem/progenitor cells, as suggested from the studies cited above, newly formed steroidogenic cells will therefore be *Cyp21a1*-deficient and mice revert to a CAH phenotype. In the future, it will be important to determine AAVs serotypes that are able to efficiently transduce stem/progenitor cells in order to offer a long-term curative solution.

In considering the applicability of animal models for gene therapy of 21OHD, it must be kept in mind that mice do not express Cyp17a1 in their adrenal glands and consequently cannot synthesize sex steroid precursors in the adrenals. Thus, mice cannot be used to model the efficacy

of suppression of adrenal androgen secretion with gene therapy. Moreover, enzyme kinetics suggest that extra-adrenal expression of CYP21A2 using gene therapy is likely to produce adequate amounts of cortisol only with very high levels of precursor steroids, which means that this approach will be of limited utility in controlling adrenal androgen secretion in humans.

F. Psychological risk factors, surveillance, and intervention

Historically, psychological studies in CAH have emphasized the role of prenatal androgens on gender development (i.e., gender identity, gender role, and sexual orientation) and other domains exhibiting sex-related variability (e.g., cognitive abilities) (521-524). Most studies only concern females with classic CAH, as only they provide opportunities to test hormonal hypotheses of gender development (525). However, this emphasis may promote the belief that atypical gender behavior or non-heterosexual attractions are causes for clinical concern to the extent that they are linked to the pathophysiology of CAH. Historically it has been assumed that men with CAH require little attention directed to their mental health because prenatal androgen exposure is typical of males.

Advances in therapeutics contribute to a disease-specific ("categorical") approach to care (526,527). In CAH, this approach has facilitated a fuller understanding of its genetics and pathophysiology and refinement of medical and surgical interventions (134,528). A disease-specific approach also emphasizes the psychosexual aspects of CAH in affected females (522). Yet, a substantial body of evidence suggests that successful developmental trajectories in people with chronic medical conditions are influenced as much by the psychosocial environment, supports, and organization of healthcare delivery as by the specific nature of the person's medical condition (526,527). A more generic (or "noncategorical") approach emphasizes the effects of repeated

hospitalizations on the person's psychosocial adaptation, irrespective of whether the hospitalizations were for asthma or CAH. Relatively neglected topics in CAH are those routinely addressed in more prevalent conditions, including effects on parenting and family, factors influencing adherence to the medical regimen, frequent doctor visits, impact on the person's body- and self-image, and transition from pediatric to adult healthcare (**Table 10**).

1. Generic (or noncategorical) factors

Parental reactions to learning that their child has a serious and chronic medical condition – expressed as shock, panic, worry, and sometimes feelings of guilt – are common generic stressors (593). The mental health of patients with CAH is another example; although most studies focus on females, increased psychiatric symptomatology in both sexes mirrors observations for a wide range of chronic medical conditions (547,594-597).

2. CAH-specific (or categorical) factors

Patient reactions to repeated genital examinations potentially threaten mental health and well-being (598). Apart from the effects of prenatal androgens on female reproductive anatomy, the influence of early androgen exposure on brain and gender development garners significant attention. Prenatal exposure to testosterone increases the expression of behaviors and interests more typical of males than females. The largest differences between females with CAH and unaffected females are observed for childhood toy preferences and adolescent and adult hobbies and interests. The majority of girls and women with CAH experience a female binary gender identity (578), yet there is evidence that the strength of that identity may be reduced (577,599). Although the sexual orientation of women with CAH is less likely to be exclusively heterosexual than is true for unaffected women, the majority are heterosexual (227,600). Though prenatal

androgen exposure may play a role in the development of these outcomes, its influence is much smaller than effects on gender-role behavior (523,596,599,601).

3. Psychological assessment and interventions

In the general pediatric population, the base rate for having a psychiatric disorder at any time is about 20% (602), and is similar in European adults (603,604), yet many with mental health problems are neither identified nor referred for specialized treatment (605,606). Specialists treating patients with CAH should consider that many of their patients (and/or their caregivers) may be struggling with mental health problems which can impact the effectiveness of medical care provided. Consequently, regular screening of patient (and family) for risk and resilience factors are indicated along with evaluating the developmental, behavioral, emotional, social, and educational status of the patient as part of ongoing clinical care. Pediatric assessments should also encompass self-perceptions of domain-specific competencies, body image, and experiences of gender typicality and contentedness (607). Comparable surveillance in adulthood is recommended (360). Psychosocial screening should be both general (psychiatric symptoms, coping with illness) and specific (negative body image related to challenges of endocrine management, anticipated or experienced stigma, distress over non-heterosexual interests or behaviors, avoidance of potential romantic relationships as maladaptive coping strategy, sexual dysfunction potentially related to genital surgery, and fertility concerns). Adult healthcare providers need to be comfortable in assessing these topics and refer to knowledgeable specialists who understand the psychological issues in CAH. A recommendation to connect with peer support can also be extremely useful although careful consideration of where to direct patients is warranted (580). There is specific guidance for clinicians regarding the psychological aspects of CAH that warrant evaluation and possible intervention (524).

Because optimal care in CAH involves multiple subspecialties, it is recommended that clinical services be comprehensive and integrated (342,608,609), but inclusion of medical psychologists in interdisciplinary healthcare teams for CAH is inconsistent. There are no mental health interventions specifically designed for CAH. Psychoeducational counseling that includes detailed discussion of CAH with the patient and caregivers should be provided in an iterative and developmentally-sensitive manner. For girls with genital virilization, such counseling necessarily involves education regarding the process of sex development and the influence of excess androgens on genital growth. A recent Cochrane review of psychological interventions for parents of youth with chronic illness provides clinicians with evidence-based strategies for managing parenting challenges and enhancing psychosocial adaptation in both the parent and the child (610). Interventions to promote treatment adherence in other chronic conditions should be transferable to CAH (611). Although preparation for and assessment of readiness for transition from pediatric to adult care (612,613) does not guarantee physical health and well-being in adulthood, reports of major morbidities in adult patients with CAH (360) warrant continued efforts to improve outcomes.

G. Urogenital surgery

1. Decisions concerning feminizing surgery

Most girls with classic CAH are born with virilized external genitalia. Virilization may consist of fusion of the outer labia, a single opening of a common urogenital sinus, a recessed vagina that enters into the common channel and clitoromegaly. The degree of virilization is variable, and is influenced by the severity of the enzymatic defect. To indicate the severity of virilization the Prader classification, or similar scales, may be used (**Figure 4**). These anatomical variations affect the

decisions regarding surgery: the timing, one or two-stage surgery, the technique and the extensiveness of the procedure and risk for complications (614).

Feminizing surgery is often performed in early infancy/childhood in order to provide a female appearance of the genitalia in childhood, and to enable sexual intercourse in adult life. This complex surgery may lead to short- and long-term complications. Early surgery for girls with CAH has become controversial. Many surgeons prefer complete surgical repair at an early age because of good elasticity of the tissue, prevention of possible hydrometrocolpos and reduction of parents' distress (615). However, concerns have been raised regarding body integrity and the inability of children to provide informed consent for early surgery. Unsatisfactory outcomes regarding genital sensation and sexual function, and greater acceptance of gender non-binary status have led some to advocate that surgery be postponed until patients can express their gender and wishes (616). However, the effects of growing up with atypical genitalia on mental health or on sexual satisfaction are unknown and may vary in different cultures. The majority of patients and their parents in an American survey endorsed early reconstructive surgery (617). Families should be informed about surgical options including avoiding or delaying surgery. There should be a shared decision-making process including the family, endocrinologist, surgeon and mental health professionals, and the surgery should be performed by an experienced surgeon (134,581,618,619).

2. Surgical techniques, outcomes and complications

Feminizing genitoplasty involves clitoroplasty, opening of the vaginal introitus and labioplasty. When the patient has a high urethra-vaginal confluence, vaginoplasty may be postponed to later in life. Surgical techniques have been adjusted for best preservation of clitoral sensitivity and least vaginal stenosis (620). However, functional results of the current techniques can only be evaluated after many years.

In tandem with the Endocrine Society's 2018 Guideline, a systematic review and meta-analysis found no data to support one approach over another (528). The data included 29 observational studies (1,178 CAH women, mean age at the time of surgery 2.7 ± 4.7 years, mostly classic CAH). After an average follow-up of 10.3 years, the majority who underwent surgery had a female gender identity (88.7%) and were heterosexual (76.2%). Women who underwent surgery reported a lower than optimal Female Sexual Function Index Score of 25.13 out of a maximum possible score of 36, with 26 being the threshold accepted for risk for sexual dysfunction (621). Many patients reported impairment of clitoral sensitivity (622,623), uncomfortable vaginal penetrative intercourse, and low frequency of intercourse (21). The majority of patients (79.4%) and treating healthcare professionals (71.8%) were satisfied with the surgical outcomes. The most common clinical finding was vaginal stenosis, whereas other surgical complications, such as fistulas, urinary incontinence and urinary tract infections, were less common (624). Data on quality of life were sparse and inconclusive. To date there are no systematic prospective studies documenting outcomes in girls and women with CAH who did not undergo urogenital reconstruction; until recently most non-operated girls have been those who were only mildly virilized. Reoperations are usually much less extensive surgical procedures than the initial genitoplasty. Most consist of widening the vaginal introitus; clitoroplasty has also been performed.

There are no studies comparing different techniques of feminizing surgery nor studies comparing early versus late surgery (528). The Endocrine Society's Guideline (134) cites urogenital mobilization with or without neurovascular-sparing clitoroplasty as the techniques now preferred by many surgeons. No evidence-based guidelines for surgical management exist, and further long-term follow-up studies are needed.

VI. LONG TERM SEQUELAE

A. Gonadal function in males

In men with CAH, gonadal and reproductive function are often impaired due to primary gonadal failure from TARTs and/or secondary gonadal failure due to suppressed hypothalamic-pituitary-gonadal axis as a consequence of high adrenal androgen concentrations (378,437,438,625).

1. Testicular adrenal rest tumors (TARTs)

TARTs are benign testicular tumors typically found in males with classic CAH (378,437). TARTs have histological similarities to adrenocortical cells and are believed to originate from aberrant adrenal like cells in the testes but the etiology is not yet fully understood (378). TARTs are usually bilateral (70-100% of the cases) and painless (129,437,438,626-631), but discomfort can occur, especially in patients with extensive tumors (632). TARTs less than 2 cm diameter are difficult to detect by palpation (437). Both ultrasound and MRI can be used to detect/confirm TARTs with similar sensitivity down to a few millimeters, but ultrasound costs less (439,630,633). The reported prevalence of TARTs in CAH ranges from 14% to 86% (437,634), with an average of 25% in adolescents, and 46% in men (378). TARTs are found occasionally in patients with NC CAH (437,439). They occur not only in 21OHD but also in 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase type 2 deficiencies (635,636).

Elevated ACTH concentrations may play an important role in the development of TARTs. Suppression of ACTH secretion by increased doses of glucocorticoid can decrease the size of TARTs in some cases and may restore fertility (637-639). However, TARTs also occur in well-controlled patients and only a few studies have found a clear association between hormonal control,

and TARTs (437,640,641). Moreover, there seems to be no correlation between TARTs and bilateral adrenalectomy, a condition that usually leads to high ACTH concentrations (642,643).

It is important to discriminate Leydig cell tumors from TARTs, due to the malignant potential of Leydig cell tumors, but this cannot be done by either palpation or imaging (632,644). TARTs are usually bilateral whereas Leydig cell tumors are mostly unilateral and often produce estrogens (378). TART size may decrease in some cases after intensified glucocorticoid dosing (588,632,637-639). Additionally, characteristic histologic structures called Reinke crystalloids can sometimes be found in Leydig cell tumors but never in TARTs (378,632,633).. Furthermore, Leydig cell tumors are very rare in CAH while TARTs are very common.

The central location of TARTs in the testes may result in mechanical obstruction of the seminiferous tubules with azoospermia and irreversible peritubular fibrosis (645). Moreover, the paracrine effects of steroids produced by TARTs may destroy the surrounding Sertoli or germ cells (378). Testis sparing surgery has been described in TARTs but usually does not improve gonadal function, probably owing to irreversible damage to the testis (637). Regular testicular ultrasound is recommended (every 2-5 years if TARTs are small and stable) and if an increased TART burden is found, glucocorticoid therapy should be optimized and cryopreservation of sperm offered (437).

2. Secondary gonadal failure

Poor hormonal control in CAH results in increased risk of hypogonadotropic hypogonadism (437,626), because high levels of adrenal androgen precursors will be aromatized to estrogens and suppress the hypothalamic-pituitary-gonadal axis. Steroids produced by TARTs can also suppress gonadotropin secretion (438). Even though most males with CAH and secondary gonadal failure will compensate for reduced testicular testosterone production with increased adrenal testosterone, low testosterone levels are found in some patients (438,646). Overtreatment with glucocorticoids

in men with CAH may also induce gonadal failure (589); optimizing glucocorticoid therapy will usually reverse this.

3. Paternity

There are few controlled studies of fertility in men with CAH. In a Finnish study of 29 young men with classic CAH a child rate of 0.07 children per adult male was reported, which was significantly lower than the 0.34 in the entire Finnish male population with a similar age distribution (647). In a similar Swedish study of 30 men with CAH the child rate was 0.9 compared to 1.8 in the entire age-matched Swedish population (437). Of 30 US men with CAH only 7% had fathered children (638), and of 22 German men with CAH, 23% had children (589). Of 65 British men with CAH, 25% had become fathers, two after fertility treatment, but only 37% had tried to become fathers (360). Finally, of 219 French men with classic CAH, 24% had children (11% after IVF), and this fertility rate was lower than the national reference population (626). Men with CAH seem to be less sexually active than matched controls (368). However, of 221 Swedish men with CAH and 22,100 matched controls, only those born before neonatal screening had a reduced child rate (odds ratio 0.5, i.e. half as likely to have fathered a child), suggesting that fertility may not be reduced for most men with CAH in the future. Men with NC CAH had a normal child rate and of those who, irrespective of genotype or phenotype, had succeeded in having children, the number of offspring was similar to controls (590). Men with CAH adopted children more often (odds ratio 2.9) (590).

B. Reproductive function in women

CAH affects gonadal function and fertility in women. In general, there is an association between the severity of the CAH phenotype and the level of gonadal dysfunction and fertility(625).

1. Pubertal development

Age of menarche is normal in well-controlled girls, with no difference between SW, SV and NC CAH (34,356,372,425,648). However, when glucocorticoid therapy is withheld or inadequate, menarche is delayed (649). Irregular menstrual patterns in CAH are associated with other hyperandrogenic signs such as acne and hirsutism and signs of insulin resistance. This clinical picture closely resembles polycystic ovary syndrome (429). Sonographic findings of polycystic ovarian appearance have been reported in adult women with CAH (about 20-50%) and in a minority of adolescent patients (650-654). Breast development can be impaired in case of inadequate androgen control (649,655). The European multicenter dsd-LIFE study showed that only 68% of adults with CAH had reached Tanner stage B5 compared to 90% in women without DSD (656).

2. Fertility

Compared to age-matched controls, women with CAH have fewer pregnancies and children. In a Finnish study, the mean child rate was 0.34 versus 0.91 in the general Finnish female population, and was lower in SW compared to non-SW women (657). In a Swedish study, the number of pregnancies was 50% lower compared to age-matched controls (372); 16 of 19 women who attempted pregnancy succeeded in becoming pregnant and there was a clear relationship between more severe genotypes and fewer children. Of 106 CAH patients in the UK, 25 considered motherhood and 23 had actively attempted conception, of whom 21 achieved 34 pregnancies (591). The pregnancy rate in this subgroup was similar to that in the normal UK population (95%), and similar in the SW (88.9%) and non-SW (92.9%) subgroups. However, women with SW were less likely to seek motherhood. More recently, the dsd-LIFE study reported that only 14.7% of 221 CAH women had one or more children without assisted reproduction techniques (ART), and 1.9%

with ART (658). In a recent Swedish epidemiological study using the national CAH registry, 272 females with CAH (aged 14 years or above) were compared to 27,200 matched controls (659). Only 25.4% of women with CAH had given birth compared to 45.8% of controls. Furthermore, mothers with CAH were older and had fewer children.

All studies have emphasized that the major cause for low child rates is that women with CAH are less likely to seek motherhood. Women with the SW phenotype show the lowest interest in motherhood. This may be caused by the effects of prenatal androgen exposure on gender role behavior, including reduced interest in infants (592,660), the lack of a partner, dissatisfaction with genital appearance, decreased sexual satisfaction, and urogenital and sexual dysfunction as a result of corrective surgery (365). When patients attempt pregnancy, the success rate seems to have increased in the last twenty years, as a result of various factors, including increased understanding of the effect of androgen and progesterone levels, and the level of mineralocorticoid substitution (35).

3. Optimizing fertility in women

A large review of case reports of women with classic CAH included 159 pregnancies since 1999. In 84 pregnancies the mode of conception was reported, and 62/84 pregnancies were spontaneous (365). When pregnancy is attempted and especially when spontaneous conception fails, the first approach is to optimize glucocorticoid therapy, aiming at normal androgen and follicular-phase progesterone levels (591,661,662). Second, optimizing mineralocorticoid treatment appears to improve fertility SW and SV patients (365,591,663), but the exact mechanism remains unknown.

If needed, and especially when the above approaches are unsuccessful, assisted reproduction techniques can be used for ovulation induction and conception (664,665).

Most (53-68%) women with NC CAH conceive spontaneously without any treatment (650,666). Of 190 women with NC CAH, 95 wanted pregnancy and 187 pregnancies occurred in 85 women. Of these pregnancies, 99 occurred before the diagnosis of NC CAH (96/99 spontaneous), and 88 (47%) after the diagnosis (11/88 spontaneous) (650). Therefore, in case of subfertility (or recurrent miscarriages) there is a clear indication for temporary glucocorticoid treatment in NC CAH (37,134). Glucocorticoid treatment shortens the time to pregnancy from about one year to less than six months (667). If conception cannot be achieved with glucocorticoids, ovulation induction is usually successful. The course of pregnancy is usually uneventful; however, the miscarriage rate in women with NC CAH is substantially higher (25%) than in the general population (6%) in some (650,666) but not all (667) studies. The miscarriage rate can be reduced to normal in women treated with low to moderate doses of HC (650), prednisolone or prednisone (666) prior to and during pregnancy.

4. **Pregnancy outcome**

Pregnancy outcome is good in women with CAH (365). Placental aromatase activity protects the fetus from maternal androgens (101). Gestational diabetes has been described relatively frequently (372,659). Adjustments in glucocorticoid (and fludrocortisone) dose are usually necessary, especially in the third trimester (134), similar to pregnancies in women with primary adrenal insufficiency (668,669). In the offspring, the rate for small-for-gestational-age seems to be increased in some (365), but not all (659) studies, and no other problems are seen at follow-up (670).

C. Cardiovascular and metabolic morbidity

1. Metabolic consequences

The prevalence of overweight and obesity are greater in adults with CAH in the UK (360) and Sweden (671) but similar to the general population in the US (217) and France (626,672). Increased abdominal adiposity, with a higher proportion of pro-inflammatory visceral adipose tissue compared to subcutaneous adipose tissue, was present in adolescents and young adults with CAH compared to age-, sex- and BMI-matched controls (673). Metabolic syndrome was observed in nearly 20% of adults in the NIH's cross-sectional study cohort (217), associated with older age but not with androgens, glucocorticoid type, or dose. The Endocrine Society's systematic review of relevant literature published through early 2016 included 20 observational studies (14 longitudinal, 6 cross-sectional) with a moderate to high risk of bias (674). The average dose of glucocorticoids (in hydrocortisone equivalents) was 9-26.5 mg/m^2/day. In the meta-analysis (416 patients, 14 months-63 years old), compared to controls, individuals with CAH had increased values for the homeostatic model assessment of insulin resistance (HOMA-IR; weighted mean difference [WMD] 0.49; 95% CI 0.02-0.96), however, no differences were noted in fasting blood glucose, insulin level, glucose or insulin level after 2-hour glucose load, or serum lipids.

2. Blood pressure

Some studies report normal resting (217,626,675) and 24-hour blood pressure profiles (676) whereas others report a slight increase of either diurnal or both diurnal and nocturnal systolic blood pressure, compared to matched controls even in childhood (677,678). There are minimal data on the prevalence of hypertension in adults with CAH (679,680), with inconsistent results in individual studies conducted in different locales (626,671,681). The systematic review and meta-analysis (674) found that individuals with CAH had modestly increased systolic blood pressure

(WMD 4.44 mm Hg; 95% CI 3.26-5.63 mm Hg) and diastolic blood pressure (WMD 2.35 mm Hg; 95% CI 0.49-4.20 mm Hg). The authors were unable to draw conclusions regarding the effects of several important variables such as sex, glucocorticoid type and dose, fludrocortisone dose, and genotype, and bias in the individual reports was moderate to high.

3. Cardiovascular consequences

Cardiovascular morbidity and mortality are difficult to assess in CAH, as few of the studied patients are older than 50 years (682). Results for carotid intima media thickness (cIMT), a surrogate marker of cardiac dysfunction, vary in existing studies (677,678,683), without correlation between cIMT and cumulative glucocorticoid doses or androgen levels (683). A systematic review and meta-analysis showed slight but significantly greater carotid intima thickness (WMD 0.08 mm; 95% CI 0.01-0.15 mm) (674). In adolescent and adult CAH patients, normal left ventricular morphology has been reported (677,684), but mild diastolic dysfunction and impaired exercise performance were shown. Recently, a French group reported the complex interactions between gonadotropins and steroid hormones on the duration of ventricular repolarization. QT interval duration was shorter in women with CAH than in control women (685). A Swedish study analyzed cardiovascular and metabolic morbidity in CAH patients, finding increases in both cardiovascular and metabolic disorders including higher frequencies of hypertension, dyslipidemia and atrial fibrillation (671). Obesity was consistently increased in all subgroups while diabetes was increased in females, SV and NC phenotypes and those above 40 years of age. However, the non-obese patients were similarly affected by hypertension and diabetes as the entire CAH cohort. This study also found an increased frequency of venous thromboembolic events, which should be studied further to determine if, as reported in both Cushing syndrome and

glucocorticoid use, there is a higher risk of venous thromboembolism due to hypercoagulability that should prompt a lower threshold for thrombosis prophylaxis in this population.

Thus, CAH may be associated with higher cardiovascular risk (686,687). Increased cardiovascular mortality has been reported in CAH in Sweden, second only to adrenal crisis as a cause of death (368). Data on cardiac events are sparse, and most of the literature has focused on surrogate outcomes, rather than episodes of acute myocardial infarction, heart failure or death. Some subgroups of patients seem to be more affected by cardiovascular risk factors. Regular follow-up is needed, along with lifestyle interventions, to limit weight gain, prevent obesity, and screen for diabetes (especially gestational diabetes), and dyslipidemia. Close monitoring of glucocorticoid and mineralocorticoid doses is important. Further prospective studies on larger cohorts are necessary to clarify the mechanisms leading to metabolic and cardiovascular abnormalities, and to understand the respective roles of adrenal sex hormones, lifelong glucocorticoid and mineralocorticoid treatment (366), and the impact of genetic background, such as glucocorticoid receptor gene polymorphisms, and other loci contributing to adverse cardio-metabolic risk profiles (688).

4. Neurological aspects

Early hormonal alterations affect the development of mammalian neural circuits. Widespread expression of androgen and glucocorticoid receptors in the brain suggest that fetal and postnatal imbalances in androgen and glucocorticoid exposure characteristic of CAH might influence brain development and function, with the potential to impact mental health (689). Compared to controls, patients with classic CAH have higher prevalence of anxiety, depression, alcohol misuse, suicidality and adjustment disorders (394,547,597)(also see section V.F). Males diagnosed beyond

1786 the neonatal period and women with the most severe null genotype are especially at risk for mental
1787 health issues (547,594,597).

1788 Neuroimaging studies in patients with CAH have revealed alterations in brain structure and
1789 function. In a functional MRI study of 14 adolescents with classic CAH compared to age-matched
1790 controls, girls with CAH showed a similar pattern of amygdala activation to control boys,
1791 suggesting an androgen effect on amygdala function in girls with CAH (690). Glucocorticoid
1792 therapy has been implicated in the development of white matter hyperintensities which reflect
1793 reduction of white matter structural integrity (691). White matter hyperintensities are found in
1794 patients with CAH, but are an uncommon finding in healthy adults aged < 45 years (692,693).
1795 Glucocorticoid therapy in CAH has been reported to affect working memory and digit span scores;
1796 patients on higher glucocorticoid doses have worse performance (691). Memory impairment is
1797 similarly found among patients with Cushing disease and Cushing syndrome (694).

1798 Structural differences in gray matter morphometry in the medial temporal lobe were found in
1799 a cross-sectional MRI study of 27 adolescents with CAH (695). Young people with classic CAH
1800 had smaller regional volumes in the prefrontal cortex, amygdala and hippocampus and overall
1801 smaller brain volumes compared to age-matched controls. In a study of 37 young adults with CAH,
1802 alterations in grey matter structure, including the middle frontal gyrus and the parietal and superior
1803 occipital cortex were found in CAH patients compared to controls (696). These regions play a role
1804 in visuospatial working memory and patients performed worse in visuospatial working memory
1805 tasks.

1806 All of the neuroimaging studies are hindered by small sample size (689). Decreased brain
1807 volume has been observed in patients compared to controls in multiple studies and this needs to
1808 be accounted for when evaluating individual brain regions. Moreover, sex matching is essential

since human male/female differences have been found in total brain volume (697), gray matter brain volume in specific regions (698) and brain connectivity (699); sexual dimorphism of the brain has also been found during childhood (700)(reviewed in (689)). In CAH, there are multiple hormonal imbalances including *in utero* glucocorticoid deficiency and androgen excess, postnatal androgen excess and iatrogenic glucocorticoid excess, and epinephrine deficiency, all possibly occurring during different developmental periods and with varying potential impact on neural circuits.

5. Bone

Since patients with CAH are on lifelong glucocorticoid supplementation, reduced bone mineral density (BMD) and osteoporosis are potential long-term outcomes. Epidemiological and other studies have demonstrated that glucocorticoids cause secondary osteoporosis and increase fracture risk (701,702). Both direct and indirect effects by glucocorticoids on bone result in initial increased resorption and later deceased bone formation leading to micro-architectural distortion and fracture risk (703). Moreover, glucocorticoids may cause secondary hyperparathyroidism by decreasing intestinal calcium absorption and increasing renal calcium excretion. Despite the known negative effects of glucocorticoids on BMD, studies with patients with CAH have reported inconsistent finding. A few studies have reported normal (704-709) or even high BMD (710), but most have shown low BMD at all or at least some sites (209,217,360,646,711-722). These differences may be due to both glucocorticoid and androgen exposure, since androgens stimulate osteoblast proliferation and differentiation in both genders (723). Adrenal androgens, including DHEAS, affect bone metabolism throughout life, especially during adrenarche, with effects mainly on cortical bone (724). Thus, late diagnosis and/or poor hormonal control may improve BMD due to high androgen concentrations (646,718). Moreover, different glucocorticoid regimens may

affect BMD differently; hydrocortisone seems to affect BMD less than longer acting glucocorticoids, especially dexamethasone (369). A recent meta-analysis comparing patients with CAH and matched controls found slightly decreased BMD in patients with CAH (725). Furthermore, adult women with CAH had more fractures than matched controls (718) whereas men with CAH did not (646). Patients with classic CAH had more nontraumatic fractures than those with NC CAH (724). However, osteoporosis-related fractures typically occur after 50 years of age and very few older patients have been included in studies of BMD and fractures. BMD screening is recommended by the Endocrine Society in adults with CAH and a prolonged period of higher-than-average glucocorticoid dosing, or in patients who have had a nontraumatic fracture (134). Others have also suggested screening any patient upon transfer to adult care and every 2-5 years thereafter (682).

6. Adrenal tumors

Approximately 20-30% of adult patients with CAH have adrenal masses (726). Almost a quarter of these are benign adrenal myelolipomas, which generally occur in patients with a history of poor hormonal control, suggesting that persistent ACTH stimulation may play a role in pathogenesis (628,726,727). There is no evidence that adrenocortical carcinoma, a rare malignancy with poor prognosis, is more prevalent in CAH. Adrenocortical carcinomas can be distinguished from benign adrenal masses by their characteristic steroid profile as assessed with mass spectrometry-based methods (728).

VII. CAH IN DEVELOPING COUNTRIES – CHALLENGES AND LIMITATIONS

CAH management in developing countries is challenging. Newborn screening for CAH is not available in many developing countries (729,730), delaying diagnosis and increasing mortality, particularly in boys who lack atypical genitalia (731,732). Pediatric endocrinologists are scarce

(560,730,733), and late referral to specialized centers may delay diagnosis and treatment. Hormonal assays for diagnosis and follow-up have limited availability and are expensive (730,733). Needed medications may be available only on the black market (137,556,733). Delayed diagnosis (557,734), emotional (731), and gender assignment problems (732,735) negatively influence quality of life (560,736).

There are also socio-economic and cultural issues (733). Myths and misconceptions about ambiguous genitalia in certain communities may lead to discrimination against patients and families (556,560). Gender reassignments in late-identified patients may be met with resistance or refusal because of social stigma and cultural pressure (730). Moreover, many developing countries also face poverty and insufficient basic medical knowledge (556,733). These issues imply the need for better primary health care education. Educational materials in the local language may increase understanding of CAH among families and communities. Clinical guidelines for developing countries are needed, along with advocacy to encourage government policy to improve access to essential medications and implementation of newborn screening.

VIII. FUTURE DIRECTIONS

A. Basic science

As discussed previously, there has been much recent progress in adrenal steroidogenesis as regards the alternative "backdoor" pathway to androgens and the importance of 11-oxo-androgens. Other unanswered questions in steroidogenesis remain (summarized in (42)). Areas requiring further study include more detailed understandings of how StAR imports cholesterol to the mitochondria inner membrane, and how the 17,20-lyase activity of CYP17A1 is regulated. Secretion of androgens and androgen precursors by the fetal adrenal gland is a key component of

the pathophysiology of CAH, yet regulation of fetal adrenal growth and postnatal involution of the fetal zone are poorly understood, and teleologically it is unclear why primate adrenal glands normally secrete DHEA and androgens either prenatally or at adrenarche. Steroid synthesizing enzymes, including CYP21A2, are found in nonglandular tissues, but the functional significance of extraglandular steroidogenesis remains uncertain.

B. Clinical management

Given the relative rarity of CAH, national and international registries are valuable in developing and testing best practices throughout the lifespan (737). Whereas it may be unrealistic to expect that every clinical site caring for CAH patients possesses a comprehensive, multidisciplinary team, networks of expert centers can ensure access to specialty care when necessary. Criteria defining a comprehensive expert level of care for CAH have been published (342,738). Surveys show that patient satisfaction, provider training, research and quality improvement activities vary among medical centers (739,740); thus, there is a need for clinical benchmarks in management. Real world data including patient and family satisfaction, as well as peer-observation of clinical care can help develop guidelines and decision-support tools. By providing robust data on epidemiology, patients' characteristics and current standard of care, registries have the potential to shape health care policy and, by engaging with patients, increase stakeholder involvement and improve the patient-centered experience (741). One example of outcomes from the I-CAH Registry has been to define acute adverse events associated with adrenal insufficiency including sick day episodes, adrenal crises and hospitalizations among CAH patients (401). The challenge for rare disease registries is to ensure that the data represent the widest range of patients, and that the data are findable, accessible, interoperable and reusable (FAIR) within a rigorous framework of data governance, integrated

with other data sources through multiomics technology (742). With anticipated therapeutic advances over the next decade, the use of registries for measuring therapeutic effectiveness, as well as maintaining clinician and patient engagement, will become imperative (401). Given the relative rarity of CAH, national and international registries are valuable in developing and testing best practices throughout the lifespan (737). As it may be unrealistic to expect that every clinical site caring for CAH patients possesses a comprehensive, multidisciplinary team, networks of expert centers can ensure access to specialty care when necessary. Criteria defining a comprehensive expert level of care for CAH have been published (342,738). Surveys show that patient satisfaction, provider training, research and quality improvement activities vary among medical centers (739,740), thus there is a need for clinical benchmarks in disease management. (401,741). Other areas that could benefit from large-scale collaborative data collection include prenatal and neonatal diagnosis and treatment. With recent data pointing to potential serious adverse outcomes, long-term follow-up studies should closely monitor both CAH patients and unaffected siblings subjected to prenatal Dex treatment. As discussed in section IV.A, the suboptimal positive predictive value for immunoassay in many newborn screening programs mandates further studies to determine the most cost effective strategies to improve screening sensitivity and specificity. Clinical trials for novel drug targets and potential gene therapy are in progress or planned that should provide additional treatment options. At the same time, more widespread availability of mass spectrometry-based assays for new steroid biomarkers, *e.g.*, 11-oxo-androgens, may improve monitoring and titrating existing medication regimens.

Long-term management should emphasize the importance of a smooth transition from pediatric to adult medical care, with continued emphasis on risk assessment for adverse reproductive, psychosexual, cardiovascular, metabolic and musculoskeletal outcomes. To this end,

implementation of telemedicine services have lately been recognized as a valuable resource in managing patients living in remote areas or lacking access to specialty centers.

Much discourse and debate has centered on whether and when surgical intervention ought to be considered. A systematic review and meta-analysis found scant sound evidence to favor early surgery, delayed surgery or no surgery (528). More work is needed to develop evidence-based guidelines for surgical treatment of CAH, including ideal timing of surgery, surgical technique, risk of incontinence, risk of additional surgery (such as repair of vaginal stenosis at puberty), risk of loss of sexual function, and extent of clitoral surgery. Given that the likelihood of performing randomized controlled trials in this area is minimal, long-term surveillance using commonly agreed and routinely collected clinical and patient reported outcome measures should be prioritized.

Not least among desired goals is for mental health professionals in collaboration with other specialists to develop and validate quality of life instruments specific to CAH. In summary, based on what has been learned from collective clinical and basic research, the outlook is optimistic for improved modes of CAH treatment and consequently better quality of life.

Acknowledgments

HC received grant support from the International Funding CAH (IFCAH) and the Innovatiefonds zorgverzekeraars for CAH related research.

PWS received grant support from the National Institutes of Health (R01HD093450 subcontract).

HF received grant support from Magnus Bergvall Foundation.

LG received grant support from the BBSRC, Barts Charity, IFCAH and Rosetrees Trust.

AH received grant support from the Deutsche Forschungsgemeinschaft (DFG, 314061271 CRC-TRR205).

This research was supported in part by the Intramural Research Program of the NIH (DPM).

AN received grant support from Stockholm County Council and Karolinska Institutet.

NR received grant support from the Deutsche Forschungsgemeinschaft (DFG Heisenberg Professorship 325768017 and 314061271 CRC-TRR205).

PT would like to thank Pr A Bachelot for her role as investigator in different studies and Jerome Dulon for his technical assistance.

SAW acknowledges support from DFG grant WU 148/7-1 and –2).

PCW is the Audry Newman Rapoport Distinguished Chair in Pediatric Endocrinology, and received grant support from the National Institutes of Health (U01-HD083493).

