Chapter 5
Genetics and Pathophysiology of Congenital Adrenal Hyperplasia

Selma Feldman Witchel

Abbreviations

17-OHP	17-hydroxyprogesterone
21-OHD	CAH due to 21-hydroxylase deficiency
ACTH	Adrenocorticotropic hormone
CAH	Congenital adrenal hyperplasia
CRH	Corticotropin-releasing hormone
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
NC-21-OHD	Nonclassic 21-hydroxylase deficiency
SULT2A1	Steroid sulfotransferase

Introduction

The virilizing congenital adrenal hyperplasias are a family of autosomal recessive disorders affecting adrenal steroidogenesis that are characterized by excessive adrenal androgen production. The most common form is 21-hydroxylase deficiency (21-OHD) due to mutations in the 21-hydroxylase (*CYP21A2*) gene. The other virilizing forms are 3β-hydroxysteroid dehydrogenase and 11β-hydroxylase deficiencies associated with mutations in the 3β-hydroxysteroid dehydrogenase (*HSD3B2*) and 11β-hydroxylase (*CYP11B1*) genes, respectively. Another form of CAH associated

S.F. Witchel, M.D.
Division of Pediatric Endocrinology, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh, 4401 Penn Avenue, Pittsburgh, PA 15224, USA
e-mail: selma.witchel@chp.edu

A.C. Levine (ed.), *Adrenal Disorders*, Contemporary Endocrinology,
DOI 10.1007/978-3-319-62470-9_5

with genital ambiguity is oxidoreductase deficiency (PORD), which is associated with mutations in the cytochrome P450 oxidoreductase (*POR*) gene. *POR* encodes a flavoprotein that serves as an electron donor for cytochrome P450 steroidogenic enzymes such as 21-hydroxylase. Congenital lipoid adrenal hyperplasia (CLAH) is associated with mutations in the steroidogenic acute regulatory protein (StAR) gene, undervirilization of male fetuses, and absence of circulating steroid hormones. Mutations in 17α-hydroxylase/17,20-lyase (*CYP17A1*) are associated with undervirilization in males, absent puberty in females, and hypertension. Mutations in the aromatase (*CYP19A1*) gene interfere with the conversion of androgens to estrogens and are characterized by maternal virilization during puberty, virilization of female fetuses, failure of epiphyseal fusion, tall stature, and hyperandrogenic symptoms in adolescent and adult females. This chapter will focus on 21-hydroxylase deficiency because it is the most common form of congenital adrenal hyperplasia. A brief outline of other defects in steroidogenesis is provided in Table 5.1.

The clinical features associated with CAH comprise a spectrum reflecting the consequences of the specific mutation. In the case of 21-OHD, the continuum ranges from salt-losing and simple virilizing forms to the milder forms. Collectively,

Table 5.1 Disorders of steroidogenesis. Gene, gene location, and typical phenotypes are listed. In general, severity of phenotype correlates with genotype

Gene	Location	Phenotype	Characteristic laboratory findings
CYP21A2 Classic forms	6p21.33	Ambiguous genitalia with virilization of females with continued postnatal virilization if undiagnosed Normal male genitalia at birth Acute adrenal insufficiency with salt-losing crises	Increased 17-OHP, P4, androstenedione, and ACTH Increased PRA
CYP21A2 Nonclassic forms	6p21.33	Premature pubic hair, tall stature, irregular menses, acne, and infertility	Increased 17-OHP, P4, androstenedione, and ACTH
HSD3B2	1p12	Ambiguous genitalia with virilization of females Ambiguous genitalia with undervirilization of male infants Acute adrenal insufficiency with salt-losing crises	Increased 17-Preg, DHEA Increased PRA in classic salt-losing forms
CYP11B2	8q24.3	Ambiguous genitalia with virilization of females with continued postnatal virilization if undiagnosed Variable hypertension	Increased 11-deoxycortisol, DOC, androstenedione, and ACTH
StAR	8p11.23	Undervirilization of male infants Acute adrenal insufficiency with salt-losing crises	All steroid hormones are low or absent
CYP17A1	10q24.3	Undervirilization of males Delayed/absent puberty in females Variable hypertension	Increased DOC and ACTH Low 17α-hydroxylated steroids Decreased PRA

Table 5.1 (continued)