REFERENCES

1. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev*. 2000;21:245-291.
2. Gidlof S, Falhammar H, Thilen A, von Dobeln U, Ritzen M, Wedell A, Nordenstrom A. One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. *Lancet Diabetes Endocrinol*. 2013;1(1):35-42.
3. Grunieiro-Papendieck L, Chiesa A, Mendez V, Prieto L. Neonatal screening for congenital adrenal hyperplasia: experience and results in Argentina. *J Pediatr Endocrinol Metab*. 2008;21(1):73-78.
4. Gleeson HK, Wiley V, Wilcken B, Elliott E, Cowell C, Thonsett M, Byrne G, Ambler G. Two-year pilot study of newborn screening for congenital adrenal hyperplasia in New South Wales compared with nationwide case surveillance in Australia. *J Paediatr Child Health*. 2008;44(10):554-559.
5. Shetty VB, Bower C, Jones TW, Lewis BD, Davis EA. Ethnic and gender differences in rates of congenital adrenal hyperplasia in Western Australia over a 21 year period. *J Paediatr Child Health*. 2012;48(11):1029-1032.
6. Nascimento ML, Cristiano AN, Campos T, Ohira M, Cechinel E, Simoni G, Lee J, Linhares RM, Silva PC. Ten-year evaluation of a Neonatal Screening Program for congenital adrenal hyperplasia. *Arq Bras Endocrinol Metabol*. 2014;58(7):765-771.
7. Silveira EL, dos Santos EP, Bachega TA, van der LN, I, Gross JL, Elnecave RH. The actual incidence of congenital adrenal hyperplasia in Brazil may not be as high as inferred--an estimate based on a public neonatal screening program in the state of Goias. *J Pediatr Endocrinol Metab*. 2008;21(5):455-460.
8. Pezzuti IL, Barra CB, Mantovani RM, Januario JN, Silva IN. A three-year follow-up of congenital adrenal hyperplasia newborn screening. *J Pediatr (Rio J)*. 2014;90(3):300-307.
9. Kopacek C, de Castro SM, Prado MJ, da Silva CM, Beltrão LA, Spritzer PM. Neonatal screening for congenital adrenal hyperplasia in Southern Brazil: a population based study with 108,409 infants. *BMC Pediatr*. 2017;17(1):22.
10. Zhong K, Wang W, He F, Wang Z. The status of neonatal screening in China, 2013. *J Med Screen*. 2016;23:59-61.
11. Gong LF, Gao X, Yang N, Zhao JQ, Yang HH, Kong YY. A pilot study on newborn screening for congenital adrenal hyperplasia in Beijing. *J Pediatr Endocrinol Metab*. 2019;32(3):253-258.
12. Dumic K, Krnic N, Skrabic V, Stipancic G, Cvijovic K, Kusec V, Stingl K. Classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency in croatia between 1995 and 2006. *Horm Res*. 2009;72(5):310-314.
13. Gonzalez EC, Carvajal F, Frometa A, Arteaga AL, Castells EM, Espinosa T, Coto R, Perez PL, Tejeda Y, Del RL, Segura MT, Almenares P, Robaina R, Fernandez JL. Newborn screening for congenital adrenal hyperplasia in Cuba: six years of experience. *Clin Chim Acta*. 2013;421:73-78.
14. David J, Chrastina P, Pešková K, Kožich V, Friedecký D, Adam T, Hlídková E, Vinohradská H, Novotná D, Hedelová M, Al Taji E, Holubová A, Skalická V, Macek M, Gaillyová R, Votava F. Epidemiology of rare diseases detected by newborn screening in the Czech Republic. *Cent Eur J Public Health*. 2019;27(2):153-159.
15. Coulm B, Coste J, Tardy V, Ecosse E, Roussey M, Morel Y, Carel JC. Efficiency of neonatal screening for congenital adrenal hyperplasia due to 21-hydroxylase deficiency in children born in mainland France between 1996 and 2003. *Arch Pediatr Adolesc Med*. 2012;166(2):113-120.
16. Odenwald B, Dorr HG, Bonfig W, Schmidt H, Fingerhut R, Wildner M, Nennstiel-Ratzel U. Classic Congenital Adrenal Hyperplasia due to 21-Hydroxylase-Deficiency: 13 Years of Neonatal Screening and Follow-up in Bavaria. *Klin Padiatr*. 2015;227(5):278-283.
17. Kaur G, Thakur K, Kataria S, Singh TR, Chavan BS, Kaur G, Atwal R. Current and future perspective of newborn screening: an Indian scenario. *J Pediatr Endocrinol Metab*. 2016;29(1):5-13.
18. Pode-Shakked N, Blau A, Pode-Shakked B, Tiosano D, Weintrob N, Eyal O, Zung A, Levy-Khademi F, Tenenbaum-Rakover Y, Zangen D, Gillis D, Pinhas-Hamiel O, Loewenthal N, de Vries L,

Landau Z, Rachmiel M, Abu-Libdeh A, Eliakim A, Strich D, Koren I, German A, Sack J, Almashanu S. Combined Gestational Age- and Birth Weight-Adjusted Cutoffs for Newborn Screening of Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2019;104(8):3172-3180.
19. Morikawa S, Nakamura A, Fujikura K, Fukushi M, Hotsubo T, Miyata J, Ishizu K, Tajima T. Results from 28 years of newborn screening for congenital adrenal hyperplasia in sapporo. *Clin Pediatr Endocrinol*. 2014;23(2):35-43.
20. Tsuji A, Konishi K, Hasegawa S, Anazawa A, Onishi T, Ono M, Morio T, Kitagawa T, Kashimada K. Newborn screening for congenital adrenal hyperplasia in Tokyo, Japan from 1989 to 2013: a retrospective population-based study. *BMC Pediatr*. 2015;15(1):209-0529.
21. Kreukels BPC, Cohen-Kettenis PT, Roehle R, van de Grift TC, Slowikowska-Hilczer J, Claahsen-van der Grinten H, Linden Hirschberg A, de Vries ALC, Reisch N, Bouvattier C, Nordenstrom A, Thyen U, Kohler B, Group O. Sexuality in Adults with Differences/Disorders of Sex Development (DSD): Findings from the dsd-LIFE Study. *J Sex Marital Ther*. 2019;45(8):688-705.
22. Heather NL, Seneviratne SN, Webster D, Derraik JG, Jefferies C, Carll J, Jiang Y, Cutfield WS, Hofman PL. Newborn screening for congenital adrenal hyperplasia in New Zealand, 1994-2013. *J Clin Endocrinol Metab*. 2015;100(3):1002-1008.
23. Guran T, Tezel B, Cakir M, Akinci A, Orbak Z, Keskin M, Selver Eklioglu B, Ozon A, Ozbek MN, Karaguzel G, Hatipoglu N, Gurbuz F, Cizmecioglu FM, Kara C, Simsek E, Bas F, Aydin M, Darendeliler F. Neonatal Screening for Congenital Adrenal Hyperplasia in Turkey: Outcomes of Extended Pilot Study with 241,083 Infants. *J Clin Res Pediatr Endocrinol*. 2020;12:287-294.
24. Al HH, Salah M, Osman HM, Farag HM, El-Assiouty L, Saade D, Hertecant J. Expanding the comprehensive national neonatal screening programme in the United Arab Emirates from 1995 to 2011. *East Mediterr Health J*. 2014;20(1):17-23.
25. Al-Harbi T, Al-Shaikh A. Apparent mineralocorticoid excess syndrome: report of one family with three affected children. *J Pediatr Endocrinol Metab*. 2012;25(11-12):1083-1088.
26. Larrandaburu M, Matte U, Noble A, Olivera Z, Sanseverino MT, Nacul L, Schuler-Faccini L. Ethics, genetics and public policies in Uruguay: newborn and infant screening as a paradigm. *J Community Genet*. 2015;6(3):241-249.
27. van der Kamp HJ, Oudshoorn CG, Elvers BH, Van BM, Otten BJ, Wit JM, Verkerk PH. Cutoff levels of 17-alpha-hydroxyprogesterone in neonatal screening for congenital adrenal hyperplasia should be based on gestational age rather than on birth weight. *J Clin Endocrinol Metab*. 2005;90(7):3904-3907.
28. Martinerie L, Pussard E, Foix-L'Helias L, Petit F, Cosson C, Boileau P, Lombes M. Physiological partial aldosterone resistance in human newborns. *Pediatr Res*. 2009;66(3):323-328.
29. Gattineni J, Baum M. Developmental changes in renal tubular transport-an overview. *Pediatr Nephrol*. 2015;30(12):2085-2098.
30. van der Straaten S, Springer A, Zecic A, Hebenstreit D, Tonnhofer U, Gawlik A, Baumert M, Szeliga K, Debulpaep S, Desloovere A, Tack L, Smets K, Wasniewska M, Corica D, Calafiore M, Ljubicic ML, Busch AS, Juul A, Nordenström A, Sigurdsson J, Flück CE, Haamberg T, Graf S, Hannema SE, Wolffenbuttel KP, Hiort O, Ahmed SF, Cools M. The External Genitalia Score (EGS): A European Multicenter Validation Study. *J Clin Endocrinol Metab*. 2020;105(3).
31. Zhao F, Franco HL, Rodriguez KF, Brown PR, Tsai MJ, Tsai SY, Yao HH. Elimination of the male reproductive tract in the female embryo is promoted by COUP-TFII in mice. *Science*. 2017;357(6352):717-720.
32. Merke DP, Auchus RJ. Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency. *N Engl J Med*. 2020;383(13):1248-1261.
33. Muthusamy K, Elamin MB, Smushkin G, Murad MH, Lampropulos JF, Elamin KB, Abu Elnour NO, Gallegos-Orozco JF, Fatourechi MM, Agrwal N, Lane MA, Albuquerque FN, Erwin PJ, Montori VM. Clinical review: Adult height in patients with congenital adrenal hyperplasia: a systematic review and metaanalysis. *J Clin Endocrinol Metab*. 2010;95(9):4161-4172.

34. Völkl TM, Öhl L, Rauh M, Schöfl C, Dörr HG. Adrenarche and puberty in children with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res Paediatr*. 2011;76(6):400-410.
35. Gomes LG, Bachega TA, Mendonca BB. Classic congenital adrenal hyperplasia and its impact on reproduction. *Fertility Sterility*. 2019;111:7-12.
36. Mendes-Dos-Santos CT, Martins DL, Guerra-Júnior G, Baptista MTM, de-Mello MP, de Oliveira LC, Morcillo AM, Lemos-Marini SHV. Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia. *Horm Res Paediatr*. 2018;90(3):161-168.
37. Falhammar H, Nordenstrom A. Nonclassic congenital adrenal hyperplasia due to 21-hydroxylase deficiency: clinical presentation, diagnosis, treatment, and outcome. *Endocrine*. 2015;50(1):32-50.
38. Hannah-Shmouni F, Morissette R, Sinaii N, Elman M, Prezant TR, Chen W, Pulver A, Merke DP. Revisiting the prevalence of nonclassic congenital adrenal hyperplasia in US Ashkenazi Jews and Caucasians. *Genet Med*. 2017;19:1276-1279.
39. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*. 2011;32(1):81-151.
40. Miller WL. Disorders in the initial steps of steroid hormone synthesis. *J Steroid Biochem Mol Biol*. 2017;165(Pt A):18-37.
41. Selvaraj V, Stocco DM, Clark BJ. Current knowledge on the acute regulation of steroidogenesis. *Biol Reprod*. 2018;99(1):13-26.
42. Miller WL. Steroidogenesis: Unanswered Questions. *Trends Endocrinol Metab*. 2017;28(11):771-793.
43. Bose HS, Sugawara T, Strauss JF, 3rd, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med*. 1996;335(25):1870-1878.
44. Lin D, Sugawara T, Strauss JF, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science*. 1995;267:1828-1831.
45. Baker BY, Lin L, Kim CJ, Raza J, Smith CP, Miller WL, Achermann JC. Nonclassic congenital lipoid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. *J Clin Endocrinol Metab*. 2006;91(12):4781-4785.
46. Rubtsov P, Karmanov M, Sverdlova P, Spirin P, Tiulpakov A. A novel homozygous mutation in CYP11A1 gene is associated with late-onset adrenal insufficiency and hypospadias in a 46,XY patient. *J Clin Endocrinol Metab*. 2009;94(3):936-939.
47. Sahakitrungruang T, Tee MK, Blackett PR, Miller WL. Partial defect in the cholesterol side-chain cleavage enzyme P450scc (CYP11A1) resembling nonclassic congenital lipoid adrenal hyperplasia. *J Clin Endocrinol Metab*. 2011;96(3):792-798.
48. Ishii T, Tajima T, Kashimada K, Mukai T, Tanahashi Y, Katsumata N, Kanno J, Hamajima T, Miyako K, Ida S, Hasegawa T. Clinical Features of 57 Patients with Lipoid Congenital Adrenal Hyperplasia: Criteria for Nonclassic Form Revisited. *J Clin Endocrinol Metab*. 2020;105(11).
49. Miller WL. Regulation of steroidogenesis by electron transfer. *Endocrinology*. 2005;146(6):2544-2550.
50. Paul A, Drecourt A, Petit F, Deguine DD, Vasnier C, Oufadem M, Masson C, Bonnet C, Masmoudi S, Mosnier I, Mahieu L, Bouccara D, Kaplan J, Challe G, Domange C, Mochel F, Sterkers O, Gerber S, Nitschke P, Bole-Feysot C, Jonard L, Gherbi S, Mercati O, Ben Aissa I, Lyonnet S, Rotig A, Delahodde A, Marlin S. FDXR Mutations Cause Sensorial Neuropathies and Expand the Spectrum of Mitochondrial Fe-S-Synthesis Diseases. *Am J Hum Genet*. 2017;101(4):630-637.
51. Peng Y, Shinde DN, Valencia CA, Mo JS, Rosenfeld J, Truitt Cho M, Chamberlin A, Li Z, Liu J, Gui B, Brockhage R, Basinger A, Alvarez-Leon B, Heydemann P, Magoulas PL, Lewis AM, Scaglia F, Gril S, Chong SC, Bower M, Monaghan KG, Willaert R, Plona MR, Dineen R, Milan F, Hoganson G, Powis Z, Helbig KL, Keller-Ramey J, Harris B, Anderson LC, Green T, Sukoff Rizzo SJ, Kaylor J, Chen J, Guan MX, Sellars E, Sparagana SP, Gibson JB, Reinholdt LG, Tang S, Huang T. Biallelic mutations in the ferredoxin reductase gene cause novel mitochondriopathy with optic atrophy. *Hum Mol Genet*. 2017;26(24):4937-4950.

52. Slone J, Peng Y, Chamberlin A, Harris B, Kaylor J, McDonald MT, Lemmon M, El-Dairi MA, Tchapyjnikov D, Gonzalez-Krellwitz LA, Sellars EA, McConkie-Rosell A, Reinholdt LG, Huang T. Biallelic mutations in FDXR cause neurodegeneration associated with inflammation. *Journal of Human Genetics*. 2018;63(12):1211-1222.
53. Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. *Endocr Rev*. 2005;26(4):525-582.
54. Thomas JL, Mason JI, Brandt S, Spencer BR, Jr., Norris W. Structure/function relationships responsible for the kinetic differences between human type 1 and type 2 3β-hydroxysteroid dehydrogenase and for the catalysis of the type 1 activity. *J Biol Chem*. 2002;277(45):42795-42801.
55. Lee TC, Miller WL, Auchus RJ. Medroxyprogesterone acetate and dexamethasone are competitive inhibitors of different human steroidogenic enzymes. *Journal of Clinical Endocrinology & Metabolism*. 1999;84(6):2104-2110.
56. Moisan AM, Ricketts ML, Tardy V, Desrochers M, Mebarki F, Chaussain JL, Cabrol S, Raux-Demay MC, Forest MG, Sippell WG, Peter M, Morel Y, Simard J. New insight into the molecular basis of 3β-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. *J Clin Endocrinol Metab*. 1999;84(12):4410-4425.
57. Mermejo LM, Elias LL, Marui S, Moreira AC, Mendonca BB, de Castro M. Refining hormonal diagnosis of type II 3β-hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on HSD3B2 genotyping. *J Clin Endocrinol Metab*. 2005;90(3):1287-1293.
58. Jeandron DD, Sahakitrungruang T. A novel homozygous Q334X mutation in the HSD3B2 gene causing classic 3β-hydroxysteroid dehydrogenase deficiency: an unexpected diagnosis after a positive newborn screen for 21-hydroxylase deficiency. *Horm Res Paediatr*. 2012;77(5):334-338.
59. Burckhardt MA, Udhane SS, Marti N, Schnyder I, Tapia C, Nielsen JE, Mullis PE, Rajpert-De Meyts E, Fluck CE. Human 3β-hydroxysteroid dehydrogenase deficiency seems to affect fertility but may not harbor a tumor risk: lesson from an experiment of nature. *Eur J Endocrinol*. 2015;173(5):K1-K12.
60. Auchus RJ, Lee TC, Miller WL. Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J Biol Chem*. 1998;273(6):3158-3165.
61. Fluck CE, Miller WL, Auchus RJ. The 17, 20-lyase activity of cytochrome P450c17 from human fetal testis favors the Δ^5 steroidogenic pathway. *J Clin Endocrinol Metab*. 2003;88(8):3762-3766.
62. Auchus RJ. The genetics, pathophysiology, and management of human deficiencies of P450c17. *Endocrinol Metab Clin North Am*. 2001;30(1):101-119.
63. Miller WL. The syndrome of 17,20 lyase deficiency. *J Clin Endocrinol Metab*. 2012;97(1):59-67.
64. Idkowiak J, Randell T, Dhir V, Patel P, Shackleton CH, Taylor NF, Krone N, Arlt W. A missense mutation in the human cytochrome b5 gene causes 46,XY disorder of sex development due to true isolated 17,20 lyase deficiency. *J Clin Endocrinol Metab*. 2012;97(3):E465-E475.
65. Marsh CA, Auchus RJ. Fertility in patients with genetic deficiencies of cytochrome P450c17 (CYP17A1): combined 17-hydroxylase/17,20-lyase deficiency and isolated 17,20-lyase deficiency. *Fertil Steril*. 2014;101(2):317-322.
66. Flück CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, Jabs EW, Mendonça BB, Fujieda K, Miller WL. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet*. 2004;36(3):228-230.
67. Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, Okuyama T, Nakai H, Soneda S, Tachibana K, Matsuo N, Sato S, Homma K, Nishimura G, Hasegawa T, Ogata T. Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *J Clin Endocrinol Metab*. 2005;90(1):414-426.

68. Huang N, Pandey AV, Agrawal V, Reardon W, Lapunzina PD, Mowat D, Jabs EW, Vliet GV, Sack J, Fluck CE, Miller WL. Diversity and function of mutations in p450 oxidoreductase in patients with antley-bixler syndrome and disordered steroidogenesis. *Am J Hum Genet*. 2005;76(5):729-749.
69. Krone N, Reisch N, Idkowiak J, Dhir V, Ivison HE, Hughes BA, Rose IT, O'Neil DM, Vijzelaar R, Smith MJ, MacDonald F, Cole TR, Adolphs N, Barton JS, Blair EM, Braddock SR, Collins F, Cragun DL, Dattani MT, Day R, Dougan S, Feist M, Gottschalk ME, Gregory JW, Haim M, Harrison R, Olney AH, Hauffa BP, Hindmarsh PC, Hopkin RJ, Jira PE, Kempers M, Kerstens MN, Khalifa MM, Kohler B, Maiter D, Nielsen S, O'Riordan SM, Roth CL, Shane KP, Silink M, Stikkelbroeck NM, Sweeney E, Szarras-Czapnik M, Waterson JR, Williamson L, Hartmann MF, Taylor NF, Wudy SA, Malunowicz EM, Shackleton CH, Arlt W. Genotype-phenotype analysis in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *J Clin Endocrinol Metab*. 2012;97(2):E257-E267.
70. Dean B, Chrisp GL, Quartararo M, Maguire AM, Hameed S, King BR, Munns CF, Torpy DJ, Falhammar H, Rushworth RL. P450 Oxidoreductase Deficiency: A Systematic Review and Meta-analysis of Genotypes, Phenotypes, and Their Relationships. *J Clin Endocrinol Metab*. 2020;105(3):e42-e52.
71. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, Chalder SM, Borucka-Mankiewicz M, Hauffa BP, Malunowicz EM, Stewart PM, Shackleton CH. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet*. 2004;363(9427):2128-2135.
72. Fukami M, Nishimura G, Homma K, Nagai T, Hanaki K, Uematsu A, Ishii T, Numakura C, Sawada H, Nakacho M, Kowase T, Motomura K, Haruna H, Nakamura M, Ohishi A, Adachi M, Tajima T, Hasegawa Y, Hasegawa T, Horikawa R, Fujieda K, Ogata T. Cytochrome P450 oxidoreductase deficiency: identification and characterization of biallelic mutations and genotype-phenotype correlations in 35 Japanese patients. *J Clin Endocrinol Metab*. 2009;94(5):1723-1731.
73. Hershkovitz E, Parvari R, Wudy SA, Hartmann MF, Gomes LG, Loewental N, Miller WL. Homozygous mutation G539R in the gene for P450 oxidoreductase in a family previously diagnosed as having 17,20-lyase deficiency. *J Clin Endocrinol Metab*. 2008;93(9):3584-3588.
74. Laue K, Pogoda HM, Daniel PB, van Haeringen A, Alanay Y, von Ameln S, Rachwalski M, Morgan T, Gray MJ, Breuning MH, Sawyer GM, Sutherland-Smith AJ, Nikkels PG, Kubisch C, Bloch W, Wollnik B, Hammerschmidt M, Robertson SP. Craniosynostosis and multiple skeletal anomalies in humans and zebrafish result from a defect in the localized degradation of retinoic acid. *Am J Hum Genet*. 2011;89(5):595-606.
75. Tomalik-Scharte D, Maiter D, Kirchheiner J, Ivison HE, Fuhr U, Arlt W. Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *Eur J Endocrinol*. 2010;163(6):919-924.
76. Sahakitrungruang T, Huang N, Tee MK, Agrawal V, Russell WE, Crock P, Murphy N, Migeon CJ, Miller WL. Clinical, genetic, and enzymatic characterization of P450 oxidoreductase deficiency in four patients. *J Clin Endocrinol Metab*. 2009;94(12):4992-5000.
77. Geller DH, Auchus RJ, Mendonca BB, Miller WL. The genetic and functional basis of isolated 17,20-lyase deficiency. *Nature Genetics*. 1997;17(2):201-205.
78. Homma K, Hasegawa T, Nagai T, Adachi M, Horikawa R, Fujiwara I, Tajima T, Takeda R, Fukami M, Ogata T. Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone. *J Clin Endocrinol Metab*. 2006;91(7):2643-2649.
79. Reisch N, Taylor AE, Nogueira EF, Asby DJ, Dhir V, Berry A, Krone N, Auchus RJ, Shackleton CHL, Hanley NA, Arlt W. Alternative pathway androgen biosynthesis and human fetal female virilization. *Proceedings of the National Academy of Sciences*. 2019;116(44):22294-22299.
80. Fluck CE, Parween S, Rojas Velazquez MN, Pandey AV. Inhibition of placental CYP19A1 activity remains as a valid hypothesis for 46,XX virilization in P450 oxidoreductase deficiency. *Proc Natl Acad Sci U S A*. 2020;117(26):14632-14633.

81. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metab*. 1999;84(12):4677-4694.
82. Reisch N, Auchus RJ, Shackleton CHL, Hanley NA, Arlt W. Reply to Flück et al.: Alternative androgen pathway biosynthesis drives fetal female virilization in P450 oxidoreductase deficiency. *Proceedings of the National Academy of Sciences*. 2020;117(26):14634-14635.
83. Huang N, Agrawal V, Giacomini KM, Miller WL. Genetics of P450 oxidoreductase: sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. *Proc Natl Acad Sci U S A*. 2008;105(5):1733-1738.
84. Gomes LG, Huang N, Agrawal V, Mendonca BB, Bachega TA, Miller WL. The common P450 oxidoreductase variant A503V is not a modifier gene for 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2008;93(7):2913-2916.
85. White PC, Curnow KM, Pascoe L. Disorders of steroid 11 beta hydroxylase isozymes. *Endocr Rev*. 1994;15:421-438.
86. Turcu AF, Rege J, Auchus RJ, Rainey WE. 11-Oxygenated androgens in health and disease. *Nat Rev Endocrinol*. 2020;16(5):284-296.
87. Curnow KM, Tusie-Luna MT, Pascoe L, Natarajan R, Gu JL, Nadler JL, White PC. The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. *Mol Endocrinol*. 1991;5:1513-1522.
88. Mulatero P, Curnow KM, Aupetit-Faisant B, Foekling M, Gomez-Sanchez C, Veglio F, Jeunemaitre X, Corvol P, Pascoe L. Recombinant CYP11B genes encode enzymes that can catalyze conversion of 11-deoxycortisol to cortisol, 18-hydroxycortisol, and 18-oxocortisol. *Journal of Clinical Endocrinology & Metabolism*. 1998;83(11):3996-4001.
89. Therrell BL, Jr, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, Gonzalez J, Gunn S. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics*. 1998;101(4 Pt 1):583-590.
90. White PC, Curnow KM, Pascoe L. Disorders of steroid 11β-hydroxylase isozymes. *Endocr Rev*. 1994;15(4):421-438.
91. Moeller G, Adamski J. Integrated view on 17β-hydroxysteroid dehydrogenases. *Mol Cell Biol*. 2009;301(1-2):7-19.
92. Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev*. 1997;18(3):281-305.
93. Tremblay Y, Ringler GE, Morel Y, Mohandas TK, Labrie F, Strauss JF, 3rd, Miller WL. Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen -) q25. *J Biol Chem*. 1989;264(34):20458-20462.
94. Moghrabi N, Andersson S. 17β-hydroxysteroid dehydrogenases: physiological roles in health and disease. *Trends Endocrinol Metab*. 1998;9(7):265-270.
95. Mendonca BB, Inacio M, Arnhold IJ, Costa EM, Bloise W, Martin RM, Denes FT, Silva FA, Andersson S, Lindqvist A, Wilson JD. Male pseudohermaphroditism due to 17β-hydroxysteroid dehydrogenase 3 deficiency. Diagnosis, psychological evaluation, and management. *Medicine (Baltimore)*. 2000;79(5):299-309.
96. Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N, Ratnam K. Human 3α-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochem J*. 2000;351(Pt 1):67-77.
97. Nakamura Y, Hornsby PJ, Casson P, Morimoto R, Satoh F, Xing Y, Kennedy MR, Sasano H, Rainey WE. Type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. *J Clin Endocrinol Metab*. 2009;94(6):2192-2198.
98. Biswas MG, Russell DW. Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid dehydrogenases from rat and human prostate. *J Biol Chem*. 1997;272(25):15959-15966.

99. Fluck CE, Meyer-Boni M, Pandey AV, Kempna P, Miller WL, Schoenle EJ, Biason-Lauber A. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet*. 2011;89(2):201-218.
100. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, Mendelson CR, Bulun SE. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev*. 1994;15:342-355.
101. Lo JC, Grumbach MM. Pregnancy outcomes in women with congenital virilizing adrenal hyperplasia. *Endocrinol Metab Clin North Am*. 2001;30(1):207-229.
102. Gomes LG, Huang N, Agrawal V, Mendonca BB, Bachega TA, Miller WL. Extraadrenal 21-hydroxylation by CYP2C19 and CYP3A4: effect on 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2009;94(1):89-95.
103. Yoshimoto FK, Zhou Y, Peng HM, Stidd D, Yoshimoto JA, Sharma KK, Matthew S, Auchus RJ. Minor activities and transition state properties of the human steroid hydroxylases cytochromes P450c17 and P450c21, from reactions observed with deuterium-labeled substrates. *Biochemistry*. 2012;51(36):7064-7077.
104. Zhao B, Lei L, Kagawa N, Sundaramoorthy M, Banerjee S, Nagy LD, Guengerich FP, Waterman MR. Three-dimensional structure of steroid 21-hydroxylase (cytochrome P450 21A2) with two substrates reveals locations of disease-associated variants. *J Biol Chem*. 2012;287(13):10613-10622.
105. Pallan PS, Lei L, Wang C, Waterman MR, Guengerich FP, Egli M. Research Resource: Correlating Human Cytochrome P450 21A2 Crystal Structure and Phenotypes of Mutations in Congenital Adrenal Hyperplasia. *Mol Endocrinol*. 2015;29(9):1375-1384.
106. Mizrachi D, Wang Z, Sharma KK, Gupta MK, Xu K, Dwyer CR, Auchus RJ. Why human cytochrome P450c21 is a progesterone 21-hydroxylase. *Biochemistry*. 2011;50(19):3968-3974.
107. Hu MC, Chung BC. Expression of human 21-hydroxylase (P450c21) in bacterial and mammalian cells: a system to characterize normal and mutant enzymes. *Mol Endocrinol*. 1990;4:893-898.
108. Tusie-Luna MT, Speiser PW, Dumic M, New MI, White PC. A mutation (Pro-30 to Leu) in CYP21 represents a potential nonclassic steroid 21-hydroxylase deficiency allele. *Mol Endocrinol*. 1991;5:685-692.
109. Tusie-Luna MT, Traktman P, White PC. Determination of functional effects of mutations in the steroid 21-hydroxylase gene (CYP21) using recombinant vaccinia virus. *J Biol Chem*. 1990;265:20916-20922.
110. Wu DA, Chung BC. Mutations of P450c21 (steroid 21-hydroxylase) at Cys428, Val281, and Ser268 result in complete, partial, or no loss of enzymatic activity, respectively. *J Clin Invest*. 1991;88:519-523.
111. Wang C, Pallan PS, Zhang W, Lei L, Yoshimoto FK, Waterman MR, Egli M, Guengerich FP. Functional analysis of human cytochrome P450 21A2 variants involved in congenital adrenal hyperplasia. *J Biol Chem*. 2017;292(26):10767-10778.
112. Peng HM, Barlow C, Auchus RJ. Catalytic modulation of human cytochromes P450 17A1 and P450 11B2 by phospholipid. *J Steroid Biochem Mol Biol*. 2018;181:63-72.
113. Auchus RJ. The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab*. 2004;15(9):432-438.
114. Wilson JD, Auchus RJ, Leihy MW, Guryev OL, Estabrook RW, Osborn SM, Shaw G, Renfree MB. 5alpha-androstane-3alpha,17beta-diol is formed in tammar wallaby pouch young testes by a pathway involving 5alpha-pregnane-3alpha,17alpha-diol-20-one as a key intermediate. *Endocrinology*. 2003;144(2):575-580.
115. Shaw G, Fenelon J, Sichlau M, Auchus RJ, Wilson JD, Renfree MB. Role of the alternate pathway of dihydrotestosterone formation in virilization of the Wolffian ducts of the tammar wallaby, Macropus eugenii. *Endocrinology*. 2006;147(5):2368-2373.

116. Wilson JD, Shaw G, Renfree MB, Auchus RJ, Leihy MW, Eckery DC. Ontogeny and pathway of formation of 5alpha-androstane-3alpha,17beta-diol in the testes of the immature brushtail possum Trichosurus vulpecula. *Reprod Fertil Dev*. 2005;17(6):603-609.
117. Wilson JD, Renfree MB, Auchus RJ, Pask AJ, Shaw G. Formation of 5alpha-reduced androgens in the testes and urogenital tract of the grey short-tailed opossum, Monodelphis domestica. *Reprod Fertil Dev*. 2009;21(5):649-654.
118. O'Shaughnessy PJ, Antignac JP, Le Bizec B, Morvan ML, Svechnikov K, Soder O, Savchuk I, Monteiro A, Soffientini U, Johnston ZC, Bellingham M, Hough D, Walker N, Filis P, Fowler PA. Alternative (backdoor) androgen production and masculinization in the human fetus. *PLoS Biol*. 2019;17(2):e3000002.
119. Bauman DR, Steckelbroeck S, Williams MV, Peehl DM, Penning TM. Identification of the major oxidative 3alpha-hydroxysteroid dehydrogenase in human prostate that converts 5alpha-androstane-3alpha,17beta-diol to 5alpha-dihydrotestosterone: a potential therapeutic target for androgen-dependent disease. *Mol Endocrinol*. 2006;20(2):444-458.
120. Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab*. 2012;97(3):E367-E375.
121. Schiffer L, Barnard L, Baranowski ES, Gilligan LC, Taylor AE, Arlt W, Shackleton CHL, Storbeck KH. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: A comprehensive review. *J Steroid Biochem Mol Biol*. 2019;194:105439.
122. Rege J, Nakamura Y, Satoh F, Morimoto R, Kennedy MR, Layman LC, Honma S, Sasano H, Rainey WE. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. *J Clin Endocrinol Metab*. 2013;98(3):1182-1188.
123. Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano TJ, Shields JJ, Merke DP, Rainey WE, Auchus RJ. Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. *Eur J Endocrinol*. 2016;174(5):601-609.
124. Swart AC, Storbeck KH. 11β-Hydroxyandrostenedione: Downstream metabolism by 11βHSD, 17βHSD and SRD5A produces novel substrates in familiar pathways. *Mol Cell Endocrinol*. 2015;408:114-123.
125. Borg B. Androgens in teleost fishes. *Comp Biochem Physiol*. 1994;109C:219-245.
126. Rege J, Turcu A, Kasa-Vubu JZ, Lerario AM, Auchus GC, Auchus RJ, Smith JM, White PC, Rainey WE. 11-ketotestosterone is the dominant circulating bioactive androgen during normal and premature adrenarche. *J Clin Endocrinol Metab*. 2018;103(12):4589-4598.
127. Barnard M, Quanson JL, Mostaghel E, Pretorius E, Snoep JL, Storbeck KH. 11-Oxygenated androgen precursors are the preferred substrates for aldo-keto reductase 1C3 (AKR1C3): Implications for castration resistant prostate cancer. *J Steroid Biochem Mol Biol*. 2018;183:192-201.
128. Barnard L, Schiffer L, Louw du-Toit R, Tamblyn JA, Chen S, Africander D, Arlt W, Foster PA, Storbeck KH. 11-Oxygenated Estrogens Are a Novel Class of Human Estrogens but Do not Contribute to the Circulating Estrogen Pool. *Endocrinology*. 2021;162(3):1-11.
129. Turcu AF, Mallappa A, Elman MS, Avila NA, Marko J, Rao H, Tsodikov A, Auchus RJ, Merke DP. 11-Oxygenated Androgens Are Biomarkers of Adrenal Volume and Testicular Adrenal Rest Tumors in 21-Hydroxylase Deficiency. *J Clin Endocrinol Metab*. 2017;102(8):2701-2710.
130. Davio A, Woolcock H, Nanba AT, Rege J, O'Day P, Ren J, Zhao L, Ebina H, Auchus R, Rainey WE, Turcu AF. Sex Differences in 11-Oxygenated Androgen Patterns Across Adulthood. *J Clin Endocrinol Metab*. 2020;105(8):e2921-e2929.
131. Turcu AF, El-Maouche D, Zhao L, Nanba AT, Gaynor A, Veeraraghavan P, Auchus RJ, Merke DP. Androgen excess and diagnostic steroid biomarkers for nonclassic 21-hydroxylase deficiency without cosyntropin stimulation. *Eur J Endocrinol*. 2020;183(1):63-71.

132. Storbeck KH, Schiffer L, Baranowski ES, Chortis V, Prete A, Barnard L, Gilligan LC, Taylor AE, Idkowiak J, Arlt W, Shackleton CHL. Steroid Metabolome Analysis in Disorders of Adrenal Steroid Biosynthesis and Metabolism. *Endocr Rev*. 2019;40(6):1605-1625.
133. Jones CM, Mallappa A, Reisch N, Nikolaou N, Krone N, Hughes BA, O'Neil DM, Whitaker MJ, Tomlinson JW, Storbeck KH, Merke DP, Ross RJ, Arlt W. Modified-Release and Conventional Glucocorticoids and Diurnal Androgen Excretion in Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2017;102(6):1797-1806.
134. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, Meyer-Bahlburg HFL, Miller WL, Murad MH, Oberfield SE, White PC. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2018;103(11):4043-4088.
135. Falhammar H, Wedell A, Nordenstrom A. Biochemical and genetic diagnosis of 21-hydroxylase deficiency. *Endocrine*. 2015;50(2):306-314.
136. Miller WL. Congenital Adrenal Hyperplasia: Time to Replace 17OHP with 21-Deoxycortisol. *Horm Res Paediatr*. 2019;91(6):416-420.
137. Engels M, Pijnenburg-Kleizen KJ, Utari A, Faradz SMH, Oude-Alink S, van Herwaarden AE, Span PN, Sweep FC, Claahsen-van der Grinten HL. Glucocorticoid Activity of Adrenal Steroid Precursors in Untreated Patients With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2019;104(11):5065-5072.
138. Pijnenburg-Kleizen KJ, Engels M, Mooij CF, Griffin A, Krone N, Span PN, van Herwaarden AE, Sweep FC, Claahsen-van der Grinten HL. Adrenal Steroid Metabolites Accumulating in Congenital Adrenal Hyperplasia Lead to Transactivation of the Glucocorticoid Receptor. *Endocrinology*. 2015;156(10):3504-3510.
139. Quinkler M, Meyer B, Bumke-Vogt C, Grossmann C, Gruber U, Oelkers W, Diederich S, Bahr V. Agonistic and antagonistic properties of progesterone metabolites at the human mineralocorticoid receptor. *Eur J Endocrinol*. 2002;146(6):789-799.
140. Mooij CF, Parajes S, Pijnenburg-Kleizen KJ, Arlt W, Krone N, Claahsen-van der Grinten HL. Influence of 17-Hydroxyprogesterone, Progesterone and Sex Steroids on Mineralocorticoid Receptor Transactivation in Congenital Adrenal Hyperplasia. *Hormone Research in Paediatrics*. 2015;83(6):414-421.
141. Pijnenburg-Kleizen KJ, Noordam C, Otten BJ, Claahsen-van der Grinten HL. A delayed diagnosis of salt-wasting congenital adrenal hyperplasia. *Clinical Endocrinology*. 2016;85(3):497-499.
142. Engels M, Pijnenburg-Kleizen K, Utari A, Faradz S, Heuvel J, van Herwaarden T, Span P, Sweep F, Claahsen-van der Grinten H. Elevated Concentrations of Adrenal Steroid Precursors with Glucocorticoid Activity Might Prevent Addisonian Crisis in Untreated Patients with Classic Congenital Adrenal Hyperplasia. *Hormone Research in Paediatrics*. 2018;90:127.
143. White PC, New MI, Dupont B. Structure of human steroid 21-hydroxylase genes. *Proc Natl Acad Sci U S A*. 1986;83:5111-5115.
144. Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y. Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a pseudogene and a genuine gene. *Proc Natl Acad Sci U S A*. 1986;83:2841-2845.
145. White PC, Grossberger D, Onufer BJ, Chaplin DD, New MI, Dupont B, Strominger JL. Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. *Proc Natl Acad Sci U S A*. 1985;82:1089-1093.
146. Bristow J, Gitelman SE, Tee MK, Staels B, Miller WL. Abundant adrenal-specific transcription of the human P450c21A "pseudogene". *J Biol Chem*. 1993;268:12919-12924.
147. Tee MK, Babalola GO, Aza-Blanc P, Speek M, Gitelman SE, Miller WL. A promoter within intron 35 of the human C4A gene initiates abundant adrenal-specific transcription of a 1 kb RNA: location of a cryptic CYP21 promoter element? *Hum Mol Genet*. 1995;4(11):2109-2116.
148. Bristow J, Tee MK, Gitelman SE, Mellon SH, Miller WL. Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. *J Cell Biol*. 1993;122:265-278.

149. Blanchong CA, Zhou B, Rupert KL, Chung EK, Jones KN, Sotos JF, Zipf WB, Rennebohm RM, Yung Yu C. Deficiencies of human complement component C4A and C4B and heterozygosity in length variants of RP-C4-CYP21-TNX (RCCX) modules in caucasians. The load of RCCX genetic diversity on major histocompatibility complex-associated disease. *J Exp Med*. 2000;191(12):2183-2196.
150. Yang Z, Mendoza AR, Welch TR, Zipf WB, Yu CY. Modular variations of the human major histocompatibility complex class III genes for serine/threonine kinase RP, complement component C4, steroid 21-hydroxylase CYP21, and tenascin TNX (the RCCX module). A mechanism for gene deletions and disease associations. *Journal of Biological Chemistry*. 1999;274(17):12147-12156.
151. Chen W, Xu Z, Nishitani M, Van Ryzin C, McDonnell NB, Merke DP. Complement component 4 copy number variation and CYP21A2 genotype associations in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hum Genet*. 2012;131(12):1889-1894.
152. Globerman H, Amor M, Parker KL, New MI, White PC. Nonsense mutation causing steroid 21-hydroxylase deficiency. *J Clin Invest*. 1988;82:139-144.
153. Higashi Y, Tanae A, Inoue H, Hiromasa T, Fujii-Kuriyama Y. Aberrant splicing and missense mutations cause steroid 21- hydroxylase [P-450(C21)] deficiency in humans: possible gene conversion products. *Proc Natl Acad Sci U S A*. 1988;85:7486-7490.
154. Amor M, Parker KL, Globerman H, New MI, White PC. Mutation in the CYP21B gene (Ile-172----Asn) causes steroid 21- hydroxylase deficiency. *Proc Natl Acad Sci U S A*. 1988;85:1600-1604.
155. Speiser PW, New MI, White PC. Molecular genetic analysis of nonclassic steroid 21-hydroxylase deficiency associated with HLA-B14,DR1. *N Engl J Med*. 1988;319:19-23.
156. Goto M, Hanley KP, Marcos J, Wood PJ, Wright S, Postle AD, Cameron IT, Mason JI, Wilson DI, Hanley NA. In human, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *J Clin Invest*. 2006;116:953-960.
157. Narasaka T, Suzuki T, Moriya T, Sasano H. Temporal and spatial distribution of corticosteroidogenic enzymes immunoreactivity in developing human adrenal. *Mol Cell Endocrinol*. 2001;174(1-2):111-120.
158. White PC. Ontogeny of adrenal steroid biosynthesis: why girls will be girls. *J Clin Invest*. 2006;116(4):872-874.
159. Nomura S. Immature adrenal steroidogenesis in preterm infants. *Early Hum Dev*. 1997;49(3):225-233.
160. Sasano H, White PC, New MI, Sasano N. Immunohistochemical localization of cytochrome P-450C21 in human adrenal cortex and its relation to endocrine function. *Hum Pathol*. 1988;19:181-185.
161. Zhou Z, Agarwal VR, Dixit N, White PC, Speiser PW. Steroid 21-hydroxylase expression and activity in human lymphocytes. *Mol Cell Endocrinol*. 1997;127(1):11-18.
162. Kayes-Wandover KM, White PC. Steroidogenic enzyme gene expression in the human heart. *J Clin Endocrinol Metab*. 2000;85(7):2519-2525.
163. Sirianni R, Rehman KS, Carr BR, Parker CR, Jr., Rainey WE. Corticotropin-releasing hormone directly stimulates cortisol and the cortisol biosynthetic pathway in human fetal adrenal cells. *J Clin Endocrinol Metab*. 2005;90(1):279-285.
164. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation [see comments]. [Review] [132 refs]. *N Engl J Med*. 1995;332(20):1351-1362.
165. Weber MM, Michl P, Auernhammer CJ, Engelhardt D. Interleukin-3 and interleukin-6 stimulate cortisol secretion from adult human adrenocortical cells. *Endocrinology*. 1997;138(5):2207-2210.
166. Bird IM, Mason JI, Rainey WE. Protein kinase A, protein kinase C, and Ca(2+)-regulated expression of 21-hydroxylase cytochrome P450 in H295R human adrenocortical cells. *J Clin Endocrinol Metab*. 1998;83(5):1592-1597.
167. Bassett MH, Suzuki T, Sasano H, White PC, Rainey WE. The orphan nuclear receptors NURR1 and NGFIB regulate adrenal aldosterone production. *Mol Endocrinol*. 2004;18(2):279-290.

168. Bird IM, Mason JI, Rainey WE. Battle of the kinases: integration of adrenal responses to cAMP, DG and Ca2+ at the level of steroidogenic cytochromes P450 and 3betaHSD expression in H295R cells. *Endocr Res*. 1998;24(3-4):345-354.
169. Kelly SN, McKenna TJ, Young LS. Modulation of steroidogenic enzymes by orphan nuclear transcriptional regulation may control diverse production of cortisol and androgens in the human adrenal. *J Endocrinol*. 2004;181(2):355-365.
170. Endoh A, Yang L, Hornsby PJ. CYP21 pseudogene transcripts are much less abundant than those from the active gene in normal human adrenocortical cells under various conditions in culture. *Mol Cell Endocrinol*. 1998;137(1):13-19.
171. Wenzel J, Grabinski N, Knopp CA, Dendorfer A, Ramanjaneya M, Randeva HS, Ehrhart-Bornstein M, Dominiak P, Jöhren O. Hypocretin/orexin increases the expression of steroidogenic enzymes in human adrenocortical NCI H295R cells. *Am J Physiol Regul Integr Comp Physiol*. 2009;297(5):R1601-1609.
172. Ding L, Murphy MB, He Y, Xu Y, Yeung LW, Wang J, Zhou B, Lam PK, Wu RS, Giesy JP. Effects of brominated flame retardants and brominated dioxins on steroidogenesis in H295R human adrenocortical carcinoma cell line. *Environ Toxicol Chem*. 2007;26(4):764-772.
173. Song R, He Y, Murphy MB, Yeung LW, Yu RM, Lam MH, Lam PK, Hecker M, Giesy JP, Wu RS, Zhang W, Sheng G, Fu J. Effects of fifteen PBDE metabolites, DE71, DE79 and TBBPA on steroidogenesis in the H295R cell line. *Chemosphere*. 2008;71(10):1888-1894.
174. Bláha L, Hilscherová K, Mazurová E, Hecker M, Jones PD, Newsted JL, Bradley PW, Gracia T, Duris Z, Horká I, Holoubek I, Giesy JP. Alteration of steroidogenesis in H295R cells by organic sediment contaminants and relationships to other endocrine disrupting effects. *Environ Int*. 2006;32(6):749-757.
175. Speek M, Barry F, Miller WL. Alternate promoters and alternate splicing of human tenascin-X, a gene with 5' and 3' ends buried in other genes. *Hum Mol Genet*. 1996;5(11):1749-1758.
176. Chang SF, Chung BC. Difference in transcriptional activity of two homologous CYP21A genes. *Molecular Endocrinology*. 1995;9(10):1330-1336.
177. Kyllo JH, Collins MM, Donohoue PA. Constitutive human steroid 21-hydroxylase promoter gene and pseudogene activity in steroidogenic and nonsteroidogenic cells with the luciferase gene as a reporter. *Endocrine Research*. 1995;21(4):777-791.
178. Chin KK, Chang SF. The -104G nucleotide of the human CYP21 gene is important for CYP21 transcription activity and protein interaction. *Nucleic Acids Research*. 1998;26(8):1959-1964.
179. Araújo RS, Mendonca BB, Barbosa AS, Lin CJ, Marcondes JA, Billerbeck AE, Bachega TA. Microconversion between CYP21A2 and CYP21A1P promoter regions causes the nonclassical form of 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2007;92(10):4028-4034.
180. Araujo RS, Billerbeck AE, Madureira G, Mendonca BB, Bachega TA. Substitutions in the CYP21A2 promoter explain the simple-virilizing form of 21-hydroxylase deficiency in patients harbouring a P30L mutation. *Clin Endocrinol (Oxf)*. 2005;62(2):132-136.
181. Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocr Rev*. 1997;18:361-377.
182. Schimmer BP, White PC. Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease. *Mol Endocrinol*. 2010;24(7):1322-1337.
183. Wijesuriya SD, Zhang G, Dardis A, Miller WL. Transcriptional regulatory elements of the human gene for cytochrome P450c21 (steroid 21-hydroxylase) lie within intron 35 of the linked C4B gene. *J Biol Chem*. 1999;274(53):38097-38106.
184. Wilson TE, Mouw AR, Weaver CA, Milbrandt J, Parker KL. The orphan nuclear receptor NGFI-B regulates expression of the gene encoding steroid 21-hydroxylase. *Mol Cell Biol*. 1993;13:861-868.
185. Crawford PA, Sadovsky Y, Woodson K, Lee SL, Milbrandt J. Adrenocortical function and regulation of the steroid 21-hydroxylase gene in NGFI-B-deficient mice. *Molecular & Cellular Biology*. 1995;15(8):4331-4316.

186. Fernandez PM, Brunel F, Jimenez MA, Saez JM, Cereghini S, Zakin MM. Nuclear receptors Nor1 and NGFI-B/Nur77 play similar, albeit distinct, roles in the hypothalamo-pituitary-adrenal axis. *Endocrinology*. 2000;141(7):2392-2400.
187. Tusie-Luna MT, White PC. Gene conversions and unequal crossovers between *CYP21* (steroid 21-hydroxylase gene) and *CYP21P* involve different mechanisms. *Proc Natl Acad Sci U S A*. 1995;92:10796-10800.
188. L'Allemand D, Tardy V, Grüters A, Schnabel D, Krude H, Morel Y. How a patient homozygous for a 30-kb deletion of the C4-CYP 21 genomic region can have a nonclassic form of 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2000;85(12):4562-4567.
189. Lee HH, Lee YJ, Wang YM, Chao HT, Niu DM, Chao MC, Tsai FJ, Lo FS, Lin SJ. Low frequency of the CYP21A2 deletion in ethnic Chinese (Taiwanese) patients with 21-hydroxylase deficiency. *Mol Genet Metab*. 2008;93(4):450-457.
190. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome [see comments]. *Nature Genetics*. 1997;17(1):104-108.
191. Merke DP, Chen W, Morissette R, Xu Z, Van Ryzin C, Sachdev V, Hannoush H, Shanbhag SM, Acevedo AT, Nishitani M, Arai AE, McDonnell NB. Tenascin-X haploinsufficiency associated with Ehlers-Danlos syndrome in patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2013;98(2):E379-387.
192. Gao Y, Lu L, Yu B, Mao J, Wang X, Nie M, Wu X. The Prevalence of the Chimeric TNXA/TNXB Gene and Clinical Symptoms of Ehlers-Danlos Syndrome with 21-Hydroxylase Deficiency. *J Clin Endocrinol Metab*. 2020;105(7):2288-2299.
193. Morissette R, Chen W, Perritt AF, Dreiling JL, Arai AE, Sachdev V, Hannoush H, Mallappa A, Xu Z, McDonnell NB, Quezado M, Merke DP. Broadening the Spectrum of Ehlers Danlos Syndrome in Patients With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2015;100(8):E1143-1152.
194. Miller WL, Merke DP. Tenascin-X, Congenital Adrenal Hyperplasia, and the CAH-X Syndrome. *Horm Res Paediatr*. 2018;89(5):352-361.
195. Speiser PW, Dupont J, Zhu D, Serrat J, Buegeleisen M, Tusie-Luna MT, Lesser M, New MI, White PC. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Invest*. 1992;90:584-595.
196. Wedell A, Thilen A, Ritzen EM, Stengler B, Luthman H. Mutational spectrum of the steroid 21-hydroxylase gene in Sweden: implications for genetic diagnosis and association with disease manifestation. *J Clin Endocrinol Metab*. 1994;78(5):1145-1152.
197. Barbat B, Bogyo A, Raux-Demay MC, Kuttenn F, Boue J, Simon-Bouy B, Serre JL, Mornet E. Screening of CYP21 gene mutations in 129 French patients affected by steroid 21-hydroxylase deficiency. *Hum Mut*. 1995;5(2):126-130.
198. Carrera P, Bordone L, Azzani T, Brunelli V, Garancini MP, Chiumello G, Ferrari M. Point mutations in Italian patients with classic, non-classic, and cryptic forms of steroid 21-hydroxylase deficiency. *Human Genetics*. 1996;98(6):662-665.
199. Jaaskelainen J, Levo A, Voutilainen R, Partanen J. Population-wide evaluation of disease manifestation in relation to molecular genotype in steroid 21-hydroxylase (CYP21) deficiency: good correlation in a well defined population. *J Clin Endocrinol Metab*. 1997;82(10):3293-3297.
200. Bachega TA, Billerbeck AE, Madureira G, Marcondes JA, Longui CA, Leite MV, Arnhold IJ, Mendonca BB. Molecular genotyping in Brazilian patients with the classical and nonclassical forms of 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 1998;83(12):4416-4419.
201. Krone N, Braun A, Roscher AA, Knorr D, Schwarz HP. Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany. *J Clin Endocrinol Metab*. 2000;85(3):1059-1065.
202. Baumgartner-Parzer SM, Schulze E, Waldhausl W, Pauschenwein S, Rondot S, Nowotny P, Meyer K, Frisch H, Waldhauser F, Vierhapper H. Mutational spectrum of the steroid 21-hydroxylase gene

in Austria: identification of a novel missense mutation. *J Clin Endocrinol Metab*. 2001;86(10):4771-4775.
203. Stikkelbroeck NM, Hoefsloot LH, de Wijs IJ, Otten BJ, Hermus AR, Sistermans EA. CYP21 gene mutation analysis in 198 patients with 21-hydroxylase deficiency in The Netherlands: six novel mutations and a specific cluster of four mutations. *J Clin Endocrinol Metab*. 2003;88(8):3852-3859.
204. Loidi L, Quinteiro C, Parajes S, Barreiro J, Leston DG, Cabezas-Agricola JM, Sueiro AM, Araujo-Vilar D, Catro-Feijoo L, Costas J, Pombo M, Dominguez F. High variability in CYP21A2 mutated alleles in Spanish 21-hydroxylase deficiency patients, six novel mutations and a founder effect. *Clinical Endocrinology*. 2006;64(3):330-336.
205. Friaes A, Rego AT, Aragues JM, Moura LF, Mirante A, Mascarenhas MR, Kay TT, Lopes LA, Rodrigues JC, Guerra S, Dias T, Teles AG, Goncalves J. CYP21A2 mutations in Portuguese patients with congenital adrenal hyperplasia: identification of two novel mutations and characterization of four different partial gene conversions. *Mol Genet Metab*. 2006;88(1):58-65.
206. Wilson RC, Nimkarn S, Dumic M, Obeid J, Azar M, Najmabadi H, Saffari F, New MI. Ethnic-specific distribution of mutations in 716 patients with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Mol Genet Metab*. 2007;90(4):414-421.
207. Bas F, Kayserili H, Darendeliler F, Uyguner O, Gunoz H, Yuksel Apak M, Atalar F, Bundak R, Wilson RC, New MI, Wollnik B, Saka N. CYP21A2 gene mutations in congenital adrenal hyperplasia: genotype-phenotype correlation in Turkish children. *J Clin Res Pediatr Endocrinol*. 2009;1(3):116-128.
208. Finkielstain GP, Chen W, Mehta SP, Fujimura FK, Hanna RM, Van RC, McDonnell NB, Merke DP. Comprehensive genetic analysis of 182 unrelated families with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2011;96(1):E161-E172.
209. Nermoen I, Bronstad I, Fougner KJ, Svartberg J, Oksnes M, Husebye ES, Lovas K. Genetic, anthropometric and metabolic features of adult Norwegian patients with 21-hydroxylase deficiency. *Eur J Endocrinol*. 2012;167(4):507-516.
210. Rabbani B, Mahdieh N, Ashtiani MT, Larijani B, Akbari MT, New M, Parsa A, Schouten JP, Rabbani A. Mutation analysis of the CYP21A2 gene in the Iranian population. *Genet Test Mol Biomarkers*. 2012;16(2):82-90.
211. Ben Charfeddine I, Riepe FG, Clauser E, Ayedi A, Makni S, Sfar MT, Sboui H, Kahloul N, Ben Hamouda H, Chouchane S, Trimech S, Zouari N, M'Rabet S, Amri F, Saad A, Holterhus PM, Gribaa M. Steroid 21-hydroxylase gene mutational spectrum in 50 Tunisian patients: characterization of three novel polymorphisms. *Gene*. 2012;507(1):20-26.
212. Krone N, Rose IT, Willis D, Hodson J, Wild SH, Doherty EJ, Hahner S, Parajes S, Stimson RH, Han TS, Carroll PV, Conway GS, Walker BR, MacDonald F, Ross RJ, Arlt W. Genotype-phenotype correlation in 153 adult patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency – analysis of the United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE) cohort. *J Clin Endocrinol Metab*. 2013;98(2):E346-E354.
213. New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, Sun L, Zaidi M, Wilson RC, Yuen T. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *ProcNatlAcadSciUSA*. 2013;110(7):2611-2616.
214. Parajes S, Quinteiro C, Dominguez F, Loidi L. High Frequency of Copy Number Variations and Sequence Variants at CYP21A2 Locus: Implication for the Genetic Diagnosis of 21-Hydroxylase Deficiency. *PLoS ONE*. 2008;3(5):e2138.
215. Kleinle S, Lang R, Fischer GF, Vierhapper H, Waldhauser F, Fodinger M, Baumgartner-Parzer SM. Duplications of the functional CYP21A2 gene are primarily restricted to Q318X alleles: evidence for a founder effect. *J Clin Endocrinol Metab*. 2009;94(10):3954-3958.
216. Xu C, Jia W, Cheng X, Ying H, Chen J, Xu J, Guan Q, Zhou X, Zheng D, Li G, Zhao J. Genotype-phenotype correlation study and mutational and hormonal analysis in a Chinese cohort with 21-hydroxylase deficiency. *Mol Genet Genomic Med*. 2019;7(6):e671.

217. Finkielstain GP, Kim MS, Sinaii N, Nishitani M, Van RC, Hill SC, Reynolds JC, Hanna RM, Merke DP. Clinical characteristics of a cohort of 244 patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2012;97(12):4429-4438.
218. Marino R, Ramirez P, Galeano J, Garrido NP, Rocco C, Ciaccio M, Warman DM, Guercio G, Chaler E, Maceiras M, Bergada I, Gryngarten M, Balbi V, Pardes E, Rivarola MA, Belgorosky A. Steroid 21-Hydroxylase Gene Mutational Spectrum in 454 Argentinean Patients: Genotype-Phenotype Correlation in a Large Cohort of Patients with Congenital Adrenal Hyperplasia. *Clin Endocrinol (Oxf)*. 2011;75:427-435.
219. Wedell A. Molecular genetics of congenital adrenal hyperplasia (21-hydroxylase deficiency): implications for diagnosis, prognosis and treatment. [Review] [30 refs]. *Acta Paediatrica*. 1998;87(2):159-164.
220. Hou L, Liang L, Lin S, Ou H, Liu Z, Huang S, Zhang L, Meng Z. Analysis of phenotypes and genotypes in 84 patients with 21-Hydroxylase deficiency in southern China. *Steroids*. 2019;151:108474.
221. Chiou SH, Hu MC, Chung BC. A missense mutation at Ile172----Asn or Arg356----Trp causes steroid 21-hydroxylase deficiency. *J Biol Chem*. 1990;265:3549-3552.
222. Kocova M, Anastasovska V, Falhammar H. Clinical outcomes and characteristics of P30L mutations in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocrine*. 2020;69(2):262-277.
223. Gurgov S, Bernabé KJ, Stites J, Cunniff CM, Lin-Su K, Felsen D, New MI, Poppas DP. Linking the degree of virilization in females with congenital adrenal hyperplasia to genotype. *Ann N Y Acad Sci*. 2017;1402(1):56-63.
224. Nordenstrom A. Adult women with 21-hydroxylase deficient congenital adrenal hyperplasia, surgical and psychological aspects. *Curr Opin Pediatr*. 2011;23(4):436-442.
225. Hall CM, Jones JA, Meyer-Bahlburg HF, Dolezal C, Coleman M, Foster P, Price DA, Clayton PE. Behavioral and physical masculinization are related to genotype in girls with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2004;89(1):419-424.
226. Balsamo A, Cicognani A, Baldazzi L, Barbaro M, Baronio F, Gennari M, Bal M, Cassio A, Kontaxaki K, Cacciari E. CYP21 genotype, adult height, and pubertal development in 55 patients treated for 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2003;88(12):5680-5688.
227. Meyer-Bahlburg HF, Dolezal C, Baker SW, New MI. Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. *Arch Sex Behav*. 2008;37(1):85-99.
228. Strandqvist A, Falhammar H, Lichtenstein P, Hirschberg AL, Wedell A, Norrby C, Nordenskjold A, Frisen L, Nordenstrom A. Suboptimal psychosocial outcomes in patients with congenital adrenal hyperplasia: epidemiological studies in a nonbiased national cohort in Sweden. *J Clin Endocrinol Metab*. 2014;99(4):1425-1432.
229. Lao Q, Jardin MD, Jayakrishnan R, Ernst M, Merke DP. Complement component 4 variations may influence psychopathology risk in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hum Genet*. 2018;137(11-12):955-960.
230. Haider S, Islam B, D'Atri V, Sgobba M, Poojari C, Sun L, Yuen T, Zaidi M, New MI. Structure-phenotype correlations of human CYP21A2 mutations in congenital adrenal hyperplasia. *Proc Natl Acad Sci U S A*. 2013;110(7):2605-2610.
231. Therrell BL, Padilla CD, Loeber JG, Kneisser I, Saadallah A, Borrajo GJC, Adams J. Current status of newborn screening worldwide: 2015. *Seminars in Perinatology*. 2015;39(3):171-187.
232. White PC. Neonatal screening for congenital adrenal hyperplasia. *Nat Rev Endocrinol*. 2009;5(9):490-498.
233. Pang S, Shook MK. Current status of neonatal screening for congenital adrenal hyperplasia. *Current Opinion in Pediatrics*. 1997;9(4):419-423.
234. Therrell BL. Newborn screening for congenital adrenal hyperplasia. [Review] [42 refs]. *Endocrinol Metab Clin North Am*. 2001;30(1):15-30.

235. van der Kamp HJ, Wit JM. Neonatal screening for congenital adrenal hyperplasia. *Eur J Endocrinol*. 2004;151 Suppl 3:U71-U75.
236. Speiser PW, Chawla R, Chen M, Diaz-Thomas A, Finlayson C, Rutter MM, Sandberg DE, Shimy K, Talib R, Cerise J, Vilain E, Délot EC. Newborn Screening Protocols and Positive Predictive Value for Congenital Adrenal Hyperplasia Vary across the United States. *Int J Neonatal Screen*. 2020;6(2):37.
237. Balsamo A, Cacciari E, Piazzi S, Cassio A, Bozza D, Pirazzoli P, Zappulla F. Congenital adrenal hyperplasia: neonatal mass screening compared with clinical diagnosis only in the Emilia-Romagna region of Italy, 1980-1995. *Pediatrics*. 1996;98(3 Pt 1):362-367.
238. Brosnan PG, Brosnan CA, Kemp SF, Domek DB, Jelley DH, Blackett PR, Riley WJ. Effect of newborn screening for congenital adrenal hyperplasia. *Arch Pediatr Adolesc Med*. 1999;153:1272-1278.
239. Thilen A, Nordenstrom A, Hagenfeldt L, von Dobeln U, Guthenberg C, Larsson A. Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. *Pediatrics*. 1998;101(4):E11.
240. Nordenstrom A, Ahmed S, Jones J, Coleman M, Price DA, Clayton PE, Hall CM. Female preponderance in congenital adrenal hyperplasia due to CYP21 deficiency in England: implications for neonatal screening. *Horm Res*. 2005;63(1):22-28.
241. Liu SY, Lee CT, Tung YC, Chien YH, Hwu WL, Tsai WY. Clinical characteristics of Taiwanese children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency detected by neonatal screening. *J Formos Med Assoc*. 2018;117(2):126-131.
242. Hird BE, Tetlow L, Tobi S, Patel L, Clayton PE. No evidence of an increase in early infant mortality from congenital adrenal hyperplasia in the absence of screening. *Arch Dis Child*. 2014;99(2):158-164.
243. Riedl S, Röhl FW, Bonfig W, Brämswig J, Richter-Unruh A, Fricke-Otto S, Bettendorf M, Riepe F, Kriegshäuser G, Schönau E, Even G, Hauffa B, Dörr HG, Holl RW, Mohnike K. Genotype/phenotype correlations in 538 congenital adrenal hyperplasia patients from Germany and Austria: discordances in milder genotypes and in screened versus prescreening patients. *Endocr Connect*. 2019;8(2):86-94.
244. Grosse SD, Van Vliet G. How Many Deaths Can Be Prevented by Newborn Screening for Congenital Adrenal Hyperplasia? *Hormone Research in Paediatrics*. 2007;67(6):284-291.
245. Dörr HG, Wollmann HA, Hauffa BP, Woelfle J. Mortality in children with classic congenital adrenal hyperplasia and 21-hydroxylase deficiency (CAH) in Germany. *BMC Endocr Disord*. 2018;18(1):37.
246. Van der Kamp HJ, Noordam K, Elvers B, Van Baarle M, Otten BJ, Verkerk PH. Newborn screening for congenital adrenal hyperplasia in the Netherlands. *Pediatrics*. 2001;108(6):1320-1324.
247. Fox DA, Ronsley R, Khowaja AR, Haim A, Vallance H, Sinclair G, Amed S. Clinical Impact and Cost Efficacy of Newborn Screening for Congenital Adrenal Hyperplasia. *J Pediatr*. 2020;220:101-108.e102.
248. Carroll AE, Downs SM. Comprehensive cost-utility analysis of newborn screening strategies. *Pediatrics*. 2006;117(5 Pt 2):S287-S295.
249. Chan CL, McFann K, Taylor L, Wright D, Zeitler PS, Barker JM. Congenital adrenal hyperplasia and the second newborn screen. *J Pediatr*. 2013;163(1):109-113.
250. Pang S, Hotchkiss J, Drash AL, Levine LS, New MI. Microfilter paper method for 17 alpha-hydroxyprogesterone radioimmunoassay: its application for rapid screening for congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1977;45:1003-1008.
251. Gonzalez RR, Maentausta O, Solyom J, Vihko R. Direct solid-phase time-resolved fluoroimmunoassay of 17 alpha-hydroxyprogesterone in serum and dried blood spots on filter paper. *Clin Chem*. 1990;36(9):1667-1672.

252. Gidlof S, Wedell A, Guthenberg C, von DU, Nordenstrom A. Nationwide neonatal screening for congenital adrenal hyperplasia in sweden: a 26-year longitudinal prospective population-based study. *JAMA Pediatr*. 2014;168(6):567-574.
253. Olgemoller B, Roscher AA, Liebl B, Fingerhut R. Screening for congenital adrenal hyperplasia: adjustment of 17-hydroxyprogesterone cut-off values to both age and birth weight markedly improves the predictive value. *J Clin Endocrinol Metab*. 2003;88(12):5790-5794.
254. Sarafoglou K, Gaviglio A, Hietala A, Frogner G, Banks K, McCann M, Thomas W. Comparison of newborn screening protocols for congenital adrenal hyperplasia in preterm infants. *J Pediatr*. 2014;164(5):1136-1140.
255. Jiang X, Tang F, Feng Y, Li B, Jia X, Tang C, Liu S, Huang Y. The adjustment of 17-hydroxyprogesterone cut-off values for congenital adrenal hyperplasia neonatal screening by GSP according to gestational age and age at sampling. *J Pediatr Endocrinol Metab*. 2019;32(11):1253-1258.
256. Hayashi GY, Carvalho DF, de Miranda MC, Faure C, Vallejos C, Brito VN, Rodrigues AS, Madureira G, Mendonca BB, Bachega TA. Neonatal 17-hydroxyprogesterone levels adjusted according to age at sample collection and birthweight improve the efficacy of congenital adrenal hyperplasia newborn screening. *Clin Endocrinol (Oxf)*. 2017;86(4):480-487.
257. Held PK, Shapira SK, Hinton CF, Jones E, Hannon WH, Ojodu J. Congenital adrenal hyperplasia cases identified by newborn screening in one- and two-screen states. *Mol Genet Metab*. 2015;116(3):133-138.
258. Gatelais F, Berthelot J, Beringue F, Descamps P, Bonneau D, Limal JM, Coutant R. Effect of single and multiple courses of prenatal corticosteroids on 17-hydroxyprogesterone levels: implication for neonatal screening of congenital adrenal hyperplasia. *Pediatr Res*. 2004;56(5):701-705.
259. King JL, Naber JM, Hopkin RJ, Repaske DR, Bailey L, Leslie ND. Antenatal corticosteroids and newborn screening for congenital adrenal hyperplasia. *Arch Pediatr Adolesc Med*. 2001;155(9):1038-1042.
260. Varness TS, Allen DB, Hoffman GL. Newborn screening for congenital adrenal hyperplasia has reduced sensitivity in girls. *J Pediatr*. 2005;147(4):493-498.
261. van der Linde AAA, Schönbeck Y, van der Kamp HJ, van den Akker ELT, van Albada ME, Boelen A, Finken MJJ, Hannema SE, Hoorweg-Nijman G, Odink RJ, Schielen P, Straetemans S, van Trotsenburg PS, Claahsen-van der Grinten HL, Verkerk PH. Evaluation of the Dutch neonatal screening for congenital adrenal hyperplasia. *Arch Dis Child*. 2019;104(7):653-657.
262. Sarafoglou K, Banks K, Kyllo J, Pittock S, Thomas W. Cases of congenital adrenal hyperplasia missed by newborn screening in Minnesota. *JAMA*. 2012;307(22):2371-2374.
263. Minutti CZ, Lacey JM, Magera MJ, Hahn SH, McCann M, Schulze A, Cheillan D, Dorche C, Chace DH, Lymp JF, Zimmerman D, Rinaldo P, Matern D. Steroid profiling by tandem mass spectrometry improves the positive predictive value of newborn screening for congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2004;89(8):3687-3693.
264. Rauh M, Groschl M, Rascher W, Dorr HG. Automated, fast and sensitive quantification of 17 alpha-hydroxy-progesterone, androstenedione and testosterone by tandem mass spectrometry with on-line extraction. *Steroids*. 2006;71(6):450-458.
265. Janzen N, Sander S, Terhardt M, Steuerwald U, Peter M, Das AM, Sander J. Rapid steroid hormone quantification for congenital adrenal hyperplasia (CAH) in dried blood spots using UPLC liquid chromatography-tandem mass spectrometry. *Steroids*. 2011;76(13):1437-1442.
266. Boelen A, Ruiter AF, Claahsen-van der Grinten HL, Endert E, Ackermans MT. Determination of a steroid profile in heel prick blood using LC-MS/MS. *Bioanalysis*. 2016;8(5):375-384.
267. Hines JM, Bancos I, Bancos C, Singh RD, Avula AV, Young WF, Grebe SK, Singh RJ. High-Resolution, Accurate-Mass (HRAM) Mass Spectrometry Urine Steroid Profiling in the Diagnosis of Adrenal Disorders. *Clin Chem*. 2017;63:1824-1835.

268. Kamrath C, Hartmann MF, Boettcher C, Zimmer KP, Wudy SA. Diagnosis of 21-hydroxylase deficiency by urinary metabolite ratios using gas chromatography-mass spectrometry analysis: Reference values for neonates and infants. *J Steroid Biochem Mol Biol*. 2016;156:10-16.
269. Matern D, Tortorelli S, Oglesbee D, Gavrilov D, Rinaldo P. Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: The Mayo Clinic experience (2004–2007). *Journal of Inherited Metabolic Disease*. 2007;30(4):585-592.
270. Lai F, Srinivasan S, Wiley V. Evaluation of a Two-Tier Screening Pathway for Congenital Adrenal Hyperplasia in the New South Wales Newborn Screening Programme. *Int J Neonatal Screen*. 2020;6(3):63.
271. Tieh PY, Yee JK, Hicks RA, Mao CS, Lee WN. Utility of a precursor-to-product ratio in the evaluation of presumptive positives in newborn screening of congenital adrenal hyperplasia. *J Perinatol*. 2017;37(3):283-287.
272. Schwarz E, Liu A, Randall H, Haslip C, Keune F, Murray M, Longo N, Pasquali M. Use of steroid profiling by UPLC-MS/MS as a second tier test in newborn screening for congenital adrenal hyperplasia: the Utah experience. *Pediatr Res*. 2009;66(2):230-235.
273. Seo JY, Park HD, Kim JW, Oh HJ, Yang JS, Chang YS, Park WS, Lee SY. Steroid profiling for congenital adrenal hyperplasia by tandem mass spectrometry as a second-tier test reduces follow-up burdens in a tertiary care hospital: a retrospective and prospective evaluation. *J Perinat Med*. 2014;42(1):121-127.
274. Lasarev MR, Bialk ER, Allen DB, Held PK. Application of Principal Component Analysis to Newborn Screening for Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2020.
275. De Jesús VR, Simms DA, Schiffer J, Kennedy M, Mei JV, Hannon WH. Pilot proficiency testing study for second tier congenital adrenal hyperplasia newborn screening. *Clinica Chimica Acta*. 2010;411(21-22):1684-1687.
276. Grecsó N, Zádori A, Szécsi I, Baráth Á, Galla Z, Bereczki C, Monostori P. Storage stability of five steroids and in dried blood spots for newborn screening and retrospective diagnosis of congenital adrenal hyperplasia. *PLoS One*. 2020;15(5):e0233724.
277. Fitness J, Dixit N, Webster D, Torresani T, Pergolizzi R, Speiser PW, Day DJ. Genotyping of CYP21, linked chromosome 6p markers, and a sex-specific gene in neonatal screening for congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1999;84:960-966.
278. Kosel S, Burggraf S, Fingerhut R, Dorr HG, Roscher AA, Olgemoller B. Rapid second-tier molecular genetic analysis for congenital adrenal hyperplasia attributable to steroid 21-hydroxylase deficiency. *Clin Chem*. 2005;51(2):298-304.
279. Nemeth S, Riedl S, Kriegshauser G, Baumgartner-Parzer S, Concolino P, Neocleous V, Phylactou LA, Borucka-Mankiewicz M, Onay H, Tukun A, Oberkanins C. Reverse-hybridization assay for rapid detection of common CYP21A2 mutations in dried blood spots from newborns with elevated 17-OH progesterone. *Clin Chim Acta*. 2012;414:211-4. doi: 10.1016/j.cca.2012.09.013. Epub;%2012 Sep 14.:211-214.
280. Nordenstrom A, Thilen A, Hagenfeldt L, Larsson A, Wedell A. Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency [see comments]. *J Clin Endocrinol Metab*. 1999;84(5):1505-1509.
281. Silveira EL, Elnecave RH, dos Santos EP, Moura V, Pinto EM, van der LN, I, Mendonca BB, Bachega TA. Molecular analysis of CYP21A2 can optimize the follow-up of positive results in newborn screening for congenital adrenal hyperplasia. *Clin Genet*. 2009;76(6):503-510.
282. Marino S, Perez Garrido N, Ramírez P, Pujana M, Dratler G, Belgorosky A, Marino R. Molecular analysis of the CYP21A2 gene in dried blood spot samples. *Medicina (B Aires)*. 2020;80(3):197-202.
283. Wudy SA, Schuler G, Sanchez-Guijo A, Hartmann MF. The art of measuring steroids: Principles and practice of current hormonal steroid analysis. *J Steroid Biochem Mol Biol*. 2018;179:88-103.

284. Wudy SA, Wachter UA, Homoki J, Teller WM. 17 alpha-hydroxyprogesterone, 4-androstenedione, and testosterone profiled by routine stable isotope dilution/gas chromatography-mass spectrometry in plasma of children. *Pediatr Res*. 1995;38(1):76-80.
285. Wong T, Shackleton CH, Covey TR, Ellis G. Identification of the steroids in neonatal plasma that interfere with 17 alpha-hydroxyprogesterone radioimmunoassays. *Clin Chem*. 1992;38(9):1830-1837.
286. Lange-Kubini K, Zachmann M, Kempken B, Torresani T. 15 beta-hydroxylated steroids may be diagnostically misleading in confirming congenital adrenal hyperplasia suspected by a newborn screening programme. *Early Human Development*. 1997;49(3):235-236.
287. Han L, Tavakoli NP, Morrissey M, Spink DC, Cao ZT. Liquid chromatography-tandem mass spectrometry analysis of 17-hydroxyprogesterone in dried blood spots revealed matrix effect on immunoassay. *Anal Bioanal Chem*. 2019;411(2):395-402.
288. al Saedi S, Dean H, Dent W, Stockl E, Cronin C. Screening for congenital adrenal hyperplasia: the Delfia Screening Test overestimates serum 17-hydroxyprogesterone in preterm infants. *Pediatrics*. 1996;97(1):100-102.
289. Wang R, Hartmann MF, Tiosano D, Wudy SA. Characterizing the steroidal milieu in amniotic fluid of mid-gestation: A GC-MS study. *J Steroid Biochem Mol Biol*. 2019;193:105412.
290. Akgun S, Ertel NH, Imperato-McGinley J, Sayli BS, Shackleton C. Familial male pseudohermaphroditism due to 5-alpha-reductase deficiency in a Turkish village. *Am J Med*. 1986;81:267-274.
291. Langhammer M, Michaelis M, Hartmann MF, Wudy SA, Sobczak A, Nurnberg G, Reinsch N, Schon J, Weitzel JM. Reproductive performance primarily depends on the female genotype in a two-factorial breeding experiment using high-fertility mouse lines. *Reproduction*. 2017;153(3):361-368.
292. Greaves RF, Jolly L, Hartmann MF, Ho CS, Kam RK, Joseph J, Boyder C, Wudy SA. Harmonisation of serum dihydrotestosterone analysis: establishment of an external quality assurance program. *Clin Chem Lab Med*. 2017;55(4):522-529.
293. Fanis P, Neocleous V, Kosta K, Karipiadou A, Hartmann MF, Wudy SA, Karantaglis N, Papadimitriou DT, Skordis N, Tsikopoulos G, Phylactou LA, Roilides E, Papagianni M. Late diagnosis of 3β-Hydroxysteroid dehydrogenase deficiency: the pivotal role of gas chromatography-mass spectrometry urinary steroid metabolome analysis and a novel homozygous nonsense mutation in the HSD3B2 gene. *J Pediatr Endocrinol Metab*. 2021;34:131-136.
294. Nguyen HH, Eiden-Plach A, Hannemann F, Malunowicz EM, Hartmann MF, Wudy SA, Bernhardt R. Phenotypic, metabolic, and molecular genetic characterization of six patients with congenital adrenal hyperplasia caused by novel mutations in the CYP11B1 gene. *J Steroid Biochem Mol Biol*. 2016;155(Pt A):126-134.
295. Koyama Y, Homma K, Fukami M, Miwa M, Ikeda K, Ogata T, Murata M, Hasegawa T. Classic and non-classic 21-hydroxylase deficiency can be discriminated from P450 oxidoreductase deficiency in Japanese infants by urinary steroid metabolites. *Clin Pediatr Endocrinol*. 2016;25(2):37-44.
296. Wudy SA, Hartmann MF, Remer T. Sexual dimorphism in cortisol secretion starts after age 10 in healthy children: urinary cortisol metabolite excretion rates during growth. *Am J Physiol Endocrinol Metab*. 2007;293(4):E970-976.
297. Maerker T, van WE, Overlack N, Kersten FF, McGee J, Goldmann T, Sehn E, Roepman R, Walsh EJ, Kremer H, Wolfrum U. A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. *Hum Mol Genet*. 2008;17(1):71-86.
298. Kamrath C, Wettstaedt L, Boettcher C, Hartmann MF, Wudy SA. The urinary steroidome of treated children with classic 21-hydroxylase deficiency. *J Steroid Biochem Mol Biol*. 2017;165(Pt B):396-406.
299. Kamrath C, Wettstaedt L, Hartmann MF, Wudy SA. Height Velocity defined metabolic Control in Children with Congenital Adrenal Hyperplasia using urinary GC-MS Analysis. *J Clin Endocrinol Metab*. 2019;104:4214-4224.

300. Kamrath C, Wettstaedt L, Boettcher C, Hartmann MF, Wudy SA. Androgen excess is due to elevated 11-oxygenated androgens in treated children with congenital adrenal hyperplasia. *J Steroid Biochem Mol Biol*. 2018;178:221-228.
301. Barnard L, Nikolaou N, Louw C, Schiffer L, Gibson H, Gilligan LC, Gangitano E, Snoep J, Arlt W, Tomlinson JW, Storbeck KH. The A-ring reduction of 11-ketotestosterone is efficiently catalysed by AKR1D1 and SRD5A2 but not SRD5A1. *J Steroid Biochem Mol Biol*. 2020;202:105724.
302. Tiosano D, Navon R, Flor O, Knopf C, Hartmann MF, Wudy SA, Yakhini Z, Hochberg Z. A steroid metabolomic approach to 17α-hydroxylase/17,20 lyase deficiency. *Metabolomics*. 2010;6(3):417-426.
303. Gawlik A, Shmoish M, Hartmann MF, Malecka-Tendera E, Wudy SA, Hochberg Z. Steroid Metabolomic Disease Signature of Nonsyndromic Childhood Obesity. *J Clin Endocrinol Metab*. 2016;101(11):4329-4337.
304. Vitkin E, Ben-Dor A, Shmoish M, Hartmann MF, Yakhini Z, Wudy SA, Hochberg Z. Peer group normalization and urine to blood context in steroid metabolomics: the case of CAH and obesity. *Steroids*. 2014;88:83-89.
305. Kamrath C, Hartmann MF, Pons-Kühnemann J, Wudy SA. Urinary GC-MS steroid metabotyping in treated children with congenital adrenal hyperplasia. *Metabolism*. 2020;112:154354.
306. Sanchez-Guijo A, Oji V, Hartmann MF, Traupe H, Wudy SA. Simultaneous quantification of cholesterol sulfate, androgen sulfates, and progestagen sulfates in human serum by LC-MS/MS. *J Lipid Res*. 2015;56(9):1843-1851.
307. Kulle A, Krone N, Holterhus PM, Schuler G, Greaves RF, Juul A, de Rijke YB, Hartmann MF, Saba A, Hiort O, Wudy SA, Action EC. Steroid hormone analysis in diagnosis and treatment of DSD: position paper of EU COST Action BM 1303 'DSDnet'. *Eur J Endocrinol*. 2017;176(5):P1-P9.
308. White PC, Vitek A, Dupont B, New MI. Characterization of frequent deletions causing steroid 21-hydroxylase deficiency. *Proc Natl Acad Sci U S A*. 1988;85:4436-4440.
309. Ghizzoni L, Cappa M, Vottero A, Ubertini G, Carta D, Di Iorgi N, Gasco V, Marchesi M, Raggi V, Ibba A, Napoli F, Massimi A, Maghnie M, Loche S, Porzio O. Relationship of CYP21A2 genotype and serum 17-hydroxyprogesterone and cortisol levels in a large cohort of Italian children with premature pubarche. *Eur J Endocrinol*. 2011;165(2):307-314.
310. Chan AO, But WM, Ng KL, Wong LM, Lam YY, Tiu SC, Lee KF, Lee CY, Loung PY, Berry IR, Brown R, Charlton R, Cheng CW, Ho YC, Tse WY, Shek CC. Molecular analysis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Hong Kong Chinese patients. *Steroids*. 2011;76(10-11):1057-1062.
311. Jang JH, Jin DK, Kim JH, Tan HK, Kim JW, Lee SY, Ki CS, Park HD. Multiplex ligation-dependent probe amplification assay for diagnosis of congenital adrenal hyperplasia. *Ann Clin Lab Sci*. 2011;41(1):44-47.
312. Simpson JL, Rechitsky S. Prenatal genetic testing and treatment for congenital adrenal hyperplasia. *Fertil Steril*. 2019;111(1):21-23.
313. Forest MG, Dorr HG. Prenatal treatment of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency: European experience in 223 pregnancies at risk. *Ped Res*. 1993;33:S3.
314. Frasier SD, Thorneycroft IH, Weiss BA, Horton R. Letter: Elevated amniotic fluid concentration of 17 alpha-hydroxyprogesterone in congenital adrenal hyperplasia. *J Pediatr*. 1975;86(2):310-312.
315. Jeffcoate TN, Fliegner JR, Russell SH, Davis JC, Wade AP. Diagnosis of the adrenogenital syndrome before birth. *Lancet*. 1965;2(7412):553-555.
316. Pollack MS, Maurer D, Levine LS, New MI, Pang S, Duchon M, Owens RP, Merkatz IR, Nitowsky BM, Sachs G, Dupont B. Prenatal diagnosis of congenital adrenal hyperplasia (21- hydroxylase deficiency) by HLA typing. *Lancet*. 1979;1:1107-1108.
317. Forest MG, Dorr HG, Espe. Prenatal therapy in congenital adrenal hyperplasia due to 21-hydroxylase deficiency: Retrospective follow-up study of 253 treated pregnancies in 215 families. *Endocrinologist*. 2003;13(3):252-259.

318. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. *Ultrasound Obstet Gynecol*. 2019;54(4):442-451.
319. Lo YM. Fetal DNA in maternal plasma: biology and diagnostic applications. *Clin Chem*. 2000;46(12):1903-1906.
320. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. *Lancet*. 1997;350(9076):485-487.
321. Simpson JL, Elias S. Isolating fetal cells from maternal blood. Advances in prenatal diagnosis through molecular technology. *JAMA*. 1993;270(19):2357-2361.
322. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A*. 1996;93(2):705-708.
323. Devaney SA, Palomaki GE, Scott JA, Bianchi DW. Noninvasive fetal sex determination using cell-free fetal DNA: a systematic review and meta-analysis. *JAMA*. 2011;306(6):627-636.
324. Tardy-Guidollet V, Menassa R, Costa JM, David M, Bouvattier-Morel C, Baumann C, Houang M, Lorenzini F, Philip N, Odent S, Guichet A, Morel Y. New management strategy of pregnancies at risk of congenital adrenal hyperplasia using fetal sex determination in maternal serum: French cohort of 258 cases (2002-2011). *J Clin Endocrinol Metab*. 2014;99(4):1180-1188.
325. New MI, Tong YK, Yuen T, Jiang P, Pina C, Chan KC, Khattab A, Liao GJ, Yau M, Kim SM, Chiu RW, Sun L, Zaidi M, Lo YM. Noninvasive prenatal diagnosis of congenital adrenal hyperplasia using cell-free fetal DNA in maternal plasma. *J Clin Endocrinol Metab*. 2014;99(6):E1022-1030.
326. Dondorp W, de Wert G. Refining the ethics of preimplantation genetic diagnosis: A plea for contextualized proportionality. *Bioethics*. 2019;33(2):294-301.
327. Simpson JL. Preimplantation genetic diagnosis at 20 years. *Prenat Diagn*. 2010;30(7):682-695.
328. Clayton PE, Miller WL, Oberfield SE, Ritzen EM, Sippell WG, Speiser PW. Consensus statement on 21-hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. *Horm Res*. 2002;58(4):188-195.
329. Merke DP, Bornstein SR, Avila NA, Chrousos GP. NIH conference. Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Ann Intern Med*. 2002;136(4):320-334.
330. Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, Hellman L. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J Clin Endocrinol Metab*. 1971;33(1):14-22.
331. Michaud K, Matheson K, Kelly O, Anisman H. Impact of stressors in a natural context on release of cortisol in healthy adult humans: a meta-analysis. *Stress*. 2008;11(3):177-197.
332. Bryan SM, Honour JW, Hindmarsh PC. Management of altered hydrocortisone pharmacokinetics in a boy with congenital adrenal hyperplasia using a continuous subcutaneous hydrocortisone infusion. *J Clin Endocrinol Metab*. 2009;94(9):3477-3480.
333. Merza Z, Rostami-Hodjegan A, Memmott A, Doane A, Ibbotson V, Newell-Price J, Tucker GT, Ross RJ. Circadian hydrocortisone infusions in patients with adrenal insufficiency and congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2006;65(1):45-50.
334. El-Maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia. *Lancet*. 2017(17):10-6736.
335. Lajic S, Nordenstrom A, Ritzen EM, Wedell A. Prenatal treatment of congenital adrenal hyperplasia. *Eur J Endocrinol*. 2004;151:Suppl-9.
336. Nordenstrom A, Marcus C, Axelson M, Wedell A, Ritzen EM. Failure of cortisone acetate treatment in congenital adrenal hyperplasia because of defective 11beta-hydroxysteroid dehydrogenase reductase activity. *J Clin Endocrinol Metab*. 1999;84(4):1210-1213.
337. Charmandari E, Chrousos GP, Merke DP. Adrenocorticotropin hypersecretion and pituitary microadenoma following bilateral adrenalectomy in a patient with classic 21-hydroxylase deficiency. *J Pediatr Endocrinol Metab*. 2005;18(1):97-101.
338. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. *Lancet*. 2005;365(9477):2125-2136.

339. Melin J, Parra-Guillen ZP, Michelet R, Truong T, Huisinga W, Hartung N, Hindmarsh P, Kloft C. Pharmacokinetic/Pharmacodynamic Evaluation of Hydrocortisone Therapy in Pediatric Patients with Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2020;105(3):e1729-e1740.
340. German A, Suraiya S, Tenenbaum-Rakover Y, Koren I, Pillar G, Hochberg Z. Control of childhood congenital adrenal hyperplasia and sleep activity and quality with morning or evening glucocorticoid therapy. *J Clin Endocrinol Metab*. 2008;93(12):4707-4710.
341. Ng SM, Stepien KM, Krishan A. Glucocorticoid replacement regimens for treating congenital adrenal hyperplasia. *Cochrane Database Syst Rev*. 2020;3:CD012517.
342. Auchus RJ, Witchel SF, Leight KR, Aisenberg J, Azziz R, Bachega TA, Baker LA, Baratz AB, Baskin LS, Berenbaum SA, Breault DT, Cerame BI, Conway GS, Eugster EA, Fracassa S, Gearhart JP, Geffner ME, Harris KB, Hurwitz RS, Katz AL, Kalro BN, Lee PA, Alger LG, Loechner KJ, Marshall I, Merke DP, Migeon CJ, Miller WL, Nenadovich TL, Oberfield SE, Pass KA, Poppas DP, Lloyd-Puryear MA, Quigley CA, Riepe FG, Rink RC, Rivkees SA, Sandberg DE, Schaeffer TL, Schlussel RN, Schneck FX, Seely EW, Snyder D, Speiser PW, Therrell BL, VanRyzin C, Vogiatzi MG, Wajnrajch MP, White PC, Zuckerman AE. Guidelines for the Development of Comprehensive Care Centers for Congenital Adrenal Hyperplasia: Guidance from the CARES Foundation Initiative. *Int J Pediatr Endocrinol*. 2010;2010:275213. Epub;%2011 Jan 10.:275213.
343. Pijnenburg-Kleizen KJ, Thomas CMG, Otten BJ, Roeleveld N, Claahsen-van der Grinten HL. Long-term follow-up of children with classic congenital adrenal hyperplasia: suggestions for age dependent treatment in childhood and puberty. *J Pediatr Endocrinol Metab*. 2019;32(10):1055-1063.
344. Barillas JE, Eichner D, Van Wagoner R, Speiser PW. Iatrogenic Cushing Syndrome in a Child With Congenital Adrenal Hyperplasia: Erroneous Compounding of Hydrocortisone. *J Clin Endocrinol Metab*. 2018;103(1):7-11.
345. Neumann U, Burau D, Spielmann S, Whitaker MJ, Ross RJ, Kloft C, Blankenstein O. Quality of compounded hydrocortisone capsules used in the treatment of children. *Eur J Endocrinol*. 2017;177(2):239-242.
346. Merke DP, Cho D, Calis KA, Keil MF, Chrousos GP. Hydrocortisone suspension and hydrocortisone tablets are not bioequivalent in the treatment of children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2001;86(1):441-445.
347. Porter J, Withe M, Ross RJ. Immediate-release granule formulation of hydrocortisone, Alkindi(R), for treatment of paediatric adrenal insufficiency (Infacort development programme). *Expert Rev Endocrinol Metab*. 2018;13(3):119-124.
348. Neumann U, Whitaker MJ, Wiegand S, Krude H, Porter J, Davies M, Digweed D, Voet B, Ross RJ, Blankenstein O. Absorption and tolerability of taste-masked hydrocortisone granules in neonates, infants and children under 6 years of age with adrenal insufficiency. *Clin Endocrinol (Oxf)*. 2018;88(1):21-29.
349. Frisch H, Battelino T, Schober E, Baumgartner-Parzer S, Nowotny P, Vierhapper H. Salt wasting in simple virilizing congenital adrenal hyperplasia. *J Pediatr Endocrinol Metab*. 2001;14(9):1649-1655.
350. Nimkarn S, Lin-Su K, Berglind N, Wilson RC, New MI. Aldosterone-to-renin ratio as a marker for disease severity in 21-hydroxylase deficiency congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2007;92(1):137-142.
351. Mullis PE, Hindmarsh PC, Brook CG. Sodium chloride supplement at diagnosis and during infancy in children with salt-losing 21-hydroxylase deficiency. *Eur J Pediatr*. 1990;150:22-25.
352. Bonfig W, Roehl F, Riedl S, Bramswig J, Richter-Unruh A, Fricke-Otto S, Hubner A, Bettendorf M, Schonau E, Dorr H, Holl RW, Mohnike K. Sodium Chloride Supplementation Is Not Routinely Performed in the Majority of German and Austrian Infants with Classic Salt-Wasting Congenital Adrenal Hyperplasia and Has No Effect on Linear Growth and Hydrocortisone or Fludrocortisone Dose. *Horm Res Paediatr*. 2018;89(1):7-12.

353. Hindmarsh PC, Charmandari E. Variation in absorption and half-life of hydrocortisone influence plasma cortisol concentrations. *Clin Endocrinol (Oxf)*. 2015;82(4):557-561.
354. Punthakee Z, Legault L, Polychronakos C. Prednisolone in the treatment of adrenal insufficiency: a re-evaluation of relative potency. *J Pediatr*. 2003;143(3):402-405.
355. Rivkees SA, Crawford JD. Dexamethasone treatment of virilizing congenital adrenal hyperplasia: the ability to achieve normal growth. *Pediatrics*. 2000;106(4):767-773.
356. Bonfig W, Bechtold S, Schmidt H, Knorr D, Schwarz HP. Reduced final height outcome in congenital adrenal hyperplasia under prednisone treatment: deceleration of growth velocity during puberty. *J Clin Endocrinol Metab*. 2007;92(5):1635-1639.
357. Charmandari E, Hindmarsh PC, Johnston A, Brook CG. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: alterations in cortisol pharmacokinetics at puberty. *J Clin Endocrinol Metab*. 2001;86(6):2701-2708.
358. Gleeson H, Davis J, Jones J, O'Shea E, Clayton PE. The challenge of delivering endocrine care and successful transition to adult services in adolescents with congenital adrenal hyperplasia: experience in a single centre over 18 years. *Clin Endocrinol (Oxf)*. 2013;78(1):23-28.
359. Bachelot A. Transition of Care from Childhood to Adulthood: Congenital Adrenal Hyperplasia. *Endocr Dev*. 2018;33:17-33.
360. Arlt W, Willis DS, Wild SH, Krone N, Doherty EJ, Hahner S, Han TS, Carroll PV, Conway GS, Rees DA, Stimson RH, Walker BR, Connell JM, Ross RJ. Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab*. 2010;95(11):5110-5121.
361. de Jesus LE, Costa EC, Dekermacher S. Gender dysphoria and XX congenital adrenal hyperplasia: how frequent is it? Is male-sex rearing a good idea? *J Pediatr Surg*. 2019;54(11):2421-2427.
362. Engberg H, Möller A, Hagenfeldt K, Nordenskjöld A, Frisén L. Identity, Sexuality, and Parenthood in Women with Congenital Adrenal Hyperplasia. *Journal of pediatric and adolescent gynecology*. 2020;33(5):470-476.
363. Jiang X, Xu C, Lei F, Liao M, Wang W, Xu N, Zhang Y, Xie W. MiR-30a targets IL-1alpha and regulates islet functions as an inflammation buffer and response factor. *Sci Rep*. 2017;7(1):5270-05560.
364. Hughes IA. Congenital adrenal hyperplasia: transitional care. *Growth Horm IGF Res*. 2004;14 Suppl A:S60-66.
365. Reisch N. Review of Health Problems in Adult Patients with Classic Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. *Exp Clin Endocrinol Diabetes*. 2019;127(2-03):171-177.
366. Paizoni L, Auer MK, Schmidt H, Hübner A, Bidlingmaier M, Reisch N. Effect of androgen excess and glucocorticoid exposure on metabolic risk profiles in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Steroid Biochem Mol Biol*. 2020;197:105540.
367. Auer MK, Paizoni L, Hofbauer LC, Rauner M, Chen Y, Schmidt H, Huebner A, Bidlingmaier M, Reisch N. Effects of androgen excess and glucocorticoid exposure on bone health in adult patients with 21-hydroxylase deficiency. *J Steroid Biochem Mol Biol*. 2020;204:105734.
368. Falhammar H, Frisen L, Norrby C, Hirschberg AL, Almqvist C, Nordenskjold A, Nordenstrom A. Increased mortality in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2014;99(12):E2715-E2721.
369. Whittle E, Falhammar H. Glucocorticoid Regimens in the Treatment of Congenital Adrenal Hyperplasia: A Systematic Review and Meta-Analysis. *J Endocr Soc*. 2019;3(6):1227-1245.
370. Riepe FG, Krone N, Viemann M, Partsch CJ, Sippell WG. Management of congenital adrenal hyperplasia: results of the ESPE questionnaire. *Horm Res*. 2002;58(4):196-205.
371. Auchus RJ. Management considerations for the adult with congenital adrenal hyperplasia. *Mol Cell Endocrinol*. 2015;408:190-197.
372. Hagenfeldt K, Janson PO, Holmdahl G, Falhammar H, Filipsson H, Frisén L, Thorén M, Nordenskjöld A. Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hum Reprod*. 2008;23(7):1607-1613.

373. Han TS, Stimson RH, Rees DA, Krone N, Willis DS, Conway GS, Arlt W, Walker BR, Ross RJ. Glucocorticoid treatment regimen and health outcomes in adults with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2013;78(2):197-203.
374. Xu L, Lin W, Cai L, Huang H, Liang J, Li L, Zong L, Wang N, Wen J, Chen G. Efficacy and safety of prenatal dexamethasone treatment in offspring at risk for congenital adrenal hyperplasia due to 21-hydroxylase deficiency: A systematic review and meta-analysis. *Clin Endocrinol (Oxf)*. 2020;92(2):109-123.
375. Miller WL. Fetal endocrine therapy for congenital adrenal hyperplasia should not be done. *Best Pract Res Clin Endocrinol Metab*. 2015;29(3):469-483.
376. New MI, Abraham M, Yuen T, Lekarev O. An update on prenatal diagnosis and treatment of congenital adrenal hyperplasia. *Semin Reprod Med*. 2012;30(5):396-399.
377. Hirvikoski T, Nordenstrom A, Wedell A, Ritzen M, Lajic S. Prenatal dexamethasone treatment of children at risk for congenital adrenal hyperplasia: the Swedish experience and standpoint. *J Clin Endocrinol Metab*. 2012;97(6):1881-1883.
378. Engels M, Span PN, van Herwaarden AE, Sweep F, Stikkelbroeck N, Claahsen-van der Grinten HL. Testicular Adrenal Rest Tumors: Current Insights on Prevalence, Characteristics, Origin, and Treatment. *Endocr Rev*. 2019;40(4):973-987.
379. Lottspeich C, Müller-Lisse U, Seiler L, Schmitt-Graeff AH, Reincke M, Reisch N. Three Cases of Testicular Adrenal Rest Tumors in Congenital Adrenal Hyperplasia-A Diagnostic and Therapeutic Challenge. *Urology*. 2019;129:24-28.
380. Johannsson G, Nilsson AG, Bergthorsdottir R, Burman P, Dahlqvist P, Ekman B, Engstrom BE, Olsson T, Ragnarsson O, Ryberg M, Wahlberg J, Biller BM, Monson JP, Stewart PM, Lennernas H, Skrtic S. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. *J Clin Endocrinol Metab*. 2012;97(2):473-481.
381. Isidori AM, Venneri MA, Graziadio C, Simeoli C, Fiore D, Hasenmajer V, Sbardella E, Gianfrilli D, Pozza C, Pasqualetti P, Morrone S, Santoni A, Naro F, Colao A, Pivonello R, Lenzi A. Effect of once-daily, modified-release hydrocortisone versus standard glucocorticoid therapy on metabolism and innate immunity in patients with adrenal insufficiency (DREAM): a single-blind, randomised controlled trial. *Lancet Diabetes Endocrinol*. 2018;6(3):173-185.
382. Plat L, Leproult R, L'Hermite-Baleriaux M, Fery F, Mockel J, Polonsky KS, Van Cauter E. Metabolic effects of short-term elevations of plasma cortisol are more pronounced in the evening than in the morning. *J Clin Endocrinol Metab*. 1999;84(9):3082-3092.
383. Debono M, Ghobadi C, Rostami-Hodjegan A, Huatan H, Campbell MJ, Newell-Price J, Darzy K, Merke DP, Arlt W, Ross RJ. Modified-release hydrocortisone to provide circadian cortisol profiles. *J Clin Endocrinol Metab*. 2009;94(5):1548-1554.
384. Whitaker M, Debono M, Huatan H, Merke D, Arlt W, Ross RJ. An oral multiparticulate, modified-release, hydrocortisone replacement therapy that provides physiological cortisol exposure. *Clin Endocrinol (Oxf)*. 2014;80(4):554-561.
385. Merke DP, Mallappa A, Arlt W, de la Perriere AB, Hirschberg AL, Juul A, Newell-Price JDC, Perry CG, Prete A, Rees A, Reisch N, Stikkelbroeck M, Touraine PA, Maltby K, Treasure P, Porter J, Ross RJM. OR25-02 A Phase 3 Study of a Modified-Release Hydrocortisone in the Treatment of Congenital Adrenal Hyperplasia. *Journal of the Endocrine Society*. 2020;4(Supplement_1).
386. Pofi R, Prete A, Thornton-Jones V, Bryce J, Ali SR, Faisal Ahmed S, Balsamo A, Baronio F, Cannuccia A, Guven A, Guran T, Darendeliler F, Higham C, Bonfig W, de Vries L, Bachega T, Miranda MC, Mendonca BB, Iotova V, Korbonits M, Krone NP, Krone R, Lenzi A, Arlt W, Ross RJ, Isidori AM, Tomlinson JW. Plasma Renin Measurements are Unrelated to Mineralocorticoid Replacement Dose in Patients With Primary Adrenal Insufficiency. *J Clin Endocrinol Metab*. 2020;105(1):314-326.

387. Turcu AF, Rege J, Chomic R, Liu J, Nishimoto HK, Else T, Moraitis AG, Palapattu GS, Rainey WE, Auchus RJ. Profiles of 21-Carbon Steroids in 21-hydroxylase Deficiency. *J Clin Endocrinol Metab*. 2015;100(6):2283-2290.
388. de Groot MJ, Pijnenburg-Kleizen KJ, Thomas CM, Sweep FC, Stikkelbroeck NM, Otten BJ, Claahsen-van der Grinten HL. Salivary morning androstenedione and 17alpha-OH progesterone levels in childhood and puberty in patients with classic congenital adrenal hyperplasia. *Clin Chem Lab Med*. 2015;53(3):461-468.
389. Bode HH, Rivkees SA, Cowley DM, Pardy K, Johnson S. Home monitoring of 17 hydroxyprogesterone levels in congenital adrenal hyperplasia with filter paper blood samples [see comments]. *Journal of Pediatrics*. 1999;134(2):185-189.
390. Wieacker I, Peter M, Borucki K, Empting S, Roehl FW, Mohnike K. Therapy monitoring in congenital adrenal hyperplasia by dried blood samples. *J Pediatr Endocrinol Metab*. 2015;28(7-8):867-871.
391. Debono M, Mallappa A, Gounden V, Nella AA, Harrison RF, Crutchfield CA, Backlund PS, Soldin SJ, Ross RJ, Merke DP. Hormonal circadian rhythms in patients with congenital adrenal hyperplasia: identifying optimal monitoring times and novel disease biomarkers. *Eur J Endocrinol*. 2015;173(6):727-737.
392. Bacila I, Adaway J, Hawley J, Mahdi S, Krone R, Patel L, Alvi S, Randell T, Gevers E, Dattani M, Cheetham T, Kyriakou A, Schiffer L, Ryan F, Crowne E, Davies JH, Ahmed SF, Keevil B, Krone N. Measurement of Salivary Adrenal-Specific Androgens as Biomarkers of Therapy Control in 21-Hydroxylase Deficiency. *J Clin Endocrinol Metab*. 2019;104(12):6417-6429.
393. Gaudl A, Kratzsch J, Ceglarek U. Advancement in steroid hormone analysis by LC-MS/MS in clinical routine diagnostics - A three year recap from serum cortisol to dried blood 17α-hydroxyprogesterone. *J Steroid Biochem Mol Biol*. 2019;192:105389.
394. Jenkins-Jones S, Parviainen L, Porter J, Withe M, Whitaker MJ, Holden SE, Morgan CL, Currie CJ, Ross RJM. Poor compliance and increased mortality, depression and healthcare costs in patients with congenital adrenal hyperplasia. *Eur J Endocrinol*. 2018;178(4):309-320.
395. Hahner S, Spinnler C, Fassnacht M, Burger-Stritt S, Lang K, Milovanovic D, Beuschlein F, Willenberg HS, Quinkler M, Allolio B. High incidence of adrenal crisis in educated patients with chronic adrenal insufficiency: a prospective study. *J Clin Endocrinol Metab*. 2015;100(2):407-416.
396. Odenwald B, Nennstiel-Ratzel U, Dorr HG, Schmidt H, Wildner M, Bonfig W. Children with classic congenital adrenal hyperplasia experience salt loss and hypoglycemia: evaluation of adrenal crises during the first 6 years of life. *Eur J Endocrinol*. 2016;174(2):177-186.
397. Reisch N, Willige M, Kohn D, Schwarz HP, Allolio B, Reincke M, Quinkler M, Hahner S, Beuschlein F. Frequency and causes of adrenal crises over lifetime in patients with 21-hydroxylase deficiency. *Eur J Endocrinol*. 2012;167(1):35-42.
398. Yang M, White PC. Risk factors for hospitalization of children with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2017;86(5):669-673.
399. Rushworth RL, Falhammar H, Munns CF, Maguire AM, Torpy DJ. Hospital Admission Patterns in Children with CAH: Admission Rates and Adrenal Crises Decline with Age. *Int J Endocrinol*. 2016;2016:5748264.
400. Ali SR, Bryce J, Haghpanahan H, Lewsey JD, Tan LE, Atapattu N, Birkebaek NH, Blankenstein O, Neumann U, Balsamo A, Ortolano R, Bonfig W, Claahsen-van der Grinten HL, Cools M, Costa EC, Darendeliler F, Poyrazoglu S, Elsedfy H, Finken MJJ, Fluck CE, Gevers E, Korbonits M, Guaragna-Filho G, Guran T, Guven A, Hannema SE, Higham C, Hughes IA, Tadokoro-Cuccaro R, Thankamony A, Iotova V, Krone NP, Krone R, Lichiardopol C, Luczay A, Mendonca BB, Bachega T, Miranda MC, Milenkovic T, Mohnike K, Nordenstrom A, Einaudi S, van der Kamp H, Vieites A, de Vries L, Ross RJM, Ahmed SF. Real World Estimates Of Adrenal Insufficiency Related Adverse Events In Children With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2021;106:e192-e203.

401. Tresoldi AS, Sumilo D, Perrins M, Toulis KA, Prete A, Reddy N, Wass JAH, Arlt W, Nirantharakumar K. Increased Infection Risk in Addison's Disease and Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2020;105(2):418-429.
402. El-Maouche D, Hargreaves CJ, Sinaii N, Mallappa A, Veeraraghavan P, Merke DP. Longitudinal Assessment of Illnesses, Stress Dosing, and Illness Sequelae in Patients With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2018;103(6):2336-2345.
403. Donaldson MD, Thomas PH, Love JG, Murray GD, McNinch AW, Savage DC. Presentation, acute illness, and learning difficulties in salt wasting 21-hydroxylase deficiency. *Arch Dis Child*. 1994;70(3):214-218.
404. Merke DP, Chrousos GP, Eisenhofer G, Weise M, Keil MF, Rogol AD, Van Wyk JJ, Bornstein SR. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *N Engl J Med*. 2000;343(19):1362-1368.
405. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2016;101(2):364-389.
406. Allolio B. Extensive expertise in endocrinology. Adrenal crisis. *Eur J Endocrinol*. 2015;172(3):R115-124.
407. Aso K, Izawa M, Higuchi A, Kotoh S, Hasegawa Y. Stress doses of glucocorticoids cannot prevent progression of all adrenal crises. *Clin Pediatr Endocrinol*. 2009;18(1):23-27.
408. Chrisp GL, Torpy DJ, Maguire AM, Quartararo M, Falhammar H, King BR, Munns CF, Hameed S, Rushworth RL. The effect of patient-managed stress dosing on electrolytes and blood pressure in acute illness in children with adrenal insufficiency. *Clin Endocrinol (Oxf)*. 2020;93(2):97-103.
409. Rushworth RL, Torpy DJ, Falhammar H. Adrenal Crisis. *N Engl J Med*. 2019;381(9):852-861.
410. Prete A, Taylor AE, Bancos I, Smith DJ, Foster MA, Kohler S, Fazal-Sanderson V, Komninos J, O'Neil DM, Vassiliadi DA, Mowatt CJ, Mihai R, Fallowfield JL, Annane D, Lord JM, Keevil BG, Wass JAH, Karavitaki N, Arlt W. Prevention of Adrenal Crisis: Cortisol Responses to Major Stress Compared to Stress Dose Hydrocortisone Delivery. *J Clin Endocrinol Metab*. 2020;105(7):2262-2274.
411. Taylor LK, Auchus RJ, Baskin LS, Miller WL. Cortisol response to operative stress with anesthesia in healthy children. *J Clin Endocrinol Metab*. 2013;98(9):3687-3693.
412. Bidet M, Bellanne-Chantelot C, Galand-Portier MB, Tardy V, Billaud L, Laborde K, Coussieu C, Morel Y, Vaury C, Golmard JL, Claustre A, Mornet E, Chakhtoura Z, Mowszowicz I, Bachelot A, Touraine P, Kuttenn F. Clinical and molecular characterization of a cohort of 161 unrelated women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency and 330 family members. *J Clin Endocrinol Metab*. 2009;94(5):1570-1578.
413. Nandagopal R, Sinaii N, Avila NA, Van Ryzin C, Chen W, Finkielstain GP, Mehta SP, McDonnell NB, Merke DP. Phenotypic profiling of parents with cryptic nonclassic congenital adrenal hyperplasia: findings in 145 unrelated families. *Eur J Endocrinol*. 2011;164(6):977-984.
414. Hahner S, Allolio B. Therapeutic management of adrenal insufficiency. *Best Pract Res Clin Endocrinol Metab*. 2009;23(2):167-179.
415. Repping-Wuts HJ, Stikkelbroeck NM, Noordzij A, Kerstens M, Hermus AR. A glucocorticoid education group meeting: an effective strategy for improving self-management to prevent adrenal crisis. *Eur J Endocrinol*. 2013;169(1):17-22.
416. Dorr HG, Schulze N, Bettendorf M, Binder G, Bonfig W, Denzer C, Dunstheimer D, Salzgeber K, Schmidt H, Schwab KO, Voss E, Wabitsch M, Wolfle J. Genotype-phenotype correlations in children and adolescents with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Mol Cell Pediatr*. 2020;7(1):8.
417. McCann-Crosby B CM, Lyons SK, Lin Y, Axelrad M, Dietrich JE, Sutton VR, Macias CG, Gunn S, Karaviti L. Non classic CAH; targets of treatment and transition. *Pediatric Endocrine Review*. 2014;12:224-238.

418. Speiser PW. Medical treatment of classic and nonclassic congenital adrenal hyperplasia. *Adv Exp Med Biol*. 2011;707:41-45.
419. Witchel SF. Non-classic congenital adrenal hyperplasia. *Steroids*. 2013;78(8):747-750.
420. New M. Extensive clinical-experience - Nonclassical 21-hydroxylase deficiency (vol 91, pg 4205, 2006). *Journal of Clinical Endocrinology & Metabolism*. 2007;92(1):142-142.
421. Pijnenburg-Kleizen KJ, Borm GF, Otten BJ, Schott DA, van den Akker EL, Stokvis-Brantsma WH, Voorhoeve PG, Bakker B, Claahsen-van der Grinten HL. Absence of Clinically Relevant Growth Acceleration in Untreated Children with Non-Classical Congenital Adrenal Hyperplasia. *Hormone Research in Paediatrics*. 2012;77(3):164-169.
422. Claahsen-van der Grinten HL, Noordam K, Borm GF, Otten BJ. Absence of increased height velocity in the first year of life in untreated children with simple virilizing congenital adrenal hyperplasia. *Journal of Clinical Endocrinology & Metabolism*. 2006;91(4):1205-1209.
423. Eyal O, Tenenbaum-Rakover Y, Shalitin S, Israel S, Weintrob N. Adult height of subjects with nonclassical 21-hydroxylase deficiency. *Acta Paediatr*. 2013;102(4):419-423.
424. New MI, Gertner JM, Speiser PW, del Balzo P. Growth and final height in classical and nonclassical 21hydroxylase deficiency. *J Endocrinol Invest*. 1989;12(S3):915-995.
425. Trinh L, Nimkarn S, New MI, Lin-Su K. Growth and pubertal characteristics in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Pediatr Endocrinol Metab*. 2007;20(8):883-891.
426. Claahsen-van der Grinten HL, Stikkelbroeck NMML, Otten BJ, Hermus ARMM. Congenital adrenal hyperplasia - Pharmacologic interventions from the prenatal phase to adulthood. *Pharmacology & Therapeutics*. 2011;132(1):1-14.
427. Moran C, Azziz R, Carmina E, Dewailly D, Fruzzetti F, Ibanez L, Knochenhauer ES, Marcondes JA, Mendonca BB, Pignatelli D, Pugeat M, Rohmer V, Speiser PW, Witchel SF. 21-Hydroxylase-deficient nonclassic adrenal hyperplasia is a progressive disorder: a multicenter study. *Am J Obstet Gynecol*. 2000;183(6):1468-1474.
428. Livadas S, Bothou C. Management of the Female With Non-classical Congenital Adrenal Hyperplasia (NCCAH): A Patient-Oriented Approach. *Front Endocrinol (Lausanne)*. 2019;10:366.
429. Nordenström A, Falhammar H. MANAGEMENT OF ENDOCRINE DISEASE: Diagnosis and management of the patient with non-classic CAH due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2019;180(3):R127-R145.
430. Buitenwerf E, Links TP, Kema IP, Haadsma ML, Kerstens MN. Congenital adrenal hyperplasia as a cause of adrenal incidentaloma. *Neth J Med*. 2017;75(7):298-300.
431. Jaresch S, Kornely E, Kley HK, Schlaghecke R. Adrenal incidentaloma and patients with homozygous or heterozygous congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1992;74(3):685-689.
432. Falhammar H, Torpy DJ. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency presenting as adrenal incidentaloma: a systematic review and meta-analysis. *Endocr Pract*. 2016;22(6):736-752.
433. van Zuuren EJ, Fedorowicz Z, Carter B, Pandis N. Interventions for hirsutism (excluding laser and photoepilation therapy alone). *Cochrane Database Syst Rev*. 2015(4):CD010334.
434. Hamzavi I, Tan E, Shapiro J, Lui H. A randomized bilateral vehicle-controlled study of eflornithine cream combined with laser treatment versus laser treatment alone for facial hirsutism in women. *J Am Acad Dermatol*. 2007;57(1):54-59.
435. Spritzer P, Billaud L, Thalabard JC, Birman P, Mowszowicz I, Raux-Demay MC, Clair F, Kuttenn F, Mauvais-Jarvis P. Cyproterone acetate versus hydrocortisone treatment in late-onset adrenal hyperplasia. *J Clin Endocrinol Metab*. 1990;70(3):642-646.
436. van Zuuren EJ, Fedorowicz Z, Carter B, Andriolo RB, Schoones J. Interventions for female pattern hair loss. *Cochrane Database Syst Rev*. 2012(5):CD007628.

437. Falhammar H, Nystrom HF, Ekstrom U, Granberg S, Wedell A, Thoren M. Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia. *Eur J Endocrinol*. 2012;166(3):441-449.
438. Engels M, Gehrmann K, Falhammar H, Webb EA, Nordenstrom A, Sweep FC, Span PN, van Herwaarden AE, Rohayem J, Richter-Unruh A, Bouvattier C, Kohler B, Kortmann BB, Arlt W, Roeleveld N, Reisch N, Stikkelbroeck N, Claahsen-van der Grinten HL, dsd Lg. Gonadal function in adult male patients with congenital adrenal hyperplasia. *Eur J Endocrinol*. 2018;178(3):285-294.
439. Kocova M, Janevska V, Anastasovska V. Testicular adrenal rest tumors in boys with 21-hydroxylase deficiency, timely diagnosis and follow-up. *Endocr Connect*. 2018;7(4):544-552.
440. Mercado AB, Wilson RC, Cheng KC, Wei JQ, New MI. Prenatal treatment and diagnosis of congenital adrenal hyperplasia owing to steroid 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 1995;80(7):2014-2020.
441. Merce Fernandez-Balsells M, Muthusamy K, Smushkin G, Lampropulos JF, Elamin MB, Abu Elnour NO, Elamin KB, Agrwal N, Gallegos-Orozco JF, Lane MA, Erwin PJ, Montori VM, Murad MH. Prenatal dexamethasone use for the prevention of virilization in pregnancies at risk for classical congenital adrenal hyperplasia because of 21-hydroxylase (CYP21A2) deficiency: a systematic review and meta-analyses. *Clin Endocrinol (Oxf)*. 2010;73(4):436-444.
442. Seckl JR, Miller WL. How safe is longterm prenatal glucocorticoid treatment? *JAMA*. 1997;277:1077-1079.
443. McCann-Crosby B, Placencia FX, Adeyemi-Fowode O, Dietrich J, Franciskovich R, Gunn S, Axelrad M, Tu D, Mann D, Karaviti L, Sutton VR. Challenges in Prenatal Treatment with Dexamethasone. *Pediatr Endocrinol Rev*. 2018;16(1):186-193.
444. Witchel SF, Miller WL. Prenatal treatment of congenital adrenal hyperplasia-not standard of care. *J Genet Couns*. 2012;21(5):615-624.
445. Dreger A, Feder EK, Tamar-Mattis A. Prenatal Dexamethasone for Congenital Adrenal Hyperplasia: An Ethics Canary in the Modern Medical Mine. *J Bioeth Inq*. 2012;9(3):277-294.
446. Heland S, Hewitt JK, McGillivray G, Walker SP. Preventing female virilisation in congenital adrenal hyperplasia: The controversial role of antenatal dexamethasone. *Aust N Z J Obstet Gynaecol*. 2016;56(3):225-232.
447. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, Meyer-Bahlburg HF, Miller WL, Montori VM, Oberfield SE, Ritzen M, White PC. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2010;95(9):4133-4160.
448. Ziejewski MK, Solomon HM, Stanislaus D, Clark RL, White TE, Apostoli AR. The potential role for corticosterone in the induction of cleft palate in mice after treatment with a selective NK-1 receptor antagonist, casopitant (GW679769B). *Birth Defects Res B Dev Reprod Toxicol*. 2012;95(1):54-62.
449. Celsi G, Kistner A, Aizman R, Eklof AC, Ceccatelli S, de Santiago A, Jacobson SH. Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. *Pediatr Res*. 1998;44(3):317-322.
450. Dickinson H, Walker DW, Wintour EM, Moritz K. Maternal dexamethasone treatment at midgestation reduces nephron number and alters renal gene expression in the fetal spiny mouse. *Am J Physiol Regul Integr Comp Physiol*. 2007;292(1):R453-461.
451. Manojlovic-Stojanoski MN, Filipovic BR, Nestorovic NM, Sosic-Jurjevic BT, Ristic NM, Trifunovic SL, Milosevic V. Morpho-functional characteristics of rat fetal thyroid gland are affected by prenatal dexamethasone exposure. *Steroids*. 2014;84:22-29.
452. Lindsay RS, Lindsay RM, Edwards CR, Seckl JR. Inhibition of 11-beta-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. *Hypertension*. 1996;27(6):1200-1204.
453. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and

glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest*. 1998;101(10):2174-2181.
454. Drake AJ, Raubenheimer PJ, Kerrigan D, McInnes KJ, Seckl JR, Walker BR. Prenatal dexamethasone programs expression of genes in liver and adipose tissue and increased hepatic lipid accumulation but not obesity on a high-fat diet. *Endocrinology*. 2010;151(4):1581-1587.
455. de Vries A, Holmes MC, Heijnis A, Seier JV, Heerden J, Louw J, Wolfe-Coote S, Meaney MJ, Levitt NS, Seckl JR. Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic pituitary-adrenal axis function. *J Clin Invest*. 2007;117(4):1058-1067.
456. Lindsay RS, Lindsay RM, Waddell BJ, Seckl JR. Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia*. 1996;39(11):1299-1305.
457. Holmes MC, Abrahamsen CT, French KL, Paterson JM, Mullins JJ, Seckl JR. The mother or the fetus? 11beta-hydroxysteroid dehydrogenase type 2 null mice provide evidence for direct fetal programming of behavior by endogenous glucocorticoids. *J Neurosci*. 2006;26(14):3840-3844.
458. Huang WL, Beazley LD, Quinlivan JA, Evans SF, Newnham JP, Dunlop SA. Effect of corticosteroids on brain growth in fetal sheep. *Obstet Gynecol*. 1999;94(2):213-218.
459. Moss TJ, Doherty DA, Nitsos I, Sloboda DM, Harding R, Newnham JP. Effects into adulthood of single or repeated antenatal corticosteroids in sheep. *Am J Obstet Gynecol*. 2005;192(1):146-152.
460. Quinlivan JA DS, Newnham JP, Evans SF, Beazley LD. Repeated, but not single, maternal administration of corticosteroids delays myelination in the brain of fetal sheep. *Perinat Neonat Med* 1999;4:47-45.
461. Uno H, Lohmiller L, Thieme C, Kemnitz JW, Engle MJ, Roecker EB, Farrell PM. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. *Brain Res Dev Brain Res*. 1990;53(2):157-167.
462. Heberden C, Meffray E, Goustard-Langelier B, Maximin E, Lavialle M. Dexamethasone inhibits the maturation of newly formed neurons and glia supplemented with polyunsaturated fatty acids. *J Steroid Biochem Mol Biol*. 2013;138:395-402.
463. Crudo A, Suderman M, Moisiadis VG, Petropoulos S, Kostaki A, Hallett M, Szyf M, Matthews SG. Glucocorticoid programming of the fetal male hippocampal epigenome. *Endocrinology*. 2013;154(3):1168-1180.
464. Iqbal M, Moisiadis VG, Kostaki A, Matthews SG. Transgenerational effects of prenatal synthetic glucocorticoids on hypothalamic-pituitary-adrenal function. *Endocrinology*. 2012;153(7):3295-3307.
465. Quinn TA, Ratnayake U, Castillo-Melendez M, Moritz KM, Dickinson H, Walker DW. Adrenal steroidogenesis following prenatal dexamethasone exposure in the spiny mouse. *J Endocrinol*. 2014;221(2):347-362.
466. Crudo A, Petropoulos S, Moisiadis VG, Iqbal M, Kostaki A, Machnes Z, Szyf M, Matthews SG. Prenatal synthetic glucocorticoid treatment changes DNA methylation states in male organ systems: multigenerational effects. *Endocrinology*. 2012;153(7):3269-3283.
467. White PC, Mune T, Agarwal AK. 11β-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev*. 1997;18:135-156.
468. Xiao WL, Liu XY, Liu YS, Zhang DZ, Xue LF. The relationship between maternal corticosteroid use and orofacial clefts-a meta-analysis. *Reprod Toxicol*. 2017;69:99-105.
469. Wapner RJ, Sorokin Y, Mele L, Johnson F, Dudley DJ, Spong CY, Peaceman AM, Leveno KJ, Malone F, Caritis SN, Mercer B, Harper M, Rouse DJ, Thorp JM, Ramin S, Carpenter MW, Gabbe SG. Long-term outcomes after repeat doses of antenatal corticosteroids. *N Engl J Med*. 2007;357(12):1190-1198.
470. Forest MG, Nicolino M, David M, Morel Y. The virilized female: endocrine background. *BJU Int*. 2004;93 Suppl 3:35-43.

471. New MI, Carlson A, Obeid J, Marshall I, Cabrera MS, Goseco A, Lin-Su K, Putnam AS, Wei JQ, Wilson RC. Prenatal diagnosis for congenital adrenal hyperplasia in 532 pregnancies. *J Clin Endocrinol Metab*. 2001;86(12):5651-5657.
472. Lajic S, Wedell A, Bui TH, Ritzen EM, Holst M. Long-term somatic follow-up of prenatally treated children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1998;83(11):3872-3880.
473. Grunt S, Steinlin M, Weisstanner C, Schöning M, Mullis PE, Flück CE. Acute encephalopathy with unilateral cortical-subcortical lesions in two unrelated kindreds treated with glucocorticoids prenatally for congenital adrenal hyperplasia due to 21-hydroxylase deficiency: established facts and novel insight. *Horm Res Paediatr*. 2013;80(1):57-63.
474. Rijk Y, van Alfen-van der Velden J, Claahsen-van der Grinten HL. Prenatal Treatment with Dexamethasone in Suspected Congenital Adrenal Hyperplasia and Orofacial Cleft: a Case Report and Review of the Literature. *Pediatr Endocrinol Rev*. 2017;15(1):21-25.
475. Trautman PD, Meyer-Bahlburg HF, Postelnek J, New MI. Effects of early prenatal dexamethasone on the cognitive and behavioral development of young children: results of a pilot study. *Psychoneuroendocrinology*. 1995;20(4):439-449.
476. Meyer-Bahlburg HF, Dolezal C, Baker SW, Carlson AD, Obeid JS, New MI. Cognitive and motor development of children with and without congenital adrenal hyperplasia after early-prenatal dexamethasone. *J Clin Endocrinol Metab*. 2004;89(2):610-614.
477. Hirvikoski T, Nordenstrom A, Lindholm T, Lindblad F, Ritzen EM, Wedell A, Lajic S. Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. *J Clin Endocrinol Metab*. 2007;92(2):542-548.
478. Hirvikoski T, Nordenstrom A, Lindholm T, Lindblad F, Ritzen EM, Lajic S. Long-term follow-up of prenatally treated children at risk for congenital adrenal hyperplasia: does dexamethasone cause behavioural problems? *Eur J Endocrinol*. 2008;159(3):309-316.
479. Hirvikoski T, Lindholm T, Lajic S, Nordenstrom A. Gender role behaviour in prenatally dexamethasone-treated children at risk for congenital adrenal hyperplasia--a pilot study. *Acta Paediatr*. 2011;100(9):e112-e119.
480. Meyer-Bahlburg HF, Dolezal C, Haggerty R, Silverman M, New MI. Cognitive outcome of offspring from dexamethasone-treated pregnancies at risk for congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2012;167(1):103-110.
481. Maryniak A, Ginalska-Malinowska M, Bielawska A, Ondruch A. Cognitive and social function in girls with congenital adrenal hyperplasia - influence of prenatally administered dexamethasone. *Child Neuropsychol*. 2014;20(1):60-70.
482. Wallensteen L, Zimmermann M, Thomsen SM, Gezelius A, Nordenstrom A, Hirvikoski T, Lajic S. Sex-Dimorphic Effects of Prenatal Treatment With Dexamethasone. *J Clin Endocrinol Metab*. 2016;101(10):3838-3846.
483. Wallensteen L, Karlsson L, Messina V, Gezelius A, Sandberg MT, Nordenström A, Hirvikoski T, Lajic S. Evaluation of behavioral problems after prenatal dexamethasone treatment in Swedish children and adolescents at risk of congenital adrenal hyperplasia. *Hormones and behavior*. 2018;98:219-224.
484. Karlsson L, Nordenstrom A, Hirvikoski T, Lajic S. Prenatal dexamethasone treatment in the context of at risk CAH pregnancies: Long-term behavioral and cognitive outcome. *Psychoneuroendocrinology*. 2018;91:68-74.
485. Karlsson L, Barbaro M, Ewing E, Gomez-Cabrero D, Lajic S. Epigenetic Alterations Associated With Early Prenatal Dexamethasone Treatment. *J Endocr Soc*. 2019;3(1):250-263.
486. Riveline JP, Baz B, Nguewa JL, Vidal-Trecan T, Ibrahim F, Boudou P, Vicaut E, Brac de la Perriere A, Fetita S, Breant B, Blondeau B, Tardy-Guidollet V, Morel Y, Gautier JF. Exposure to Glucocorticoids in the First Part of Fetal Life is Associated with Insulin Secretory Defect in Adult Humans. *J Clin Endocrinol Metab*. 2020;105(3).

487. Wallensteen L, Karlsson L, Messina V, Nordenstrom A, Lajic S. Perturbed Beta-Cell Function and Lipid Profile After Early Prenatal Dexamethasone Exposure in Individuals Without CAH. *J Clin Endocrinol Metab*. 2020;105(7):e2439-e2448.
488. Karlsson L, Gezelius A, Nordenstrom A, Hirvikoski T, Lajic S. Cognitive impairment in adolescents and adults with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2017;87:651-659.
489. Van't Westeinde A, Karlsson L, Nordenström A, Padilla N, Lajic S. First trimester prenatal dexamethasone treatment is associated with alterations in brain structure at adult age. *J Clin Endocrinol Metab*. 2020;105(8):2575-2586.
490. Ilg L, Kirschbaum C, Li SC, Rosenlöcher F, Miller R, Alexander N. Persistent Effects of Antenatal Synthetic Glucocorticoids on Endocrine Stress Reactivity From Childhood to Adolescence. *J Clin Endocrinol Metab*. 2019;104(3):827-834.
491. Räikkönen K, Gissler M, Kajantie E. Associations Between Maternal Antenatal Corticosteroid Treatment and Mental and Behavioral Disorders in Children. *JAMA*. 2020;323(19):1924-1933.
492. Mallappa A, Sinaii N, Kumar P, Whitaker MJ, Daley LA, Digweed D, Eckland DJ, Van Ryzin C, Nieman LK, Arlt W, Ross RJ, Merke DP. A phase 2 study of Chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2015;100(3):1137-1145.
493. Nella AA, Mallappa A, Perritt AF, Gounden V, Kumar P, Sinaii N, Daley LA, Ling A, Liu CY, Soldin SJ, Merke DP. A Phase 2 Study of Continuous Subcutaneous Hydrocortisone Infusion in Adults With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2016;101(12):4690-4698.
494. Merke DP, Keil MF, Jones JV, Fields J, Hill S, Cutler GB, Jr. Flutamide, testolactone, and reduced hydrocortisone dose maintain normal growth velocity and bone maturation despite elevated androgen levels in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2000;85(3):1114-1120.
495. Auchus RJ, Sharifi N. Sex Hormones and Prostate Cancer. *Annu Rev Med*. 2020;71(Jan 27):33-45.
496. Auchus RJ, Osborne E, Hammer GD, Chang AY, Ramm C, Madrigal D, Yu M. Abiraterone acetate added to physiologic hydrocortisone dosing safely controls androgen excess in adult women with classical 21-hydroxylase deficiency. *Endocr Rev*. 2012;33:SUN-LB2.
497. Wright C, O'Day P, Alyamani M, Sharifi N, Auchus R. Abiraterone acetate treatment lowers 11-oxygenated androgens. *Eur J Endocrinol*. 2020;182(Apr(4)):413-421.
498. Turcu AF, Spencer-Segal JL, Farber RH, Luo R, Grigoriadis DE, Ramm CA, Madrigal D, Muth T, O'Brien CF, Auchus RJ. Single-Dose Study of a Corticotropin-Releasing Factor Receptor-1 Antagonist in Women With 21-Hydroxylase Deficiency. *J Clin Endocrinol Metab*. 2016;101(3):1174-1180.
499. Gehrand AL, Phillips J, Malott K, Raff H. A Long-Acting Neutralizing Monoclonal ACTH Antibody Blocks Corticosterone and Adrenal Gene Responses in Neonatal Rats. *Endocrinology*. 2019;160(7):1719-1730.
500. Sanders K, Mol JA, Kooistra HS, Galac S. Melanocortin 2 receptor antagonists in canine pituitary-dependent hypercortisolism: in vitro studies. *Vet Res Commun*. 2018;42(4):283-288.
501. Turcu AF, Auchus RJ. Novel treatment strategies in congenital adrenal hyperplasia. *Curr Opin Endocrinol Diabetes Obes*. 2016;23(3):225-232.
502. Van Wyk JJ, Gunther DF, Ritzén EM, Wedell A, Cutler GB, Jr, Migeon CJ, New MI. The use of adrenalectomy as a treatment for congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1996;81:3180-3190.
503. MacKay D, Nordenström A, Falhammar H. Bilateral Adrenalectomy in Congenital Adrenal Hyperplasia: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2018;103(5):1767-1778.
504. Crocker MK, Barak S, Millo CM, Beall SA, Niyyati M, Chang R, Avila NA, Van Ryzin C, Segars J, Quezado M, Merke DP. Use of PET/CT with cosyntropin stimulation to identify and localize adrenal rest tissue following adrenalectomy in a woman with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2012;97(11):E2084-E2089.

505. Burman P, Falhammar H, Waldenström E, Sundin A, Bitzén U. 11C-metomidate PET/CT detected multiple ectopic adrenal rest tumors in a woman with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2020:e675-e679.
506. Bry-Gauillard H, Cartes A, Young J. Mitotane for 21-hydroxylase deficiency in an infertile man. *N Engl J Med*. 2014;371(21):2042-2044.
507. El-Maouche D, Merke DP, Vogiatzi MG, Chang AY, Turcu AF, Joyal EG, Lin VH, Weintraub L, Plaunt MR, Mohideen P, Auchus RJ. A Phase 2, Multicenter Study of Nevanimibe for the Treatment of Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2020;105(8):2771-2778.
508. Quintos JB, Vogiatzi MG, Harbison MD, New MI. Growth hormone therapy alone or in combination with gonadotropin-releasing hormone analog therapy to improve the height deficit in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2001;86(4):1511-1517.
509. Lin-Su K, Harbison MD, Lekarev O, Vogiatzi MG, New MI. Final adult height in children with congenital adrenal hyperplasia treated with growth hormone. *J Clin Endocrinol Metab*. 2011;96(6):1710-1717.
510. Lin-Su K, Vogiatzi MG, Marshall I, Harbison MD, Macapagal MC, Betensky B, Tansil S, New MI. Treatment with growth hormone and luteinizing hormone releasing hormone analog improves final adult height in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2005;90(6):3318-3325.
511. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663-676.
512. Aghazadeh Y, Nostro MC. Cell Therapy for Type 1 Diabetes: Current and Future Strategies. *Curr Diab Rep*. 2017;17(6):37.
513. Vierbuchen T, Wernig M. Direct lineage conversions: unnatural but useful? *Nat Biotechnol*. 2011;29(10):892-907.
514. Ruiz-Babot G, Hadjidemetriou I, King PJ, Guasti L. New directions for the treatment of adrenal insufficiency. *Front Endocrinol (Lausanne)*. 2015;6:70.
515. Ruiz-Babot G, Balyura M, Hadjidemetriou I, Ajodha SJ, Taylor DR, Ghataore L, Taylor NF, Schubert U, Ziegler CG, Storr HL, Druce MR, Gevers EF, Drake WM, Srirangalingam U, Conway GS, King PJ, Metherell LA, Bornstein SR, Guasti L. Modeling Congenital Adrenal Hyperplasia and Testing Interventions for Adrenal Insufficiency Using Donor-Specific Reprogrammed Cells. *Cell Rep*. 2018;22(5):1236-1249.
516. Shiroishi T, Sagai T, Natsuume-Sakai S, Moriwaki K. Lethal deletion of the complement component C4 and steroid 21-hydroxylase genes in the mouse H-2 class III region, caused by meiotic recombination. *Proc Natl Acad Sci U S A*. 1987;84(9):2819-2823.
517. Tajima T, Okada T, Ma XM, Ramsey W, Bornstein S, Aguilera G. Restoration of adrenal steroidogenesis by adenovirus-mediated transfer of human cytochromeP450 21-hydroxylase into the adrenal gland of21-hydroxylase-deficient mice. *Gene Ther*. 1999;6(11):1898-1903.
518. Naiki Y, Miyado M, Horikawa R, Katsumata N, Onodera M, Pang S, Ogata T, Fukami M. Extra-adrenal induction of Cyp21a1 ameliorates systemic steroid metabolism in a mouse model of congenital adrenal hyperplasia. *Endocr J*. 2016;63(10):897-904.
519. Perdomini M, Dos Santos C, Goumeaux C, Blouin V, Bougneres P. An AAVrh10-CAG-CYP21-HA vector allows persistent correction of 21-hydroxylase deficiency in a Cyp21(-/-) mouse model. *Gene Ther*. 2017;24(5):275-281.
520. Markmann S, De BP, Reid J, Jose CL, Rosenberg JB, Leopold PL, Kaminsky SM, Sondhi D, Pagovich O, Crystal RG. Biology of the Adrenal Gland Cortex Obviates Effective Use of Adeno-Associated Virus Vectors to Treat Hereditary Adrenal Disorders. *Hum Gene Ther*. 2018;29(4):403-412.
521. Jordan-Young RM. Hormones, context, and "brain gender": a review of evidence from congenital adrenal hyperplasia. *Soc Sci Med*. 2012;74(11):1738-1744.
522. Hines M. Human gender development. *Neuroscience & Biobehavioral Reviews*. 2020;118:89-96.

523. Berenbaum SA. Beyond Pink and Blue: The Complexity of Early Androgen Effects on Gender Development. *Child Development Perspectives*. 2018;12(1):58-64.
524. Meyer-Bahlburg HFL. Psychoendocrinology of congenital adrenal hyperplasia. In: New MI, Lekarev O, Parsa A, O'Malley B, Hammer GD, eds. Genetic Steroid Disorders. London: Elsevier; 2014:285-300.
525. Stout SA, Litvak M, Robbins NM, Sandberg DE. Congenital adrenal hyperplasia: classification of studies employing psychological endpoints. *Int J Pediatr Endocrinol*. 2010;2010:191520.
526. Stein RE. The 1990s: a decade of change in understanding children with ongoing conditions. *Arch Pediatr Adolesc Med*. 2011;165(10):880-883.
527. Sandberg DE, Mazur T. A noncategorical approach to the psychosocial care of persons with DSD and their families. In: Kreukels BPC, Steensma TD, de Vries ALC, eds. Gender Dysphoria and Disorders of Sex Development: Springer US; 2014:93-114.
528. Almasri J, Zaiem F, Rodriguez-Gutierrez R, Tamhane SU, Iqbal AM, Prokop LJ, Speiser PW, Baskin LS, Bancos I, Murad MH. Genital Reconstructive Surgery in Females With Congenital Adrenal Hyperplasia: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2018;103(11):4089-4096.
529. Cousino MK, Hazen RA. Parenting stress among caregivers of children with chronic illness: a systematic review. *J Pediatr Psychol*. 2013;38(8):809-828.
530. Driscoll KA, Johnson SB, Barker D, Quittner AL, Deeb LC, Geller DE, Gondor M, Silverstein JH. Risk factors associated with depressive symptoms in caregivers of children with type 1 diabetes or cystic fibrosis. *J Pediatr Psychol*. 2010;35(8):814-822.
531. Hansen JA, Weissbrod C, Schwartz DD, Taylor WP. Paternal involvement in pediatric Type 1 diabetes: fathers' and mothers' psychological functioning and disease management. *Fam Syst Health*. 2012;30(1):47-59.
532. Barakat LP, Patterson CA, Daniel LC, Dampier C. Quality of life among adolescents with sickle cell disease: mediation of pain by internalizing symptoms and parenting stress. *Health Qual Life Outcomes*. 2008;6:60.
533. Helgeson VS, Becker D, Escobar O, Siminerio L. Families with children with diabetes: implications of parent stress for parent and child health. *J Pediatr Psychol*. 2012;37(4):467-478.
534. Colletti CJ, Wolfe-Christensen C, Carpentier MY, Page MC, McNall-Knapp RY, Meyer WH, Chaney JM, Mullins LL. The relationship of parental overprotection, perceived vulnerability, and parenting stress to behavioral, emotional, and social adjustment in children with cancer. *Pediatr Blood Cancer*. 2008;51(2):269-274.
535. Mullins LL, Wolfe-Christensen C, Hoff Pai AL, Carpentier MY, Gillaspy S, Cheek J, Page M. The Relationship of Parental Overprotection, Perceived Child Vulnerability, and Parenting Stress to Uncertainty in Youth with Chronic Illness. *Journal of Pediatric Psychology*. 2007;32(8):973-982.
536. Tluczek A, McKechnie AC, Brown RL. Factors associated with parental perception of child vulnerability 12 months after abnormal newborn screening results. *Res Nurs Health*. 2011;34(5):389-400.
537. Bourdeau TL, Mullins LL, Carpentier MY, Colletti CJM, Wolfe-Christensen C. An examination of parenting variables and child self-care behavior across disease groups. *Journal of Developmental and Physical Disabilities*. 2007;19(2):125-134.
538. Litzelman K, Catrine K, Gangnon R, Witt WP. Quality of life among parents of children with cancer or brain tumors: the impact of child characteristics and parental psychosocial factors. *Qual Life Res*. 2011;20(8):1261-1269.
539. Monaghan MC, Hilliard ME, Cogen FR, Streisand R. Nighttime caregiving behaviors among parents of young children with Type 1 diabetes: associations with illness characteristics and parent functioning. *Fam Syst Health*. 2009;27(1):28-38.
540. Pinquart M. Body image of children and adolescents with chronic illness: a meta-analytic comparison with healthy peers. *Body Image*. 2013;10(2):141-148.

541. Pinquart M. Self-esteem of children and adolescents with chronic illness: a meta-analysis. *Child Care Health Dev*. 2013;39(2):153-161.
542. Brady AM, Deighton J, Stansfeld S. Chronic illness in childhood and early adolescence: A longitudinal exploration of co-occurring mental illness. *Dev Psychopathol*. 2020;May 4:1-14.
543. Sentenac M, Arnaud C, Gavin A, Molcho M, Gabhainn SN, Godeau E. Peer victimization among school-aged children with chronic conditions. *Epidemiol Rev*. 2012;34(1):120-128.
544. Lum A, Wakefield CE, Donnan B, Burns MA, Fardell JE, Marshall GM. Understanding the school experiences of children and adolescents with serious chronic illness: a systematic meta-review. *Child Care Health Dev*. 2017;43(5):645-662.
545. Pinquart M, Shen Y. Behavior problems in children and adolescents with chronic physical illness: a meta-analysis. *J Pediatr Psychol*. 2011;36(9):1003-1016.
546. Secinti E, Thompson EJ, Richards M, Gaysina D. Research Review: Childhood chronic physical illness and adult emotional health - a systematic review and meta-analysis. *J Child Psychol Psychiatry*. 2017;58(7):753-769.
547. Engberg H, Butwicka A, Nordenstrom A, Hirschberg AL, Falhammar H, Lichtenstein P, Nordenskjold A, Frisen L, Landen M. Congenital adrenal hyperplasia and risk for psychiatric disorders in girls and women born between 1915 and 2010: A total population study. *Psychoneuroendocrinology*. 2015;60:195-205.
548. Gray WN, Schaefer MR, Resmini-Rawlinson A, Wagoner ST. Barriers to Transition From Pediatric to Adult Care: A Systematic Review. *J Pediatr Psychol*. 2018;43(5):488-502.
549. Fleming L, Knafl K, Van Riper M. How the Child's Gender Matters for Families Having a Child With Congenital Adrenal Hyperplasia. *J Fam Nurs*. 2017;23(4):516-533.
550. Meyer-Bahlburg HFL, Khuri J, Reyes-Portillo J, New MI. Stigma in Medical Settings As Reported Retrospectively by Women With Congenital Adrenal Hyperplasia (CAH) for Their Childhood and Adolescence. *J Pediatr Psychol*. 2017;42(5):496-503.
551. Beatty JE. Career Barriers Experienced by People with Chronic Illness: A U.S. Study. *Employee Responsibilities and Rights Journal*. 2011;24(2):91-110.
552. Crissman HP, Warner L, Gardner M, Carr M, Schast A, Quittner AL, Kogan B, Sandberg DE. Children with disorders of sex development: A qualitative study of early parental experience. *Int J Pediatr Endocrinol*. 2011;2011(1):10.
553. Meyer-Bahlburg HFL, Khuri J, Reyes-Portillo J, Ehrhardt AA, New MI. Stigma Associated with Classical Congenital Adrenal Hyperplasia in Women's Sexual Lives. *Archives of sexual behavior*. 2018;47(4):943-951.
554. Meyer-Bahlburg HFL, Khuri J, Reyes-Portillo J, Ehrhardt AA, New MI. Stigma Associated with Classical Congenital Adrenal Hyperplasia in Women's Sexual Lives. *Arch Sex Behav*. 2017:10-1003.
555. Rolston AM, Gardner M, Vilain E, Sandberg DE. Parental Reports of Stigma Associated with Child's Disorder of Sex Development. *Int J Endocrinol*. 2015;2015:980121.
556. Armstrong KL, Henderson C, Hoan NT, Warne GL. Living with congenital adrenal hyperplasia in Vietnam: a survey of parents. *J Pediatr Endocrinol Metab*. 2006;19(10):1207-1223.
557. Ediati A, Juniarto AZ, Birnie E, Okkerse J, Wisniewski A, Drop S, Faradz SMH, Dessens A. Social stigmatisation in late identified patients with disorders of sex development in Indonesia. *BMJ Paediatrics Open*. 2017;1(1):e000130.
558. de Silva KSH, de Zoysa P, Dilanka WMS, Dissanayake BS. Psychological impact on parents of children with congenital adrenal hyperplasia: a study from Sri Lanka. *J Pediatr Endocrinol Metab*. 2014;27(5-6):475.
559. Zainuddin AA, Grover SR, Soon CH, Ghani NAA, Mahdy ZA, Manaf MRA, Shamsuddin K. A Multicenter Cross-Sectional Study of Malaysian Females With Congenital Adrenal Hyperplasia: Their Body Image and Their Perspectives on Feminizing Surgery. *Journal of pediatric and adolescent gynecology*. 2020;33:477-483.

560. Zainuddin AA, Grover SR, Shamsuddin K, Mahdy ZA. Research on quality of life in female patients with congenital adrenal hyperplasia and issues in developing nations. *Journal of pediatric and adolescent gynecology*. 2013;26(6):296-304.
561. Zainuddin AA, Grover SR, Soon CH, Nur Azurah AG, Mahdy ZA, Wu LL, Rasat R, Harun F, Chia WY, Shamsuddin K. Malaysian Females With Congenital Adrenal Hyperplasia: Surgical Outcomes and Attitudes. *Front Pediatr*. 2019;7(144):144.
562. Weidler EM, Peterson KE. The impact of culture on disclosure in differences of sex development. *Semin Pediatr Surg*. 2019;28(5):150840.
563. Qadir F, Khan MM, Medhin G, Prince M. Male gender preference, female gender disadvantage as risk factors for psychological morbidity in Pakistani women of childbearing age - a life course perspective. *BMC Public Health*. 2011;11(1):745.
564. Schoer MB, Nguyen PN, Merritt DF, Wesevich VG, Hollander AS. The Role of Patient Advocacy and the Declining Rate of Clitoroplasty in 46,XX Patients With Congenital Adrenal Hyperplasia. *Clin Pediatr (Phila)*. 2018;57(14):1664-1671.
565. Crocetti D, Arfini EAG, Monro S, Yeadon-Lee T. 'You're basically calling doctors torturers': stakeholder framing issues around naming intersex rights claims as human rights abuses. *Sociol Health Illn*. 2020;42(4):943-958.
566. Kon AA. Ethical issues in decision-making for infants with disorders of sex development. *Horm Metab Res*. 2015;47(5):340-343.
567. Chase C. What is the Agenda of the Intersex Patient Advocacy Movement? *The Endocrinologist*. 2003;13(3):240-242.
568. Lampalzer U, Briken P, Schweizer K. Dealing With Uncertainty and Lack of Knowledge in Diverse Sex Development: Controversies on Early Surgery and Questions of Consent. *Sex Med*. 2020;8:472-489.
569. Lossie AC, Green J. Building Trust: The History and Ongoing Relationships Amongst DSD Clinicians, Researchers, and Patient Advocacy Groups. *Horm Metab Res*. 2015;47(5):344-350.
570. Austin J, Tamar-Mattis A, Mazur T, Henwood M, Rossi W. Disorders of sex development-when and how to tell the patient. *Pediatr Endocrinol Rev*. 2011;8(3):213-217.
571. Roen K. Intersex or Diverse Sex Development: Critical Review of Psychosocial Health Care Research and Indications for Practice. *J Sex Res*. 2019;56(4-5):511-528.
572. Lee PA, Houk CP. Review of Outcome Information in 46,XX Patients with Congenital Adrenal Hyperplasia Assigned/Reared Male: What Does It Say about Gender Assignment? *Int J Pediatr Endocrinol*. 2010;2010:982025.
573. Lee PA, Houk CP, Husmann DA. Should male gender assignment be considered in the markedly virilized patient With 46,XX and congenital adrenal hyperplasia? *J Urol*. 2010;184(4 Suppl):1786-1792.
574. Houk CP, Lee PA. Approach to Assigning Gender in 46,XX Congenital Adrenal Hyperplasia with Male External Genitalia: Replacing Dogmatism with Pragmatism. *J Clin Endocrinol Metab*. 2010;95(10):4501-4508.
575. Sandberg DE, Gardner M, Kopec K, Urbanski M, Callens N, Keegan CE, Yashar BM, Fechner PY, Shnorhavorian M, Vilain E, Timmermans S, Siminoff LA. Development of a decision support tool in pediatric Differences/Disorders of Sex Development. *Semin Pediatr Surg*. 2019;28(5):150838.
576. Bougneres P, Bouvattier C, Cartigny M, Michala L. Deferring surgical treatment of ambiguous genitalia into adolescence in girls with 21-hydroxylase deficiency: a feasibility study. *Int J Pediatr Endocrinol*. 2017;2017:3.
577. Pasterski V, Zucker KJ, Hindmarsh PC, Hughes IA, Acerini C, Spencer D, Neufeld S, Hines M. Increased Cross-Gender Identification Independent of Gender Role Behavior in Girls with Congenital Adrenal Hyperplasia: Results from a Standardized Assessment of 4- to 11-Year-Old Children. *Archives of sexual behavior*. 2015;44(5):1363-1375.
578. Dessens AB, Slijper FM, Drop SL. Gender dysphoria and gender change in chromosomal females with congenital adrenal hyperplasia. *Archives of sexual behavior*. 2005;34(4):389-397.

579. Liao L-M, Audi L, Magritte E, Bahlburg HFL, Quigley CA. Determinant factors of gender identity: A commentary. *J Pediatr Urol*. 2012;8(6):597-601.
580. Baratz AB, Sharp MK, Sandberg DE. Disorders of sex development peer support. In: Hiort O, Ahmed S, eds. Understanding Differences and Disorders of Sex Development (DSD). Endocrine Development. Basel: Karger; 2014:99-112.
581. Lee PA, Fuqua JS, Houk CP, Kogan BA, Mazur T, Caldamone A. Individualized care for patients with intersex (disorders/differences of sex development): part I. *J Pediatr Urol*. 2020;16:230-237.
582. Lin-Su K, Lekarev O, Poppas DP, Vogiatzi MG. Congenital adrenal hyperplasia patient perception of 'disorders of sex development' nomenclature. *Int J Pediatr Endocrinol*. 2015;2015(1):9.
583. Johnson EK, Rosoklija I, Finlayson C, Chen D, Yerkes EB, Madonna MB, Holl JL, Baratz AB, Davis G, Cheng EY. Attitudes towards "disorders of sex development" nomenclature among affected individuals. *J Pediatr Urol*. 2017;13(6):608 e601-608 e608.
584. Bennecke E, Kohler B, Rohle R, Thyen U, Gehrmann K, Lee P, Nordenstrom A, Cohen-Kettenis P, Bouvattier C, Wiesemann C. Disorders or Differences of Sex Development? Views of Affected Individuals on DSD Terminology. *J Sex Res*. 2020:1-10.
585. Collaer ML, Hindmarsh PC, Pasterski V, Fane BA, Hines M. Reduced short term memory in congenital adrenal hyperplasia (CAH) and its relationship to spatial and quantitative performance. *Psychoneuroendocrinology*. 2016;64:164-173.
586. Johannsen TH, Ripa CP, Reinisch JM, Schwartz M, Mortensen EL, Main KM. Impaired cognitive function in women with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2006;91(4):1376-1381.
587. Hamed SA, Metwalley KA, Farghaly HS. Cognitive function in children with classic congenital adrenal hyperplasia. *Eur J Pediatr*. 2018;177(11):1633-1640.
588. King TF, Lee MC, Williamson EE, Conway GS. Experience in optimizing fertility outcomes in men with congenital adrenal hyperplasia due to 21 hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 2016;84(6):830-836.
589. Reisch N, Flade L, Scherr M, Rottenkolber M, Pedrosa Gil F, Bidlingmaier M, Wolff H, Schwarz HP, Quinkler M, Beuschlein F, Reincke M. High prevalence of reduced fecundity in men with congenital adrenal hyperplasia. *The Journal of clinical endocrinology and metabolism*. 2009;94(5):1665-1670.
590. Falhammar H, Frisen L, Norrby C, Almqvist C, Hirschberg AL, Nordenskjold A, Nordenstrom A. Reduced Frequency of Biological and Increased Frequency of Adopted Children in Males With 21-Hydroxylase Deficiency: A Swedish Population-Based National Cohort Study. *J Clin Endocrinol Metab*. 2017;102(11):4191-4199.
591. Casteras A, De SP, Rumsby G, Conway GS. Reassessing fecundity in women with classical congenital adrenal hyperplasia (CAH): normal pregnancy rate but reduced fertility rate. *Clin Endocrinol (Oxf)*. 2009;70(6):833-837.
592. Meyer-Bahlburg HF. What causes low rates of child-bearing in congenital adrenal hyperplasia?. [Review] [58 refs]. *J Clin Endocrinol Metab*. 1999;84(6):1844-1847.
593. Salm N, Yetter E, Tluczek A. Informing parents about positive newborn screen results: parents' recommendations. *J Child Health Care*. 2012;16(4):367-381.
594. Mueller SC, Ng P, Sinaii N, Leschek EW, Green-Golan L, VanRyzin C, Ernst M, Merke DP. Psychiatric characterization of children with genetic causes of hyperandrogenism. *Eur J Endocrinol*. 2010;163(5):801-810.
595. Falhammar H, Nyström H, Thorén M. Quality of life, social situation, and sexual satisfaction, in adult males with congenital adrenal hyperplasia. *Endocrine*. 2014:1-9.
596. Daae E, Feragen KB, Nermoen I, Falhammar H. Psychological adjustment, quality of life, and self-perceptions of reproductive health in males with congenital adrenal hyperplasia: a systematic review. *Endocrine*. 2018;62(1):3-13.

597. Falhammar H, Butwicka A, Landen M, Lichtenstein P, Nordenskjold A, Nordenstrom A, Frisen L. Increased psychiatric morbidity in men with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2014;99(3):E554-560.
598. Lee P, Houk C, Ahmed S, Hughes I, the International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. . *Pediatrics*. 2006;118(2):e488-e500.
599. Meyer-Bahlburg HF, Dolezal C, Baker SW, Ehrhardt AA, New MI. Gender development in women with congenital adrenal hyperplasia as a function of disorder severity. *Archives of sexual behavior*. 2006;35(6):667-684.
600. Frisen L, Nordenstrom A, Falhammar H, Filipsson H, Holmdahl G, Janson PO, Thoren M, Hagenfeldt K, Moller A, Nordenskjold A. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. *J Clin Endocrinol Metab*. 2009;94(9):3432-3439.
601. Nordenström A, Servin A, Bohlin G, Larsson A, Wedell A. Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2002;87(11):5119-5124.
602. Costello EJ, Angold A. Developmental Epidemiology. In: Cicchetti D, ed. Developmental Psychopathology. Vol 1: John Wiley & Sons, Inc.; 2016:1-35.
603. Wittchen HU, Jacobi F. Size and burden of mental disorders in Europe--a critical review and appraisal of 27 studies. *Eur Neuropsychopharmacol*. 2005;15(4):357-376.
604. Gustavson K, Knudsen AK, Nesvag R, Knudsen GP, Vollset SE, Reichborn-Kjennerud T. Prevalence and stability of mental disorders among young adults: findings from a longitudinal study. *BMC Psychiatry*. 2018;18(1):65.
605. Bernal P. Hidden morbidity in pediatric primary care. *Pediatr Ann*. 2003;32(6):413-418; quiz 421-412.
606. Centers for Disease Control and Prevention. Mental Health in the United States. 2019. https://www.cdc.gov/workplacehealthpromotion/tools-resources/workplace-health/infographics/mental-health-united-states.html.
607. Sandberg DE, Gardner M, Callens N, Mazur T, DSD-TRN Psychosocial Workgroup, the DSD-TRN Advocacy Advisory Network, Accord Alliance. Interdisciplinary care in disorders/differences of sex development (DSD): The psychosocial component of the DSD-Translational research network. *Am J Med Genet C Semin Med Genet*. 2017;175(2):279-292.
608. Schaeffer TL, Tryggestad JB, Mallappa A, Hanna AE, Krishnan S, Chernausek SD, Chalmers LJ, Reiner WG, Kropp BP, Wisniewski AB. An Evidence-Based Model of Multidisciplinary Care for Patients and Families Affected by Classical Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. *Int J Pediatr Endocrinol*. 2010;2010(2010):692439.
609. Witchel SF. The medical home concept and congenital adrenal hyperplasia: a comfortable habitat! *Int J Pediatr Endocrinol*. 2010;2010(1):561526.
610. Law E, Fisher E, Eccleston C, Palermo TM. Psychological interventions for parents of children and adolescents with chronic illness. *Cochrane Database Syst Rev*. 2019;3(3):CD009660.
611. Pai ALH, McGrady M. Systematic Review and Meta-Analysis of Psychological Interventions to Promote Treatment Adherence in Children, Adolescents, and Young Adults With Chronic Illness. *J Pediatr Psychol*. 2014;39(8):918-931.
612. Davis AM, Brown RF, Taylor JL, Epstein RA, McPheeters ML. Transition Care for Children With Special Health Care Needs. *Pediatrics*. 2014;134(5):900-908.
613. Stinson J, Kohut SA, Spiegel L, White M, Gill N, Colbourne G, Sigurdson S, Duffy KW, Tucker L, Stringer E, Hazel B, Hochman J, Reiss J, Kaufman M. A systematic review of transition readiness and transfer satisfaction measures for adolescents with chronic illness. *Int J Adolesc Med Health*. 2014;26(2):159-174.

614. van der Zwan YG, Janssen EH, Callens N, Wolffenbuttel KP, Cohen-Kettenis PT, van den Berg M, Drop SL, Dessens AB, Beerendonk C. Severity of virilization is associated with cosmetic appearance and sexual function in women with congenital adrenal hyperplasia: a cross-sectional study. *J Sex Med*. 2013;10(3):866-875.
615. Yankovic F, Cherian A, Steven L, Mathur A, Cuckow P. Current practice in feminizing surgery for congenital adrenal hyperplasia; a specialist survey. *J Pediatr Urol*. 2013;9(6 Pt B):1103-1107.
616. Jesus LE. Feminizing genitoplasties: Where are we now? *J Pediatr Urol*. 2018;14(5):407-415.
617. Binet A, Lardy H, Geslin D, Francois-Fiquet C, Poli-Merol ML. Should we question early feminizing genitoplasty for patients with congenital adrenal hyperplasia and XX karyotype? *J Pediatr Surg*. 2016;51(3):465-468.
618. Bangalore Krishna K, Kogan BA, Ernst MM, Romao RL, Mohsin F, Serrano-Gonzalez M, Quintos JB, Phornphutkul C, Aguiar L, Lee PA. Individualized care for patients with intersex (disorders/differences of sex development): Part 3. *J Pediatr Urol*. 2020;16(5):598-605.
619. Ernst MM, Kogan BA, Lee PA. Gender identity: A psychosocial primer for providing care to patients with a disorder/difference of sex development and their families [individualized care for patients with intersex (Disorders/differences of sex development): Part 2]. *J Pediatr Urol*. 2020;16(5):606-611.
620. Braga LH, Pippi Salle JL. Congenital adrenal hyperplasia: a critical appraisal of the evolution of feminizing genitoplasty and the controversies surrounding gender reassignment. *Eur J Pediatr Surg*. 2009;19(4):203-210.
621. Boehmer U, Timm A, Ozonoff A, Potter J. Applying the Female Sexual Functioning Index to sexual minority women. *J Womens Health (Larchmt)*. 2012;21(4):401-409.
622. Crouch NS, Liao LM, Woodhouse CR, Conway GS, Creighton SM. Sexual function and genital sensitivity following feminizing genitoplasty for congenital adrenal hyperplasia. *J Urol*. 2008;179(2):634-638.
623. Nordenskjold A, Holmdahl G, Frisen L, Falhammar H, Filipsson H, Thoren M, Janson PO, Hagenfeldt K. Type of mutation and surgical procedure affect long-term quality of life for women with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2008;93(2):380-386.
624. Creighton SM, Minto CL, Steele SJ. Objective cosmetic and anatomical outcomes at adolescence of feminising surgery for ambiguous genitalia done in childhood. *Lancet*. 2001;358(9276):124-125.
625. Claahsen-van der Grinten HL, Stikkelbroeck N, Falhammar H, Reisch N. MANAGEMENT OF ENDOCRINE DISEASE: Gonadal dysfunction in congenital adrenal hyperplasia. *Eur J Endocrinol*. 2021;184(3):R85-r97.
626. Bouvattier C, Esterle L, Renoult-Pierre P, de la Perriere AB, Illouz F, Kerlan V, Pascal-Vigneron V, Drui D, Christin-Maitre S, Galland F, Brue T, Reznik Y, Schillo F, Pinsard D, Piguel X, Chabrier G, Decoudier B, Emy P, Tauveron I, Raffin-Sanson ML, Bertherat J, Kuhn JM, Caron P, Cartigny M, Chabre O, Dewailly D, Morel Y, Touraine P, Tardy-Guidollet V, Young J. Clinical Outcome, Hormonal Status, Gonadotrope Axis, and Testicular Function in 219 Adult Men Born With Classic 21-Hydroxylase Deficiency. A French National Survey. *J Clin Endocrinol Metab*. 2015;100(6):2303-2313.
627. Reisch N, Arlt W. Fine tuning for quality of life: 21st century approach to treatment of Addison's disease. *Endocrinol Metab Clin North Am*. 2009;38(2):407-440x.
628. Nermoen I, Rorvik J, Holmedal SH, Hykkerud DL, Fougner KJ, Svartberg J, Husebye ES, Lovas K. High frequency of adrenal myelolipomas and testicular adrenal rest tumours in adult Norwegian patients with classical congenital adrenal hyperplasia because of 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 2011;75(6):753-759.
629. Mouritsen A, Jorgensen N, Main KM, Schwartz M, Juul A. Testicular adrenal rest tumours in boys, adolescents and adult men with congenital adrenal hyperplasia may be associated with the CYP21A2 mutation. *Int J Androl*. 2010;33(3):521-527.

630. Yilmaz R, Sahin D, Aghayev A, Erol OB, Poyrazoglu S, Saka N, Yekeler E. Sonography and Magnetic Resonance Imaging Characteristics of Testicular Adrenal Rest Tumors. *Pol J Radiol*. 2017;82:583-588.
631. Kim MS, Goodarzian F, Keenan MF, Geffner ME, Koppin CM, De Filippo RE, Kokorowski PJ. Testicular Adrenal Rest Tumors in Boys and Young Adults with Congenital Adrenal Hyperplasia. *J Urol*. 2017;197(3 Pt 2):931-936.
632. Lolis E, Juhlin CC, Nordenstrom A, Falhammar H. Extensive Bilateral Adrenal Rest Testicular Tumors in a Patient With 3beta-Hydroxysteroid Dehydrogenase Type 2 Deficiency. *J Endocr Soc*. 2018;2(6):513-517.
633. Avila NA, Shawker TS, Jones JV, Cutler GB, Jr, Merke DP. Testicular adrenal rest tissue in congenital adrenal hyperplasia: serial sonographic and clinical findings. *Am J Roentgenol*. 1999;172(5):1235-1238.
634. Kim JH, Choi JH, Kang E, Kim YM, Lee BH, Yoo HW. Long-term Consequences of Congenital Adrenal Hyperplasia due to Classic 21-hydroxylase Deficiency in Adolescents and Adults. *Exp Clin Endocrinol Diabetes*. 2017;125(3):196-201.
635. Bulsari K, Falhammar H. Clinical perspectives in congenital adrenal hyperplasia due to 11beta-hydroxylase deficiency. *Endocrine*. 2017;55(1):19-36.
636. Al Alawi AM, Nordenstrom A, Falhammar H. Clinical perspectives in congenital adrenal hyperplasia due to 3beta-hydroxysteroid dehydrogenase type 2 deficiency. *Endocrine*. 2019;63(3):407-421.
637. Claahsen-van der Grinten HL, Sweep FC, Blickman JG, Hermus AR, Otten BJ. Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2007;157(3):339-344.
638. Cabrera MS, Vogiatzi MG, New MI. Long term outcome in adult males with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2001;86(7):3070-3078.
639. Tanaka M, Enatsu N, Chiba K, Fujisawa M. Two cases of reversible male infertility due to congenital adrenal hyperplasia combined with testicular adrenal rest tumor. *Reprod Med Biol*. 2018;17(1):93-97.
640. Reisch N, Rottenkolber M, Greifenstein A, Krone N, Schmidt H, Reincke M, Schwarz HP, Beuschlein F. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2013;98(11):E1820-E1826.
641. Mazzilli R, Stigliano A, Delfino M, Olana S, Zamponi V, Iorio C, Defeudis G, Cimadomo D, Toscano V, Mazzilli F. The High Prevalence of Testicular Adrenal Rest Tumors in Adult Men With Congenital Adrenal Hyperplasia Is Correlated With ACTH Levels. *Front Endocrinol (Lausanne)*. 2019;10:335.
642. MacKay D, Nordenstrom A, Falhammar H. Bilateral Adrenalectomy in Congenital Adrenal Hyperplasia: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2018.
643. Verhees MJM, Kamphuis-Van Ulzen K, Hermus A, Stikkelbroeck N, Mooij CF, Claahsen-van der Grinten HL. Re: Testicular Adrenal Rest Tumors in Boys and Young Adults with Congenital Adrenal Hyperplasia: M. S. Kim, F. Goodarzian, M. F. Keenan, M. E. Geffner, C. M. Koppin, R. E. De Filippo and P. J. Kokorowski J Urol 2017;197:931-936. *J Urol*. 2018;199(5):1357-1358.
644. Altieri VM, Altieri B, Castellucci R, Alba S, Bottone F, Fragala E, Mirone V, Greco F. Leydig cell tumour and giant adrenal myelolipoma associated with adrenogenital syndrome: a case report with a review of the literature. *Urologia*. 2016;83(1):43-48.
645. Claahsen-van der Grinten HL, Otten BJ, Hermus AR, Sweep FC, Hulsbergen-van de Kaa CA. Testicular adrenal rest tumors in patients with congenital adrenal hyperplasia can cause severe testicular damage. *Fertil Steril*. 2008;89(3):597-601.
646. Falhammar H, Filipsson Nystrom H, Wedell A, Brismar K, Thoren M. Bone mineral density, bone markers, and fractures in adult males with congenital adrenal hyperplasia. *Eur J Endocrinol*. 2013;168(3):331-341.

647. Jaaskelainen J, Kiekara O, Hippelainen M, Voutilainen R. Pituitary gonadal axis and child rate in males with classical 21-hydroxylase deficiency. *J Endocrinol Invest*. 2000;23(1):23-27.
648. Manoli I, Kanaka-Gantenbein C, Voutetakis A, Maniati-Christidi M, Dacou-Voutetakis C. Early growth, pubertal development, body mass index and final height of patients with congenital adrenal hyperplasia: factors influencing the outcome. *Clin Endocrinol (Oxf)*. 2002;57(5):669-676.
649. Kulshreshtha B, Eunice M, Ammini AC. Pubertal development among girls with classical congenital adrenal hyperplasia initiated on treatment at different ages. *Indian J Endocrinol Metab*. 2012;16(4):599-603.
650. Bidet M, Bellanne-Chantelot C, Galand-Portier MB, Golmard JL, Tardy V, Morel Y, Clauin S, Coussieu C, Boudou P, Mowzowicz I, Bachelot A, Touraine P, Kuttenn F. Fertility in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2010;95(3):1182-1190.
651. Carmina E, Dewailly D, Escobar-Morreale HF, Kelestimur F, Moran C, Oberfield S, Witchel SF, Azziz R. Non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency revisited: an update with a special focus on adolescent and adult women. *Hum Reprod Update*. 2017;23(5):580-599.
652. Hague WM, Adams J, Rodda C, Brook CG, de Bruyn R, Grant DB, Jacobs HS. The prevalence of polycystic ovaries in patients with congenital adrenal hyperplasia and their close relatives. *Clin Endocrinol (Oxf)*. 1990;33(4):501-510.
653. Pall M, Azziz R, Beires J, Pignatelli D. The phenotype of hirsute women: a comparison of polycystic ovary syndrome and 21-hydroxylase-deficient nonclassic adrenal hyperplasia. *Fertil Steril*. 2010;94(2):684-689.
654. Stikkelbroeck NM, Hermus AR, Schouten D, Suliman HM, Jager GJ, Braat DD, Otten BJ. Prevalence of ovarian adrenal rest tumours and polycystic ovaries in females with congenital adrenal hyperplasia: results of ultrasonography and MR imaging. *Eur Radiol*. 2004;14(10):1802-1806.
655. Kulshreshtha B, Marumudi E, Khurana ML, Kriplani A, Kinra G, Gupta DK, Kucheria K, Khadgawat R, Gupta N, Seith A, Ammini AC. Fertility among women with classical congenital adrenal hyperplasia: report of seven cases where treatment was started after 9 years of age. *Gynecol Endocrinol*. 2008;24(5):267-272.
656. van de Grift TC, Kreukels BPC. Breast development and satisfaction in women with disorders/differences of sex development. *Hum Reprod*. 2019;34(12):2410-2417.
657. Jaaskelainen J, Hippelainen M, Kiekara O, Voutilainen R. Child rate, pregnancy outcome and ovarian function in females with classical 21-hydroxylase deficiency. *Acta Obstet Gynecol Scand*. 2000;79(8):687-692.
658. Słowikowska-Hilczer J, Hirschberg AL, Claahsen-van der Grinten H, Reisch N, Bouvattier C, Thyen U, Cohen Kettenis P, Roehle R, Köhler B, Nordenström A. Fertility outcome and information on fertility issues in individuals with different forms of disorders of sex development: findings from the dsd-LIFE study. *Fertil Steril*. 2017;108(5):822-831.
659. Hirschberg AL, Gidlöf S, Falhammar H, Frisén L, Almqvist C, Nordenskiöld A, Nordenström A. Reproductive and perinatal outcomes in women with congenital adrenal hyperplasia - a population-based cohort study. *J Clin Endocrinol Metab*. 2021;106:e957-e965.
660. Leveroni C, Berenbaum SA. Early androgen effects on interest in infants: evidence from children with congenital adrenal hyperplasia. *Dev Neuropsychol*. 1998;14:321-340.
661. Helleday J, Siwers B, Ritzen EM, Carlstrom K. Subnormal androgen and elevated progesterone levels in women treated for congenital virilizing 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 1993;76:933-936.
662. Holmes-Walker DJ, Conway GS, Honour JW, Rumsby G, Jacobs HS. Menstrual disturbance and hypersecretion of progesterone in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 1995;43(3):291-296.

663. Hoepffner W, Schulze E, Bennek J, Keller E, Willgerodt H. Pregnancies in patients with congenital adrenal hyperplasia with complete or almost complete impairment of 21-hydroxylase activity. *Fertil Steril*. 2004;81(5):1314-1321.
664. Reichman DE, White PC, New MI, Rosenwaks Z. Fertility in patients with congenital adrenal hyperplasia. *Fertil Steril*. 2014;101(2):301-309.
665. Reisch N. Pregnancy in Congenital Adrenal Hyperplasia. *Endocrinol Metab Clin North Am*. 2019;48(3):619-641.
666. Moran C, Azziz R, Weintrob N, Witchel SF, Rohmer V, Dewailly D, Marcondes JA, Pugeat M, Speiser PW, Pignatelli D, Mendonca BB, Bachega TA, Escobar-Morreale HF, Carmina E, Fruzzetti F, Kelestimur F. Reproductive outcome of women with 21-hydroxylase-deficient nonclassic adrenal hyperplasia. *J Clin Endocrinol Metab*. 2006;91(9):3451-3456.
667. Eyal O, Ayalon-Dangur I, Segev-Becker A, Schachter-Davidov A, Israel S, Weintrob N. Pregnancy in women with nonclassic congenital adrenal hyperplasia: Time to conceive and outcome. *Clin Endocrinol (Oxf)*. 2017;87(5):552-556.
668. Husebye ES, Allolio B, Arlt W, Badenhoop K, Bensing S, Betterle C, Falorni A, Gan EH, Hulting AL, Kasperlik-Zaluska A, Kämpe O, Løvås K, Meyer G, Pearce SH. Consensus statement on the diagnosis, treatment and follow-up of patients with primary adrenal insufficiency. *J Intern Med*. 2014;275(2):104-115.
669. Bothou C, Anand G, Li D, Kienitz T, Seejore K, Simeoli C, Ebbehoj A, Ward EG, Paragliola RM, Ferrigno R, Badenhoop K, Bensing S, Oksnes M, Esposito D, Bergthorsdottir R, Drake W, Wahlberg J, Reisch N, Hahner S, Pearce S, Trainer P, Etzrodt-Walter G, Thalmann SP, Saevik AB, Husebye E, Isidori AM, Falhammar H, Meyer G, Corsello SM, Pivonello R, Murray R, Bancos I, Quinkler M, Beuschlein F. Current Management and Outcome of Pregnancies in Women With Adrenal Insufficiency: Experience from a Multicenter Survey. *J Clin Endocrinol Metab*. 2020;105(8):e2853-e2863.
670. Krone N, Wachter I, Stefanidou M, Roscher AA, Schwarz HP. Mothers with congenital adrenal hyperplasia and their children: outcome of pregnancy, birth and childhood. *Clin Endocrinol (Oxf)*. 2001;55(4):523-529.
671. Falhammar H, Frisen L, Hirschberg AL, Norrby C, Almqvist C, Nordenskjold A, Nordenstrom A. Increased Cardiovascular and Metabolic Morbidity in Patients With 21-Hydroxylase Deficiency: A Swedish Population-Based National Cohort Study. *J Clin Endocrinol Metab*. 2015;100(9):3520-3528.
672. Bachelot A, Golmard JL, Dulon J, Dahmoune N, Leban M, Bouvattier C, Cabrol S, Leger J, Polak M, Touraine P. Determining clinical and biological indicators for health outcomes in adult patients with childhood onset of congenital adrenal hyperplasia. *Eur J Endocrinol*. 2015;173(2):175-184.
673. Kim B, Lee MN, Park HD, Kim JW, Chang YS, Park WS, Lee SY. Dried blood spot testing for seven steroids using liquid chromatography-tandem mass spectrometry with reference interval determination in the Korean population. *Ann Lab Med*. 2015;35(6):578-585.
674. Tamhane S, Rodriguez-Gutierrez R, Iqbal AM, Prokop LJ, Bancos I, Speiser PW, Murad MH. Cardiovascular and Metabolic Outcomes in Congenital Adrenal Hyperplasia: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2018;103(11):4097-4103.
675. Falhammar H, Filipsson H, Holmdahl G, Janson PO, Nordenskjold A, Hagenfeldt K, Thoren M. Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2007;92(1):110-116.
676. Falhammar H, Filipsson NH, Wedell A, Thoren M. Cardiovascular risk, metabolic profile, and body composition in adult males with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2011;164(2):285-293.
677. Mooij CF, Kroese JM, HL C-vdG, Tack CJ, Hermus AR. Unfavourable trends in cardiovascular and metabolic risk in paediatric and adult patients with congenital adrenal hyperplasia? *Clin Endocrinol (Oxf)*. 2010;73(2):137-146.

678. Ubertini G, Bizzarri C, Grossi A, Gimigliano F, Rava L, Fintini D, Cappa M. Blood Pressure and Left Ventricular Characteristics in Young Patients with Classical Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. *Int J Pediatr Endocrinol*. 2009;2009:383610.
679. Vijayan R, Bhavani N, Pavithran PV, Nair V, Menon UV, Menon AS, Abraham N, Bhadran K, Narayanan P, Kumar H. Metabolic profile, cardiovascular risk factors and health-related quality of life in children, adolescents and young adults with congenital adrenal hyperplasia. *J Pediatr Endocrinol Metab*. 2019;32(8):871-877.
680. Subbarayan A, Dattani MT, Peters CJ, Hindmarsh PC. Cardiovascular risk factors in children and adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 2014;80(4):471-477.
681. Rosenbaum D, Gallo A, Lethielleux G, Bruckert E, Levy BI, Tanguy ML, Dulon J, Dahmoune N, Salem JE, Bittar R, Leban M, Girerd X, Touraine P, Bachelot A, group Cs. Early central blood pressure elevation in adult patients with 21-hydroxylase deficiency. *J Hypertens*. 2019;37(1):175-181.
682. Falhammar H, Thoren M. Clinical outcomes in the management of congenital adrenal hyperplasia. *Endocrine*. 2012;41(3):355-373.
683. Sartorato P, Zulian E, Benedini S, Mariniello B, Schiavi F, Bilora F, Pozzan G, Greggio N, Pagnan A, Mantero F, Scaroni C. Cardiovascular risk factors and ultrasound evaluation of intima-media thickness at common carotids, carotid bulbs, and femoral and abdominal aorta arteries in patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2007;92(3):1015-1018.
684. Marra AM, Improda N, Capalbo D, Salzano A, Arcopinto M, De Paulis A, Alessio M, Lenzi A, Isidori AM, Cittadini A, Salerno M. Cardiovascular abnormalities and impaired exercise performance in adolescents with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2015;100(2):644-652.
685. Salem JE, Nguyen LS, Hammoudi N, Preud'homme G, Hulot JS, Leban M, Funck-Brentano C, Touraine P, Isnard R, Bachelot A, Group CS. Complex Association of Sex Hormones on Left Ventricular Systolic Function: Insight into Sexual Dimorphism. *J Am Soc Echocardiogr*. 2018;31(2):231-240 e231.
686. Improda N, Barbieri F, Ciccarelli GP, Capalbo D, Salerno M. Cardiovascular Health in Children and Adolescents With Congenital Adrenal Hyperplasia Due to 21-Hydroxilase Deficiency. *Front Endocrinol (Lausanne)*. 2019;10:212.
687. Gomes LG, Mendonca BB, Bachega T. Long-term cardio-metabolic outcomes in patients with classical congenital adrenal hyperplasia: is the risk real? *Curr Opin Endocrinol Diabetes Obes*. 2020;27(3):155-161.
688. Moreira RP, Gomes LG, Mendonca BB, Bachega TA. Impact of glucocorticoid receptor gene polymorphisms on the metabolic profile of adult patients with the classical form of 21-hydroxylase deficiency. *PLoS One*. 2012;7(9):e44893.
689. Bhargava A, Arnold AP, Bangasser DA, Denton KM, Gupta A, Hilliard Krause LM, Mayer EA, McCarthy M, Miller WL, Raznahan A, Verma R. Considering Sex as a Biological Variable in Basic and Clinical Studies: An Endocrine Society Scientific Statement. *Endocr Rev*. 2021;Mar 11;bnaa034. doi: 10.1210/endrev/bnaa034. Online ahead of print.
690. Ernst M, Maheu FS, Schroth E, Hardin J, Golan LG, Cameron J, Allen R, Holzer S, Nelson E, Pine DS, Merke DP. Amygdala function in adolescents with congenital adrenal hyperplasia: a model for the study of early steroid abnormalities. *Neuropsychologia*. 2007;45(9):2104-2113.
691. Webb EA, Elliott L, Carlin D, Wilson M, Hall K, Netherton J, Reed J, Barrett TG, Salwani V, Clayden JD, Arlt W, Krone N, Peet AC, Wood AG. Quantitative Brain MRI in Congenital Adrenal Hyperplasia: In Vivo Assessment of the Cognitive and Structural Impact of Steroid Hormones. *J Clin Endocrinol Metab*. 2018;103(4):1330-1341.
692. Nass R, Heier L, Moshang T, Oberfield S, George A, New MI, Speiser PW. Magnetic resonance imaging in the congenital adrenal hyperplasia population: increased frequency of white-matter abnormalities and temporal lobe atrophy. *J Child Neurol*. 1997;12(3):181-186.

693. Mnif MF, Kamoun M, Mnif F, Charfi N, Kallel N, Rekik N, Naceur BB, Fourati H, Daoud E, Mnif Z, Sfar MH, Younes-Mhenni S, Sfar MT, Hachicha M, Abid M. Brain magnetic resonance imaging findings in adult patients with congenital adrenal hyperplasia: Increased frequency of white matter impairment and temporal lobe structures dysgenesis. *Indian J Endocrinol Metab*. 2013;17(1):121-127.
694. Siegel S, Kirstein CF, Grzywotz A, Hütter BO, Wrede KH, Kuhna V, Kreitschmann-Andermahr I. Neuropsychological Functioning in Patients with Cushing's Disease and Cushing's Syndrome. *Exp Clin Endocrinol Diabetes*. 2020;129:194-202.
695. Herting MM, Azad A, Kim R, Tyszka JM, Geffner ME, Kim MS. Brain Differences in the Prefrontal Cortex, Amygdala, and Hippocampus in Youth with Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab*. 2020;105(4):1098-1111.
696. Van't Westeinde A, Karlsson L, Thomsen Sandberg M, Nordenström A, Padilla N, Lajic S. Altered Gray Matter Structure and White Matter Microstructure in Patients with Congenital Adrenal Hyperplasia: Relevance for Working Memory Performance. *Cereb Cortex*. 2020;30(5):2777-2788.
697. Ritchie SJ, Cox SR, Shen X, Lombardo MV, Reus LM, Alloza C, Harris MA, Alderson HL, Hunter S, Neilson E, Liewald DCM, Auyeung B, Whalley HC, Lawrie SM, Gale CR, Bastin ME, McIntosh AM, Deary IJ. Sex Differences in the Adult Human Brain: Evidence from 5216 UK Biobank Participants. *Cereb Cortex*. 2018;28(8):2959-2975.
698. Lotze M, Domin M, Gerlach FH, Gaser C, Lueders E, Schmidt CO, Neumann N. Novel findings from 2,838 Adult Brains on Sex Differences in Gray Matter Brain Volume. *Sci Rep*. 2019;9(1):1671.
699. Ingalhalikar M, Smith A, Parker D, Satterthwaite TD, Elliott MA, Ruparel K, Hakonarson H, Gur RE, Gur RC, Verma R. Sex differences in the structural connectome of the human brain. *Proc Natl Acad Sci U S A*. 2014;111(2):823-828.
700. Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, Blumenthal JD, Lerch J, Zijdenbos AP, Evans AC, Thompson PM, Giedd JN. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage*. 2007;36(4):1065-1073.
701. Van Staa TP, Leufkens HG, Abenhaim L, Zhang B, Cooper C. Use of oral corticosteroids and risk of fractures. *J Bone Miner Res*. 2000;15(6):993-1000.
702. van Staa TP, Leufkens HG, Cooper C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. *Osteoporos Int*. 2002;13(10):777-787.
703. Chotiyarnwong P, McCloskey EV. Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. *Nat Rev Endocrinol*. 2020;16(8):437-447.
704. Girgis R, Winter JS. The effects of glucocorticoid replacement therapy on growth, bone mineral density, and bone turnover markers in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1997;82(12):3926-3929.
705. Fleischman A, Ringelheim J, Feldman HA, Gordon CM. Bone mineral status in children with congenital adrenal hyperplasia. *J Pediatr Endocrinol Metab*. 2007;20(2):227-235.
706. Stikkelbroeck NM, Oyen WJ, van der Wilt GJ, Hermus AR, Otten BJ. Normal bone mineral density and lean body mass, but increased fat mass, in young adult patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2003;88(3):1036-1042.
707. Christiansen P, Molgaard C, Muller J. Normal bone mineral content in young adults with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res*. 2004;61(3):133-136.
708. Gussinye M, Carrascosa A, Potau N, Enrubia M, Vicens-Calvet E, Ibanez L, Yeste D. Bone mineral density in prepubertal and in adolescent and young adult patients with the salt-wasting form of congenital adrenal hyperplasia. *Pediatrics*. 1997;100(4):671-674.
709. Guo CY, Weetman AP, Eastell R. Bone turnover and bone mineral density in patients with congenital adrenal hyperplasia. *Clinical Endocrinology*. 1996;45(5):535-541.
710. Arisaka O, Hoshi M, Kanazawa S, Numata M, Nakajima D, Kanno S, Negishi M, Nishikura K, Nitta A, Imataka M, Kuribayashi T, Kano K. Preliminary report: effect of adrenal androgen and estrogen on bone maturation and bone mineral density. *Metabolism: clinical and experimental*. 2001;50(4):377-379.

711. Zimmermann A, Sido PG, Schulze E, Al Khzouz C, Lazea C, Coldea C, Weber MM. Bone mineral density and bone turnover in Romanian children and young adults with classical 21-hydroxylase deficiency are influenced by glucocorticoid replacement therapy. *Clinical endocrinology*. 2009;71(4):477-484.
712. Cameron FJ, Kaymakci B, Byrt EA, Ebeling PR, Warne GL, Wark JD. Bone mineral density and body composition in congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1995;80(7):2238-2243.
713. de Almeida Freire PO, de Lemos-Marini SH, Maciel-Guerra AT, Morcillo AM, Matias Baptista MT, de Mello MP, Guerra G, Jr. Classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: a cross-sectional study of factors involved in bone mineral density. *J Bone Miner Metab*. 2003;21(6):396-401.
714. Paganini C, Radetti G, Livieri C, Braga V, Migliavacca D, Adami S. Height, bone mineral density and bone markers in congenital adrenal hyperplasia. *Horm Res*. 2000;54(4):164-168.
715. Jaaskelainen J, Voutilainen R. Bone mineral density in relation to glucocorticoid substitution therapy in adult patients with 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 1996;45(6):707-713.
716. Sciannamblo M, Russo G, Cuccato D, Chiumello G, Mora S. Reduced bone mineral density and increased bone metabolism rate in young adult patients with 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2006;91(11):4453-4458.
717. King JA, Wisniewski AB, Bankowski BJ, Carson KA, Zacur HA, Migeon CJ. Long-term corticosteroid replacement and bone mineral density in adult women with classical congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2006;91(3):865-869.
718. Falhammar H, Filipsson H, Holmdahl G, Janson PO, Nordenskjold A, Hagenfeldt K, Thoren M. Fractures and bone mineral density in adult women with 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2007;92(12):4643-4649.
719. Bachelot A, Plu-Bureau G, Thibaud E, Laborde K, Pinto G, Samara D, Nihoul-Fekete C, Kuttenn F, Polak M, Touraine P. Long-term outcome of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res*. 2007;67(6):268-276.
720. Koetz KR, Ventz M, Diederich S, Quinkler M. Bone mineral density is not significantly reduced in adult patients on low-dose glucocorticoid replacement therapy. *J Clin Endocrinol Metab*. 2012;97(1):85-92.
721. Ceccato F, Barbot M, Albiger N, Zilio M, De Toni P, Luisetto G, Zaninotto M, Greggio NA, Boscaro M, Scaroni C, Camozzi V. Long-term glucocorticoid effect on bone mineral density in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2016;175(2):101-106.
722. Riehl G, Reisch N, Roehle R, Claahsen van der Grinten H, Falhammar H, Quinkler M. Bone mineral density and fractures in congenital adrenal hyperplasia: Findings from the dsd-LIFE study. *Clin Endocrinol (Oxf)*. 2020;92:284-294.
723. Kasperk CH, Wakley GK, Hierl T, Ziegler R. Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro. *J Bone Miner Res*. 1997;12(3):464-471.
724. El-Maouche D, Collier S, Prasad M, Reynolds JC, Merke DP. Cortical bone mineral density in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 2015;82(3):330-337.
725. Rangaswamaiah S, Gangathimmaiah V, Nordenstrom A, Falhammar H. Bone Mineral Density in Adults With Congenital Adrenal Hyperplasia: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne)*. 2020;11:493.
726. Nermoen I, Falhammar H. Prevalence and characteristics of adrenal tumors and myelolipomas in congenital adrenal hyperplasia: a systematic review and meta-analysis. *Endocr Pract*. 2020.
727. Hamidi O, Raman R, Lazik N, Iniguez-Ariza N, McKenzie TJ, Lyden ML, Thompson GB, Dy BM, Young WF, Jr., Bancos I. Clinical course of adrenal myelolipoma: A long-term longitudinal follow-up study. *Clin Endocrinol (Oxf)*. 2020;93(1):11-18.

728. Bancos I, Taylor AE, Chortis V, Sitch AJ, Jenkinson C, Davidge-Pitts CJ, Lang K, Tsagarakis S, Macech M, Riester A, Deutschbein T, Pupovac ID, Kienitz T, Prete A, Papathomas TG, Gilligan LC, Bancos C, Reimondo G, Haissaguerre M, Marina L, Grytaas MA, Sajwani A, Langton K, Ivison HE, Shackleton CHL, Erickson D, Asia M, Palimeri S, Kondracka A, Spyroglou A, Ronchi CL, Simunov B, Delivanis DA, Sutcliffe RP, Tsirou I, Bednarczuk T, Reincke M, Burger-Stritt S, Feelders RA, Canu L, Haak HR, Eisenhofer G, Dennedy MC, Ueland GA, Ivovic M, Tabarin A, Terzolo M, Quinkler M, Kastelan D, Fassnacht M, Beuschlein F, Ambroziak U, Vassiliadi DA, O'Reilly MW, Young WF, Jr., Biehl M, Deeks JJ, Arlt W. Urine steroid metabolomics for the differential diagnosis of adrenal incidentalomas in the EURINE-ACT study: a prospective test validation study. *Lancet Diabetes Endocrinol*. 2020;8(9):773-781.
729. Juniarto AZ, Ulfah M, Ariani MD, Utari A, Faradz SMH. Phenotypic Variation of 46,XX Late Identified Congenital Adrenal Hyperplasia among Indonesians. *Journal of the ASEAN Federation of Endocrine Societies*. 2018;33(1):6-11.
730. Raza J, Mazen I. Achieving diagnostic certainty in resource-limited settings. *Endocr Dev*. 2014;27:257-267.
731. Ediati A, Faradz SM, Juniarto AZ, van der Ende J, Drop SL, Dessens AB. Emotional and behavioral problems in late-identified Indonesian patients with disorders of sex development. *Journal of psychosomatic research*. 2015;79(1):76-84.
732. Chowdhury TK, Kabir M, Chowdhury MZ, Hutson JM, Banu T. The challenges in diagnosis and gender assignment in disorders of sex development presenting to a pediatric surgical unit in a developing country: the role of laparoscopy and simple tests for gender identity. *J Pediatr Urol*. 2014;10(6):1255-1260.
733. Warne GL, Raza J. Disorders of sex development (DSDs), their presentation and management in different cultures. *Reviews in Endocrine and Metabolic Disorders*. 2008;9(3):227-236.
734. Ediati A, Maharani N, Utari A. Sociocultural aspects of disorders of sex development. *Birth defects research Part C, Embryo today : reviews*. 2016;108(4):380-383.
735. Ediati A, Juniarto AZ, Birnie E, Drop SL, Faradz SM, Dessens AB. Gender Development in Indonesian Children, Adolescents, and Adults with Disorders of Sex Development. *Archives of sexual behavior*. 2015;44(5):1339-1361.
736. Ediati A, Verrips GHW, Juniarto AZ, Faradz SMH, Drop SLS, Dessens AB. Quality of Life in Late-Treated Patients With Disorders of Sex Development: Insights for Patient-Centered Care. *Front Pediatr*. 2019;6:434-434.
737. Boulanger V, Schlemmer M, Rossov S, Seebald A, Gavin P. Establishing Patient Registries for Rare Diseases: Rationale and Challenges. *Pharmaceut Med*. 2020;34(3):185-190.
738. Taruscio D, Gentile AE, Evangelista T, Frazzica RG, Bushby K, Montserrat AM. Centres of Expertise and European Reference Networks: key issues in the field of rare diseases. The EUCERD Recommendations. *Blood Transfus*. 2014;12 Suppl 3:s621-625.
739. Kyriakou A, Dessens A, Bryce J, Iotova V, Juul A, Krawczynski M, Nordenskjöld A, Rozas M, Sanders C, Hiort O, Ahmed SF. Current models of care for disorders of sex development - results from an International survey of specialist centres. *Orphanet J Rare Dis*. 2016;11(1):155.
740. Rolston AM, Gardner M, van Leeuwen K, Mohnach L, Keegan C, Delot E, Vilain E, Sandberg DE, members of the DSD-TRN Advocacy Advisory Network, Accord Alliance. Disorders of sex development (DSD): Clinical service delivery in the United States. *Am J Med Genet C Semin Med Genet*. 2017;175(2):268-278.
741. Sanders C, Hall J, Dessens A, Bryce J, Callens N, Cools M, Kourime M, Kyriakou A, Springer A, Audi L, Balsamo A, Iotova V, Mladenov V, Krawczynski M, Nordenskjöld A, Rozas M, Claahsen-van der Grinten H, Hiort O, Riedl S, Ahmed SF. Involving Individuals with Disorders of Sex Development and Their Parents in Exploring New Models of Shared Learning: Proceedings from a DSDnet COST Action Workshop. *Sex Dev*. 2018.
742. Brasil S, Pascoal C, Francisco R, Dos Reis Ferreira V, Videira PA, Valadão AG. Artificial Intelligence (AI) in Rare Diseases: Is the Future Brighter? *Genes (Basel)*. 2019;10(12):978.

Figure 1. Adrenal steroidogenesis. Enzymes are boxed with dotted lines extending to arrows denoting each enzymatic conversion; two enzymes, CYP11B2 and CYP17, catalyze several successive enzymatic conversions. Accessory proteins required for activity of cytochrome P450 enzymes are shown next to each such enzyme: POR, P450 oxidoreductase, required by CYP enzymes in the endoplasmic reticulum; FDXR/FDX1, ferredoxin reductase and ferredoxin, required by mitochondrial CYP enzymes. Cytochrome B5 (CYP5A) is required for full 17,20-lyase activity of CYP17A1. There are two 11β-hydroxysteroid dehydrogenase isozymes; HSD11B1, expressed mainly in the liver, catalyzes reduction (e.g., cortisone to cortisol), whereas HSD11B2, expressed mainly in the kidney, catalyzes oxidation (e.g., cortisol to cortisone). The steps affected by 21OHD, including steroids secreted in increased amounts in this disease, are denoted by red lines and red lettering. Steps taking place only in the adrenal glands are in unshaded boxes; steps taking place partly or predominantly outside the adrenal cortex are denoted by shaded boxes. Planar structures of cholesterol, aldosterone, cortisol and testosterone are illustrated; the position of the 11-oxo (11-keto) group in 11-ketotestosterone is illustrated in green. Colored rectangles indicate the following: grey, early steps of steroidogenesis common to all zones of the cortex; orange, steps in the zona glomerulosa leading to aldosterone; blue, steps in the zona fasciculata leading to cortisol; magenta; steps in the zona reticularis and extra-adrenal tissues leading to androgens; purple, the "backdoor" or alternative pathway from 17-OH progesterone to dihydrotestosterone (for clarity, the alternative pathway from progesterone is not shown); green, conversions leading to 11-oxo androgens.

Figure 2. Genetics of the CYP21 genes. **A**, the genetic region on chromosome 6p21.3, using data from the Human Genome database (http://genome.ucsc.edu/). The location of this region is

indicated on a schematic of the entire chromosome. A scale is marked every 10 kb, with positions in the genome assembly numbered every 0.1 Mb. Genes transcribed in the telomeric-to-centromeric direction (left to right) are on the strand denoted by a right-facing arrow: *SKIV2L*, Ski2 like RNA helicase; *STK19*, serine/threonine kinase 19; *C4A*, complement component C4A; *CYP21A1P*, cytochrome P450 family 21 subfamily A member 1 (21-hydroxylase) pseudogene; *STK19B*, serine/threonine kinase 19 pseudogene; *C4B*, complement component C4B, *CYP21A2*, cytochrome P450 family 21 subfamily A member 2 (21-hydroxylase). Genes transcribed from the opposite strand (right to left in the figure) are immediately below: *ATF6B*, activating transcription factor 6 beta; *TNXB*, tenascin XB; ; *TNXA*, tenascin XA pseudogene; *DXO*, Decapping and exoribonuclease protein. ZA and ZB are adrenal-specific noncoding transcripts overlapping the C4 genes in the sense direction (146,147); additional transcripts exist but are not shown. The 30 kb duplication of part of STK19, all of C4, all of CYP21, and part of TNX (a so-called RCCX module) is indicated.

B, an illustration of unequal meiotic crossing-over generating a deletion representing a salt-wasting 21-hydroxylase deficiency allele. The other chromosome has 3 copies of the RCCX tandem and is not associated with disease. The scale is expanded from Figure 1A. For clarity, only the C4 and CYP21 genes are illustrated.

Figure 3. A, Structure of the *CYP21* genes. Exons are numbered. Mutations affecting enzymatic function that are normally present in the *CYP21A1P* pseudogene are shown. They are positioned vertically to show the severity of CAH they cause when transferred to *CYP21A2* in gene conversion events. These are grouped into 4 mutation groups (0, A-C) and are associated with

particular forms of CAH, as indicated. **B,** associations between mutation groups and forms of CAH. These are displayed in tabular form on the left and as histograms on the right.

Figure 4. Genital development. Top, Differentiation of male and female reproductive systems are illustrated in schematic cross-section (not to scale). Bottom, the Prader scale of genital virilization.

Figure 5. New therapeutic approaches target different aspects of the pathophysiology of CAH. Circadian cortisol replacement with a modified-release glucocorticoid or subcutaneous hydrocortisone infusion aim to control corticotropin-driven hyperandrogenism by replacing cortisol in a physiological manner. Other approaches to reduce androgen production without chronic supraphysiological glucocorticoid exposure include corticotropin-releasing hormone receptor-1 antagonists, adrenocorticotropic hormone (corticotropin, ACTH) antibodies, adrenocorticotropic hormone receptor (MC2R) antagonists, adrenolytic agents, adrenalectomy, and pharmacological inhibition of steroidogenic enzymes or steroid receptors in the adrenal or peripheral tissues. Since CAH owing to 21OHD is a monogenic disorder, gene therapy with cell-based and gene-editing technologies may be able to restore defective steroidogenesis. CRH denotes corticotropin-releasing hormone (sometimes referred to as corticotropin-releasing factor [CRF]). From New England Journal of Medicine, Merke DP, Auchus RJ, Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency, Volume 83, Page 1258. Copyright © (2020) Massachusetts Medical Society. Reprinted with permission.

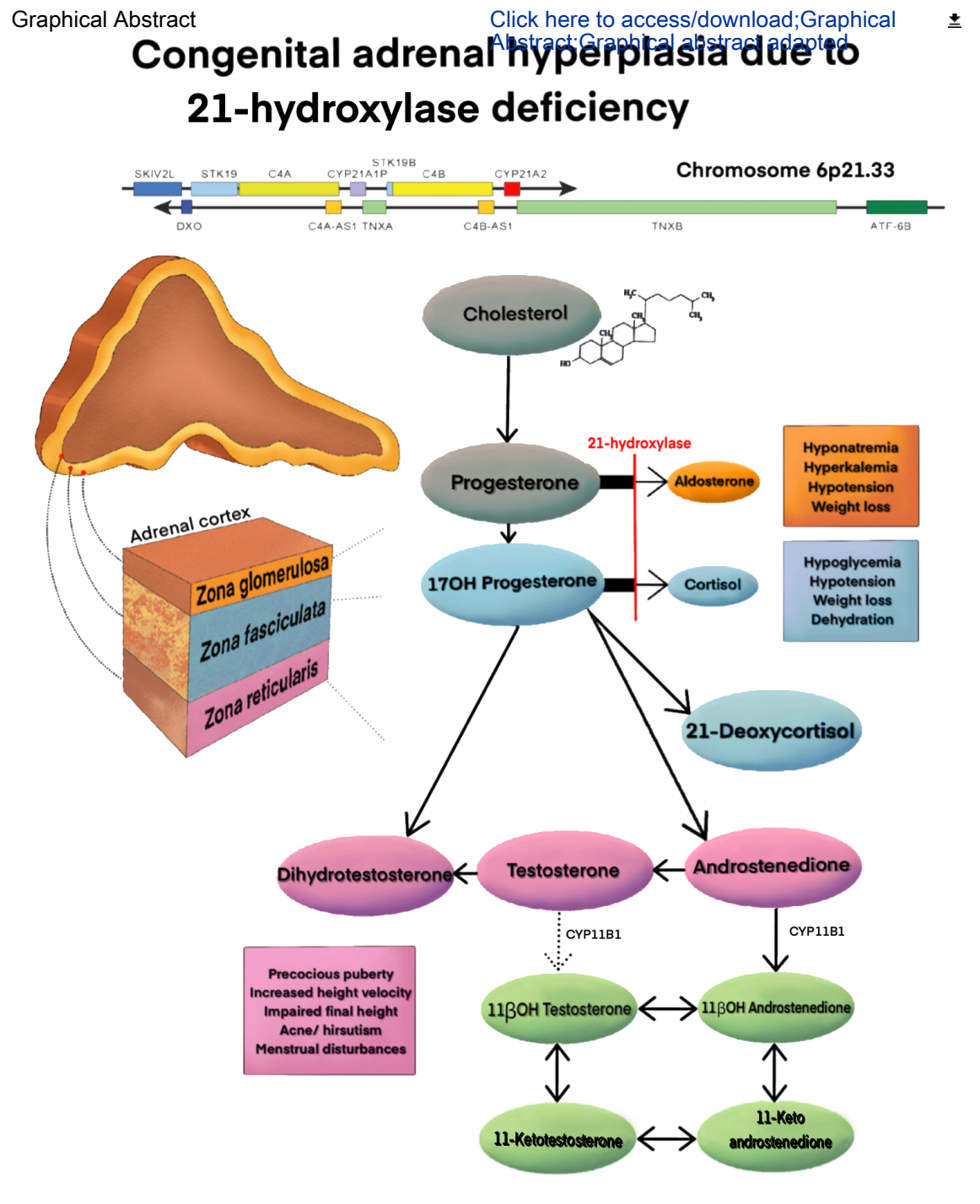


Figure 1

Click here to access/download;Figure;ERCAH Fig 1 color background C.jpg

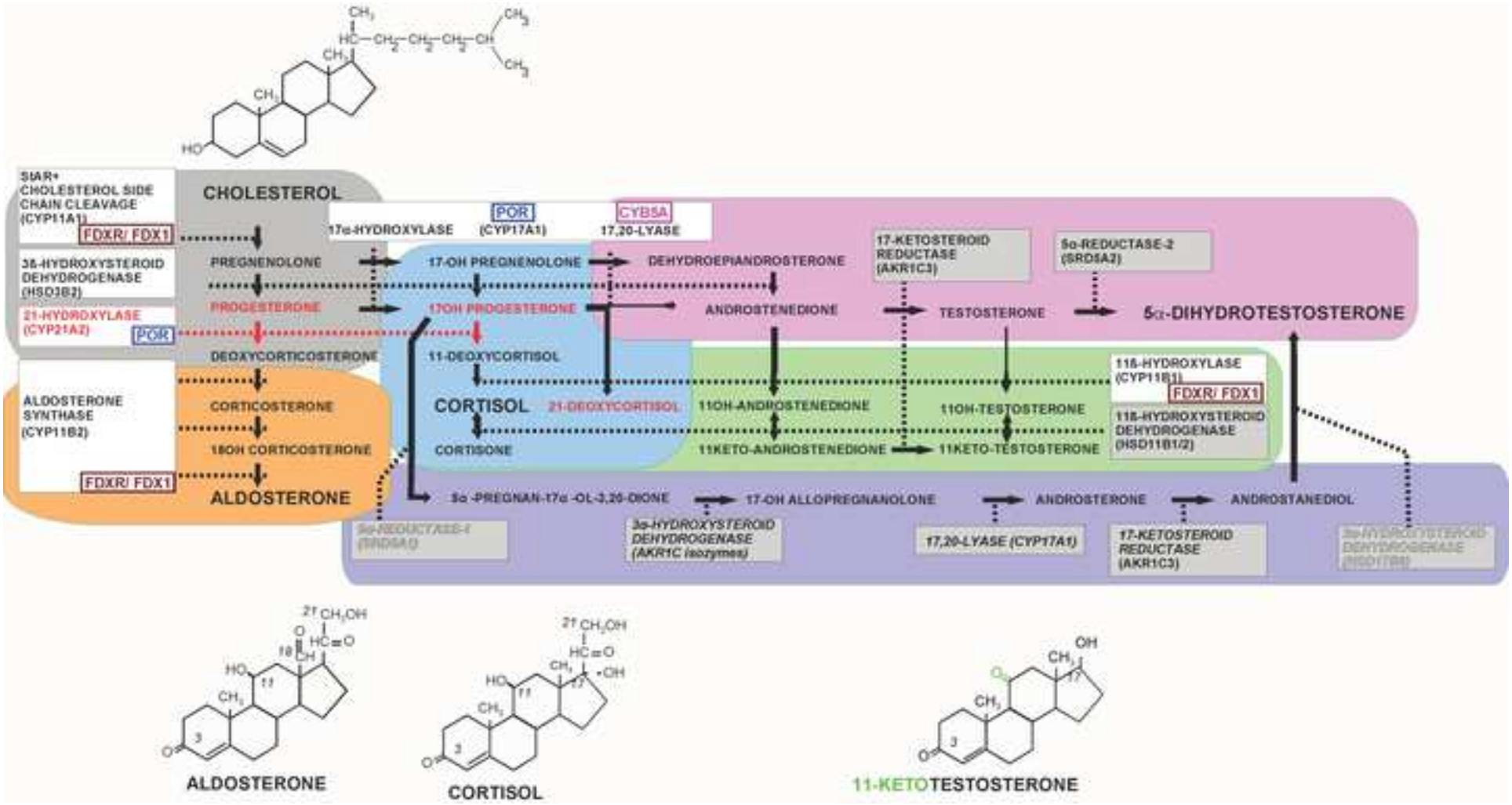


Figure 2

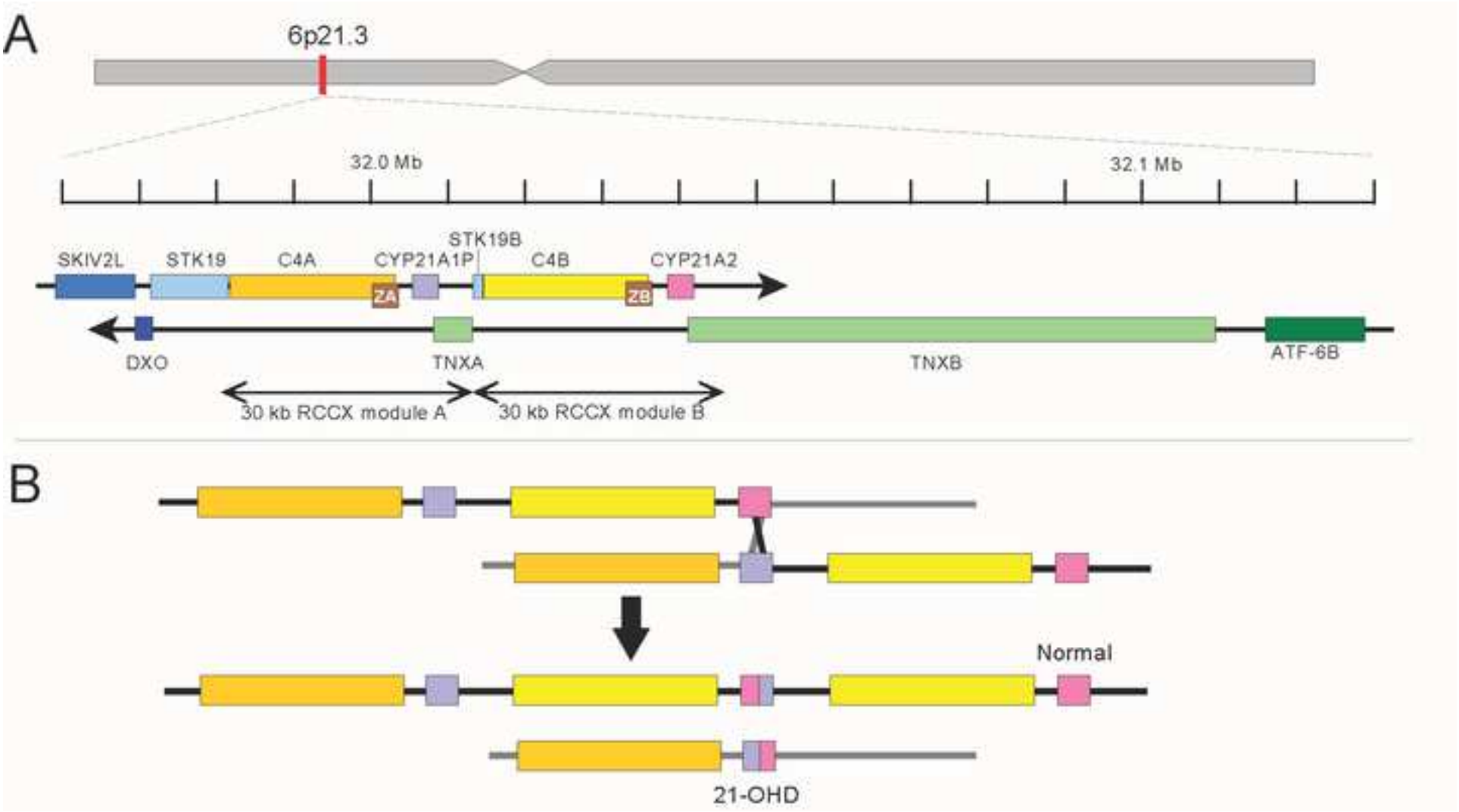
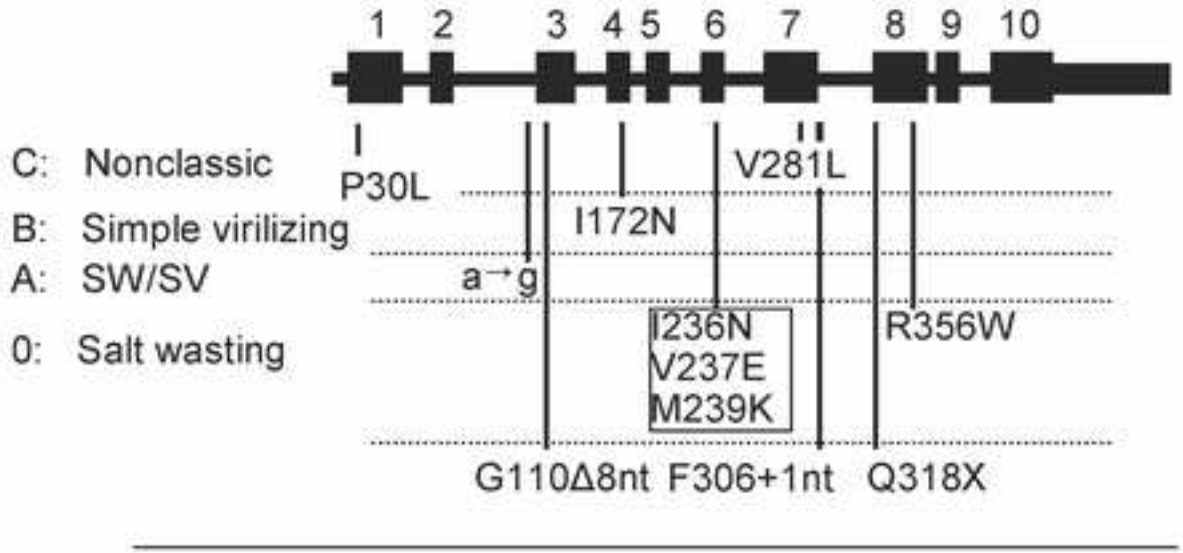


Figure 3



	SW	SV	NC	Predictive value
0	505	20	0	0.96
A	735	84	3	0.89
B	90	394	16	0.79
(P30L)	5	31	62	0.63
C	13	12	700	0.97

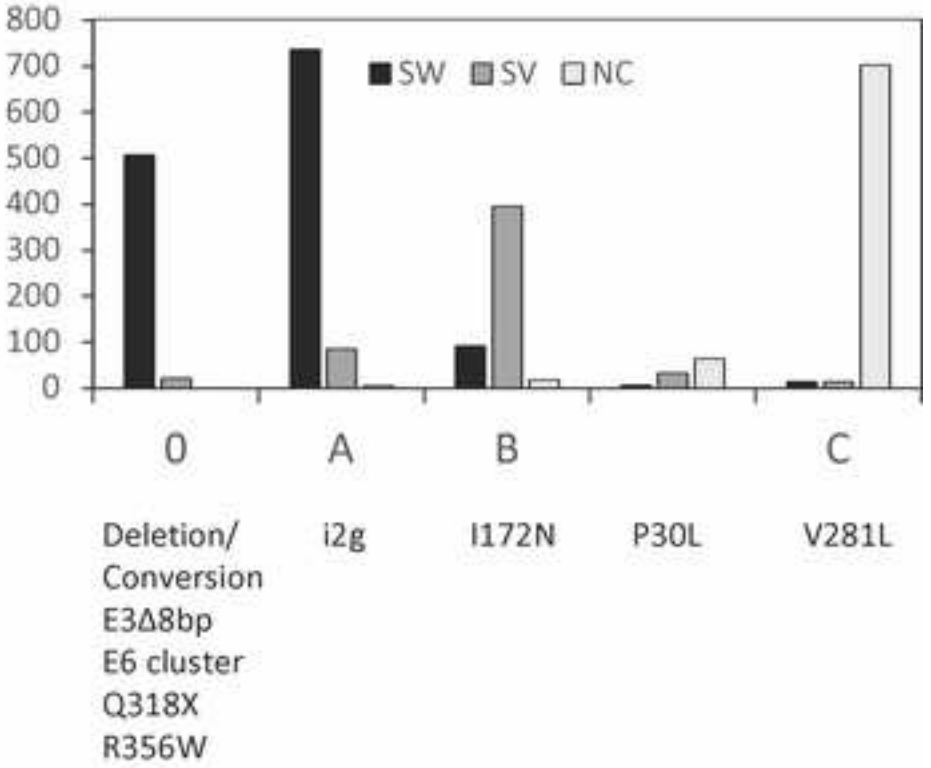


Figure 4

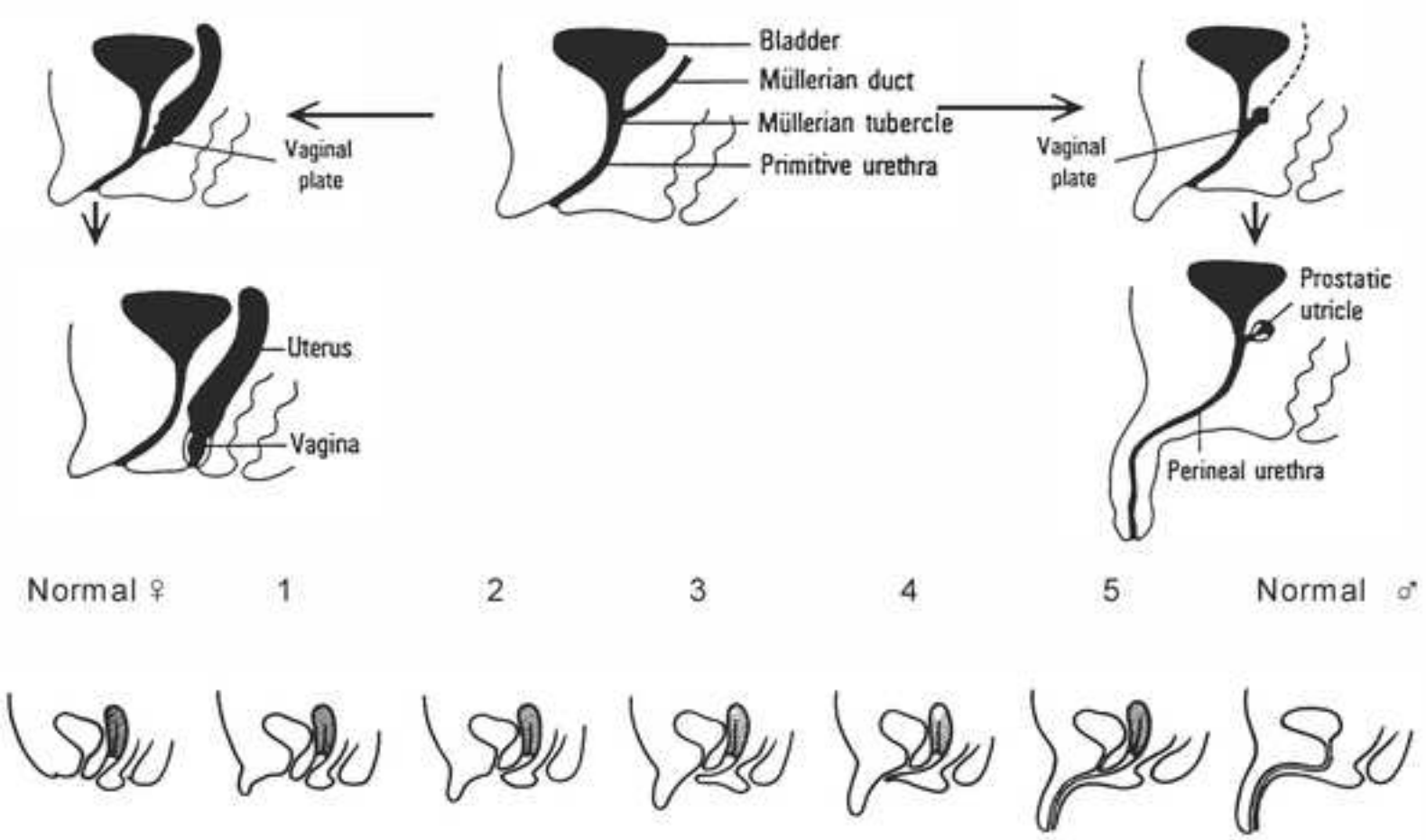


Figure 5

Click here to access/download;Figure;ERCAH Figure 5_Merke.jpg

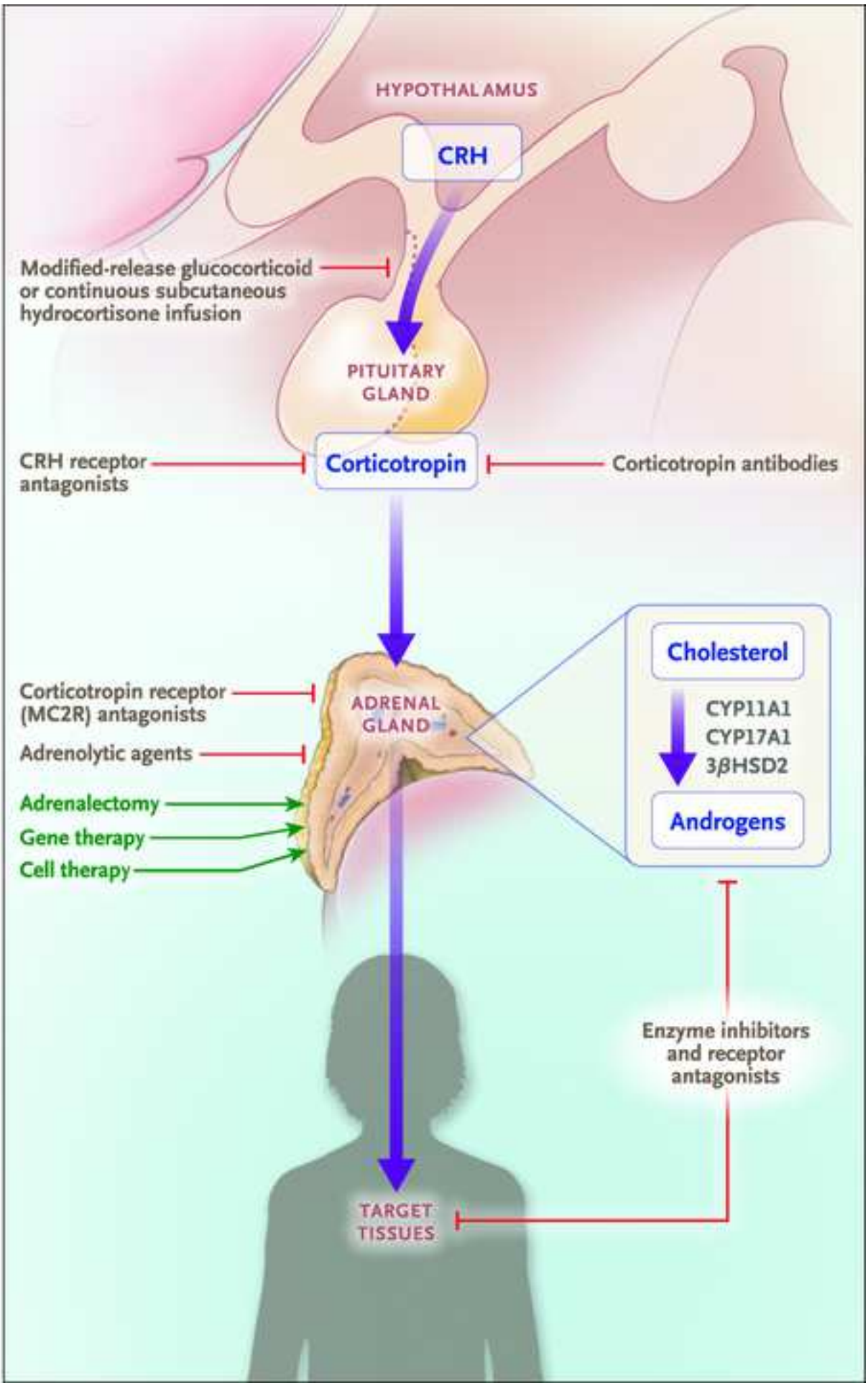


Table 1. Incidence of CAH in different countries

Country	Complete national data?	Sample size	1/Incidence	PPV (term infants or overall)	Reference
Argentina (Buenos Aires)	No	80,436	8,937	50	(3)
Australia*	Yes		18,034	N/A	(4)
Australia (New South Wales)	No	185,854	15 488	1.8	(4)
Australia (Western Australia)*	No	550,153	14,869	N/A	(5)
Brazil	No	748,350	14,967		(6)
Brazil (Goias state)	No	82,603	10,325	28.6	(7)
Brazil (Minas Gerais state)	No	159,415	19,927	2.1	(8)
Brazil (Rio Grande do Sul state)	No	108,409	13,551	1.6	(9)
China	No	30,000	6,084		(10)
China (Beijing)	No	44,360	7,393	3.0	(11)
Croatia	Yes	532,942	14,403		(12)
Cuba	Yes	621,303	15,931	0.3	(13)
Czech Republic	Yes	888,891	12,520	1.6	(14)
France	Yes	6,012,798	15,699	2.3	(15)
Germany (Bavaria)	No	1,420,102	12,457	5	(16)
India	No	55,627	6,334		(17)
Israel	Yes	1,378,132	16,910	16.5	(18)
Japan (Sapporo)	No	498,147	20,756	8	(19)
Japan (Tokyo)	No	2,105,108	21,264	25.8	(20)
Netherlands	Yes	2,235,931	17,468	24.7	(21)
New Zealand	Yes	1,175,988	26,727		(22)
Sweden	Yes	2,737,932	14,260	25.1	(2)
Turkey	No	241,083	15,067	1.9	(23)
United Arab Emirates	Yes	750,365	9,030		(24)
United Kingdom*	Yes		18,248	N/A	(25)
Uruguay	Yes	190,053	15,800		(26)

Data are from studies published in 2008 and later; Earlier studies are summarized by (27) and (2).

Data are from newborn screening except those marked (*) which are from national case registries.

PPV, positive predictive value.

Table 2. Allele frequencies in various regions

Allele	North America	South America	Europe	China	Total
References	**(195,208,213,217)**	**(200,218)**	**(197,203,212,219)**	**(216,220)**	
Deletion/ conversion	21.1%	11.1%	28.8%	21.9%	**21.5%**
P30L*	2.4%	1.0%	1.2%	1.1%	**1.8%**
I2G	23.1%	20.6%	26.7%	33.8%	**25.3%**
E3Δ8bp	2.3%	0.9%	2.4%	0.3%	**1.8%**
I172N	9.0%	9.4%	15.6%	15.1%	**11.4%**
E6	2.2%	1.8%	2.4%	1.9%	**2.1%**
V281L *	22.2%	24.5%	6.2%	1.4%	**15.7%**
Q318X	3.6%	6.5%	3.5%	5.3%	**4.2%**
R356W	3.8%	4.8%	4.3%	6.6%	**4.5%**
Other	10%	10%	8.9%	12.4%	**10.2%**
Alleles analyzed	3527	1094	1338	1142	**7101**

Gene conversion mutations occur with similar frequencies in most populations (**Table 2**).

*P30L and V281L are found mainly in patients with nonclassic CAH and therefore their allele frequencies depend on the proportions of nonclassic patients included in each study.

Table 3. Maintenance therapy in patients with CAH classic CAH		
Drug	**Recommended total daily dose**	**Divided dosing frequency (times daily)**
Children		
Hydrocortisone	10-15 mg/m^2	3-4
Fludrocortisone	0.05-0.2 mg	1-2
Sodium chloride supplements	1-2 g (17-24 mEq/day) in infancy	Several
Adults		
Glucocorticoids		
Hydrocortisone	15-25 mg	2-3
Prednisone	5-7.5 mg	2
Prednisolone	4-6 mg	2
Methylprednisolone	4-6 mg	2
Modified-release hydrocortisone (Plenadren®)	15-25 mg	*no published data in CAH patients, clinical experience shows that in addition to the morning dose a second GC dose is required in the evening
Modified- and delayed-release hydrocortisone (Chronocort®)[a]	15-25 mg	2 (2/3 of dose at 2300 and 1/3 of dose at 0700)[a]
Dexamethasone[b]	0.25-0.5 mg	1
Fludrocortisone	0.05-0.2 mg	1

[a] Not yet launched, currently only available within the extension phase of the phase III study.

[b] Avoid if possible or limit to a short time. Adapted from Speiser et al. (134).

Table 4. Indications for different glucocorticoid (GC) preparations

Steroid	Clinical indication	Pros	Cons
Hydrocortisone	Preferred option for GC replacement.	Best long-term outcome with regard to metabolic, cardiovascular and bone health.	Short half-life. Needs to be given three times daily. Adrenal androgen suppression overnight may escape.
Prednisolone (Prednisone)	Might be a preferred option for regulation of menstrual cycles or fertility induction, or if patient adherence is poor with thrice daily HC.	Longer half-life, twice daily regimen. Potentially better patient adherence compared to three times daily regimen.	Potential higher rate of adverse effects on metabolic, cardiovascular and bone health compared to HC.
Dexamethasone	Fertility induction TART treatment	Strong adrenal suppressive effect, longest half-life, once daily regimen often possible.	Highest rate of adverse effects on metabolism, bone health. Traverses placenta barrier.

Table 5. Biochemical monitoring of glucocorticoid replacement in children and adults

Sample	Variable	Goals and Comments*
Serum	Androstenedione	Normal values for sex and age *(often useful to assess together with testosterone in males)*
	Testosterone	Normal values for sex and age *(assess in the context of gonadotropins and androstenedione)*
	Sex hormone-binding globulin	For calculation of free and bioavailable testosterone
	DHEAS	Low to suppressed, not a good marker of disease control, but can be used to check for compliance/adherence
	17OHP	Normal values indicate overtreatment, aim at ULN to 400-1200 ng/dl (12-36 nmol/l)
	ACTH	Not a useful parameter for disease control; normal values indicate overtreatment
	Androstenedione/Testosterone ratio	Healthy woman: <2 Women with CAH: >4 indicates testosterone mainly of adrenal origin Healthy Males: <0.2 Men with CAH: >0.5 indicates testosterone mainly of adrenal origin Men with CAH: >1.0 + LH, FSH suppressed indicates testosterone only of adrenal origin due to poor disease control
	Progesterone (Females)	Goal is <2 nmol/l (<0.6 ng/ml) in follicular phase for women trying to conceive
	11-oxygenated C19 steroids (11-ketotestosterone, 11-hydroxytestosterone, 11-hydroxyandrostenedione, 11-ketoandrostenedione)	Translational method; not yet established in clinical care
Saliva	Androstenedione	Normal values for sex and age
	17OHP	Up to ~3 times upper normal limit
Urine	GC-MS urinary steroid metabolome analysis (C_{21}-, C_{19}-, C_{18}-steroids)	Translational method; not yet established in clinical care

*These goals are derived from clinical experience and based on expert opinion as there are no established optimal biomarkers nor target values for treatment monitoring.

Table 6. Monitoring glucocorticoid replacement by history and clinical/technical examination (generally every 4 – 6 months in adults, every 3 – 4 months in children >18 months old)

Parameter	Goals and Comments
History	
Symptoms of adrenal insufficiency (fatigue, headache, nausea, abdominal pain, postural dizziness, frequent stress dosing)	No signs of adrenal insufficiency
Adrenal crisis prevention	Well-educated and equipped patient with knowledge of sick day rules, and possession of steroid emergency card and injection kit; medical alert identification worn at all times.
Menstrual cycle	Regular menstrual cycles
Libido, erections (males)	Normal
Sexual health (females)	Pain-free intercourse
Physical examination	
Height (children)	Linear growth within target range
Pubertal development/Tanner stage (children and adolescents)	Normal pubertal development
Blood pressure	Within age- and sex-dependent reference range
BMI	Within age- and sex-dependent reference range
Cushingoid features, Striae distensae	No clinical signs of hypercortisolism
Gynecological assessment only if indicated	
Imaging	
Bone age yearly (children >2 years old /adolescents)	Bone age within 2 SD
Scrotal ultrasound every 2 – 5 years	No gonadal masses
Ovarian ultrasound only indicated in unexplained hyperandrogenism	
Bone mineral density every 3 – 5 years (adults treated with high GC doses)	Within age- and sex-dependent reference range
Others	
Semen analysis if indicated i.e presence of TARTs (males)	Normal results (WHO guideline)
Genetic assessment and counselling	Confirmation diagnosis CAH; counselling for family planning

Table 7. Suggested Management and Glucocorticoid Stress Dosing for patients with Adrenal Insufficiency due to Congenital Adrenal Hyperplasia (395,398,399,402)

Clinical Scenario	Glucocorticoid Management	Additional considerations
At home		
Major illness or high-grade fever (> 39°C children)	Children: Three times the usual dose of hydrocortisone divided into 4 doses (given every 6 hours). Adults: 20 mg of hydrocortisone orally 3 times daily in addition to usual glucocorticoid or triple usual glucocorticoid.	Drink regularly and increase fluid* intake for concentrated (dark) urine. Eat regularly simple and complex carbohydrates. 15 g (children) or 30 g (adults.) Adults with severe infections should divide dose every 6 hours.
Gastroenteritis with diarrhea ± vomiting (with or without fever)	Children: Three times the usual dose of hydrocortisone divided into 4 doses (given every 6 hours); Adults: 10-20 mg of hydrocortisone 3 to 4 times daily in addition to usual glucocorticoid or double or triple usual glucocorticoid; Dose depends on severity of diarrhea. Repeat oral dose if vomiting occurs within 1 hour of medication. If vomiting reoccurs, parenteral hydrocortisone 100 mg (children 50-100 mg/m^2).	Consider early parenteral hydrocortisone. If unable to tolerate fluids, call emergency services for evaluation following glucocorticoid injection. Return to usual dose within 1-2 days of recovery with return to usual diet.
Minor illness or low-grade fever (>38°C in children)	Children: two to three times the usual dose of hydrocortisone divided into 3-4 doses (given every 6-8 hours). Adults: 10 mg of hydrocortisone orally 3 times daily in addition to usual glucocorticoid, or double usual glucocorticoid.	Drink regularly and increase fluid* intake for concentrated (dark) urine. Eat regularly simple and complex carbohydrates. 15 g (children) or 30 g (adults). Return to usual dose within 1 day of recovery.

Exhausting physical exercise	Add one usual dose (children) or 10 mg hydrocortisone (adults) 30 to 60 minutes before exercise.	For unusual activities beyond normal routines. Not for routine use. Can repeat dose(s) if extended time period of strenuous exercise (e.g., marathon)
Procedures		
Major Surgery	Hydrocortisone intravenous bolus 50-100 mg (children 50-100 mg/m^2) followed by continuous intravenous hydrocortisone infusion 100-200 mg (children 100 mg/m^2) over 24 hours. Alternatively, divided doses every 6 hours, intravenous hydrocortisone 100-200 mg/day (children 100 mg/m^2/day)	Taper over 2-3 days with return to usual dose
Short Surgeries	Hydrocortisone intramuscularly or intravenous bolus 50-100 mg (children 50 mg/m^2) just before general anesthesia. Alternatively, give triple the usual morning dose before oral intake is held.	Rapid return to oral regimen
Labor and Delivery	As for surgical procedures	
Bowel procedures requiring overnight laxative	Double or triple usual glucocorticoid dose prior to laxative and repeat every 6 hours if oral medication tolerable and allowed. Alternatively, hydrocortisone 50-100 mg (children 50 mg/m^2) intramuscularly with laxative Hydrocortisone 50 mg (children 50 mg/m^2) intramuscularly or intravenous prior to procedure	
Dental surgery	Extra morning dose 1 hour prior to surgery	Can repeat dose depending on recovery No additional doses for routine dental procedures

Minor procedures with no sedation	No adjustment needed	
Acute emergency	Rapid infusion of Intravenous fluids: 1000 ml of 0.9% sodium chloride (children 20mL/kg normal saline, repeat up to 60 mL/kg) during the first 60 minutes, further fluid administration guided by individual patient needs Hydrocortisone bolus 100 mg (children 50-100 mg/m^2) followed by continuous intravenous infusion 200 mg over 24 hours or 50 mg every 6 hours (children 50-100 mg/m^2/day divided every 6 hours). Reduce to 100 mg (children 50 mg/m^2/day) over 24 hours the following day. For hypoglycemia: dextrose 0.5-1g/kg dextrose or 2-4 ml/kg of D25W (maximum single dose 25 g) infused slowly at rate of 2 to 3 mL/min. Alternatively, 5-10 mL/kg of D10W for children < 12yrs old	Measurement of glucose and electrolytes Cardiac monitoring Consider antibiotics Rapid hydrocortisone tapering and switch to oral regimen depending on clinical status

- *electrolyte-and sugar-containing fluids recommended. If hydrocortisone sodium succinate is unavailable, another parenteral glucocorticoid, such as dexamethasone, methylprednisolone, or prednisolone may be used in equivalent doses. Fludrocortisone replacement is not required if hydrocortisone doses exceed 50 mg every 24 hours but is generally administered, in those normally on fludrocortisone, when oral hydrocortisone is started.

Table 8. Animal models of prenatal glucocorticoid exposure

Animal	Medication/Dosing [1]	Outcome	Reference
Mouse	NK1R antagonists 30 – 300 mg/kg/day[2]	9% cleft palate in higher dosage	(448)
Rat	Dex 0.1 mg/kg/day during the whole pregnancy	Lower body weight and kidney size, postnatal hypertension, albuminuria, sodium retention, and decreased glomerular filtration	(449)
Spiny mouse	Mini osmotic pump with dex 125 mcg/kg	Decreased the number of nephrons and altered expression of genes involved in nephron development in the spiny mouse	(450)
Rat	Dex	Impaired thyroid development with fewer follicular cells and C cells	(451)
Rat	Carbenoxolone[3] 12.5 mg/day	Lower birth weight and increased blood pressure	(452)
Rat	Dex 100 mcg/kg/day sc in late pregnancy	Glucose intolerance, 25% increase in hepatic expression of glucocorticoid receptor	(453)
Rat	Dex 100 mcg/kg/day sc in late pregnancy	Lower birth weight, fatty acid esterification and triglyceride synthesis	(454)
Rodents	Dex 50-120-200 mcg/kg/day	Impaired glucose tolerance, hyperinsulinism increased blood pressure, reduced postnatal growth at 1 year of age despite normal birth weight	(455)
Rat	Carbenoxolone 12.5 mg/day	Reduced birth weight	(456)
11 β HSD mutant mouse	None	Mice lacking Hsd11b2 had lower birthweights and increased anxiety compared to wild type littermates	(457)
Sheep	Betamethasone 0.5 mg/kg during 3 days	Retardation of fetal brain development	(458)
Sheep	Single or repeated betamethasone injections	Reduced brain weight	(459)

Sheep	Repeated betamethasone injections	Reduced neuronal myelinization	(460)
Rhesus macaque	Dex 0.5 or 5 or 10 mg/kg or repeated injections	Decreased numbers of pyramidal neurons in the hippocampal CA regions	(461)
Neural stem cells of newborn rat	Dex in vitro	Impairment of neuron and oligodendrocyte size and differentiation	(462)
Fetal guinea pig	Betamethasone 1 mg/kg for 4 days	Changes in GR DNA binding and DNA methylation in the fetal hippocampus	(463)
Guinea pig	Betamethasone 1 mg/kg for 4 days	Reduced locomotor activity; effect on programming HPA axis and hippocampal glucocorticoid feedback	(464)
Spiny mouse	125 mcg/kg Dex sc for 60 hours using mini pump	Reduction of adrenal steroidogenesis, decrease in plasma DHEA reduced adrenal expression of steroidogenic enzymes in adulthood	(465)
Guinea pig	Betamethasone 1 mg/kg for 4 days	Altered DNA methylation underlies both the long-term effects of glucocorticoids and of maternal stress on the fetus	(466)

[1] Note that the doses given to animals exceed the typical doses given in pregnancies at risk for CAH.

[2]NK1R antagonists modulate the hypothalamic-pituitary-adrenal axis leading to increased corticosterone secretion.

[3]Carbenoxolone is a glycerrhetinic acid derivative with a steroid-like structure. It inhibits placental Hsd11b2 activity, thereby increasing fetal exposure to maternal glucocorticoids (467).

Table 9. Studies of human prenatal exposure to glucocorticoids

Study group			Results			
Dex exposed	**Controls**	**Age at study**	**Questionnaire findings**	**Psychological tests**	**Laboratory and MRI**	**Reference**
***First trimester exposure only*[1]**						
26 total 3 with CAH	**14 total** 3 with CAH	6 mo-5.5 Yrs Mean 2.5±1.3 yrs	NS overall development Dex treated higher shyness, emotionality, lower sociability (EAS), internalizing (CBCL) (parental Q)			(475)
174 total 48 with CAH	**313 total** 195 with CAH	1-12 yrs 3 diff age groups	No developmental differences NS CBCL school scale (parental Q)			(476)
22 total 10 F 7 M and 5 M with CAH	**35 total** all healthy	7-17 yrs Median 11 yrs	NS CBCL school scale (parental Q) Poorer scholastic competence (self-reported)	NS IQ, but Poorer working memory (WISC-III) NS learning, memory (NEPSY)		(477)
Same study Population			NS behavior (CBCL), or shyness (SPAI-CP). Higher scores sociability (EAS) (parental) more social anxiety (self-reported Q)			(478)

Same study population			M reported more neutral behavior KI-GRB (self reported Q) F, NS			(479)
<u>Study 1</u> **67 total** 51non-CAH (35 F, 16 M) 8 M CAH (8 F full term Dex) <u>Study 2</u> **7 total** (1 CAH F Full term Dex)	**73 total** 31 F 16 M 15 CAH F 11 CAH M **13 total** 2 F 1 M 4 CAH F 6 CAH M	<u>Study 1</u> 5-12 yrs <u>Study 2</u> 11-24 yrs		<u>Study 1</u> Few significant findings K-ABC Sequential Processing positive for M alone p=0.095 <u>Study 2</u> Non-CAH F performed significantly less well on Faces & Places M, NSerence.		(480)
9 F	8 non-CAH 9 CAH non-DEX All F	Mean 12 yrs But CAH untreated 16 yrs	NS psychopathology (CBCL) (parental Q)	Lower scores in non-CAH Dex treated F (WAIS-R-PL, WISC-R)		(481)
34 total 16 F 18 M	**66 total** 36 F 30 M	7-17 yrs Mean age 10.5 yrs		Poorer working memory (WISC-III) Sex difference with larger neg effects in F for executive functions and psychometric intelligence (WISC-III, WMS-III)		(482)

34 15 F 19 M	**67 total** 36 F 31 M	7-17 yrs Mean age 10.5 yrs	CBCL, SPAI-R and EAS (parental Q) SASC-R (self reported Q))	NS. Generally well adjusted.		(483)
23 Adults 12 F 11 M	**Population controls** 31 F 27 M	16-24 yrs Mean age 20-21		No significant neuropsychological changes; no increase in anxiety, depression or autistic traits.		(484)
29 total 12 F 17 M	**37 total** 18 F 19 M	Mean age 16.5-17 yrs		Methylation in BDNF, FKBP5, and NR3C1 genes were associated with the performance on WAIS	Altered DNA methylation in peripheral CD4+ T-cells	(485)
16 total 9 F 7 M	**15 total** 8 F 7 M	Mean 24 yrs			Lower insulin secretion by 17%-22%. Lower glucagon	(486)
40 total 18 F 22 M	**75 total** 35 F 40 M	Mean age 16.3 ± 6.2 Age groups Young<16 Older $\geq$16			HOMA-β index, lower β-cell function in younger F. Glucose level, higher in younger age group	(487)

					Higher cholesterol, and LDL in older age group	
19 total 9 F 10 M	**43 total** 26 F 17 M	16-26 yrs			MRI Alterations in brain structure Enlarged amygdala, surface area and volume of left sup frontal gyrus, widespread white matter changes	(483)
Girls with CAH, Dex-treated until term						
4				1 not able to perform neuropsychological testing Generally low IQ		(477)
Study 1 8 Study 2 1	Study 1 15 CAH girls Study 2 4 CAH F	Study 1 5-12 yrs Study 2 11-24 yrs		Performed more poorly on K-ABC Mental Processing Composite (p=0.09) Performed (marginally) less well on Hand Movements (subtest Sequential Processing) and Spatial Memory (Simultaneous Processing).		(480)
9	8 non-CAH	Mean 12 yrs	NS psychopathology (CBCL) (parental Q)	Higher scores IQ in Dex treated F.		(481)

	9 CAH untreated	and CAH untreated 16 yrs		Lower in non-CAH Dex treated F (WAIS-R-PL, WISC-R)		
4	25 F CAH		Diff in self-perceived deficits in executive function (B-DEFS-SF)	Broad deficits in most measures of cognition in Dex treated F (WAIS-IV, WMS-III)		(488)

[1] The earlier studies reported results from mixed cohorts, short-term treated boys and girls without CAH and boys with CAH, while more recent studies have assessed individuals with and without CAH, males and females, separately.

Abbreviations

NS, not significantly different; Dex, dexamethasone; CAH, congenital adrenal hyperplasia; CBCL, Child Behavior Checklist; EAS Temperament Survey for Children; WISC-III, Wechsler Intelligence Scales for Children; NEPSY, Developmental Neuropsychological Assessment; SPPC, Self-Perception SPAI-C-P, Social Phobia and Anxiety Inventory for Children – Parent Report; K-ABC, Kaufman Assessment Battery for Children; WAIS, Wechsler Adult Intelligence Scale; WMS, Wechsler Memory Scale; KI-GRB, The Karolinska Inventory of Gender Role Behavior; B-DEFS-SF, Barkley Deficit in Executive Functioning Scale – Short Form.

HOMA-β homeostasis model assessment of beta cell function; LDL, low density lipoprotein; MRI magnetic resonance imaging.

Table 10. Selected generic and CAH-specific risk factors for mental health, psychosocial/psychosexual adaptation and well-being.

A. **Generic (noncategorical)**	**Illustrative studies**
Males and Females	
Challenges to parenting with …	(529)
… accompanying caregiver psychological distress	(530,531)
… negative emotional spillover effects from parent to child	(532-534)
… perceived child vulnerability and overprotectiveness	(535,536)
Burdens of clinic visits and adherence to sometimes complex and changing treatment regimens; emergency room visits and hospitalizations	(537-539)
Threats to body-image and self-esteem	(540,541)
Higher rates of missed school and peer victimization	(542,543)
Academic challenges	(544)
Problems of psychosocial adaptation (i.e., increased psychological symptomatology in youth and adults compared to healthy comparison groups	(542,545-547)
Systemic weaknesses in the process of transitioning from pediatric to adult care	(548-550)
Career barriers for people with chronic illness	(551)
B. **CAH-specific (categorical)**	
Female-specific	
Early reactions to newborn with atypical genitalia (experiences in medical environment)	(549,552)
Stigma (anticipated or experienced) stemming from atypical genitalia and its modulation by culture	(553-563)
Tension between person-first (i.e., CAH as a medical condition) versus identity-first (intersex and LGBT advocacy); and related human rights perspectives	(564-566)
Secrecy	(562,567-570)

Genital examinations and medical photography	(550,571)
Gender of rearing in Prader V cases	(134,572-574)
Genital surgery decision making and …	(575)
… consequences for sexual function	(528)
… outcomes of postponing surgery	(576)
Gender identity	(577-579)
Effects on social support	(580,581)
Model of care	(342)
Males and Females	
Terminology	(582-584)
Early puberty/attenuated adult height; growth hormone therapy	(33,509)
Neurocognitive sequelae	(488,585,586)
… prenatal dexamethasone	(374,482,489)
… hyponatremic episodes	(587)
Fertility problems (testicular adrenal rest tumors in males; low levels of fecundity in females)	(588-592)

Essential points:

- Congenital adrenal hyperplasia (CAH) is most often caused by deficiency of steroid 21 hydroxylase encoded by *CYP21A2*.
- Allelic variants are associated with a spectrum of phenotypes.
- CAH in its severe, classic form includes cortisol and aldosterone deficiencies, as well as androgen excess.
- Newer concepts in steroid biosynthesis, hormonal and genetic diagnostic tools, and novel therapeutics have expanded our understanding of CAH.
- Long term sequelae of this disease have been reported in detail and strategies are being developed to improve quality of life for these patients.