Gene	Location	Phenotype	Characteristic laboratory findings
POR	7q11.23	Ambiguous genitalia in males and females Antley-Bixler skeletal anomalies, i.e., craniosynostosis, radiohumeral synostosis, midface hypoplasia, and femoral bowing Infertility	Increased 17-OHP, P4, and ACTH Decreased DHEA, androstenedione, testosterone Normal electrolytes
CYP19A1	15q21.2	Virilization of female infants Maternal virilization during pregnancy Delayed puberty with hypogonadotropic hypogonadism and multicystic ovaries in females Delayed/failed epiphyseal fusion Osteopenia/osteoporosis Impaired glucose tolerance/insulin resistance Decreased sperm number and impaired motility	Increased androgens and P4 Increased LH and FSH
CYB5	18q22.3	Undervirilization of male infants because cytochrome b_5 is requisite cofactor for P450c17	Decreased testosterone Methemoglobinemia

Key: *17-OHP* 17-Hydroxyprogesterone, *P4* progesterone, *DHEA* dehydroepiandrosterone, *PRA* plasma renin activity

the salt-losing and simple virilizing forms are considered to be the classic forms. The mild form is also known as the late-onset or nonclassic form (NCAH). This classification system is somewhat contrived because disease severity is better represented as a continuum based on residual enzyme activity. The incidence of the classic forms ranges from 1:5000 to 1:15,000 with variation among ethnic/racial backgrounds [1]. The prevalence of 21-OHD is lower among African-Americans than Caucasians in the United States [2]. Incomplete ascertainment muddies accurate determination of the incidence of NCAH. However, available data indicate that NCAH may occur in 1:1000 with increased frequency among Hispanics, Yugoslavs, and Ashkenazi Jews [3].

Pathophysiology

In these disorders, the loss of cortisol negative feedback inhibition leads to increased hypothalamic corticotrophin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH) secretion. The excessive ACTH secretion leads to accumulation of steroid hormone intermediates proximal to the deficient enzyme and hyperplasia of the zona fasciculata and zona reticularis.

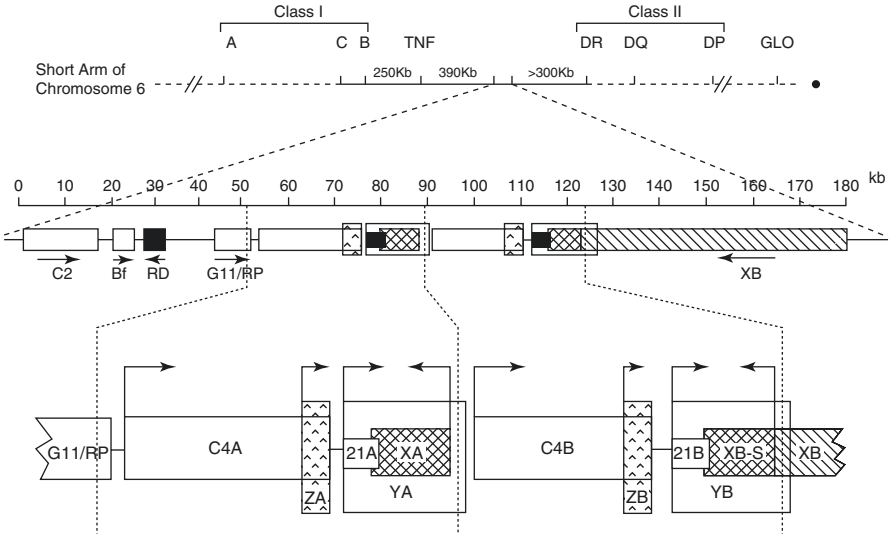

Fig. 5.1 Pathways of adrenal steroid hormone synthesis particularly relevant for 21-OHD. *CYP11A1* cytochrome P450 cholesterol side-chain cleavage, *StAR* steroidogenic acute regulatory protein, *CYP17A1* 17α-hydroxylase/17,20-lyase, *HSD3B2* 3β-hydroxysteroid dehydrogenase type 2, *P450oxido* P450-oxidoreductase, *CYB5A* cytochrome b_5, type A, *SULT2A1* sulfotransferase 2A1, *CYP21A2* 21-hydroxylase, *CYP11B1* 11β-hydroxylase, *CYP11B2* aldosterone synthase, *AKR1C3* 17β-hydroxysteroid dehydrogenase type 5, *SRD5A* 5α-reductase

In the classical pathway of adrenal steroidogenesis (Fig. 5.1), cholesterol is converted to pregnenolone by the P450 side-chain cleavage enzyme encoded by *CYP11A1*. Most steroidogenic enzymes are cytochrome P450 enzymes which acquired their family name because they absorb light at 450 nm when reduced with carbon monoxide [4]. In the zona glomerulosa, pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase type 2 encoded by *HSD3B2*. Progesterone is converted to dexoxycorticosterone by 21-hydroxylase and subsequently to aldosterone by aldosterone synthase encoded by *CYP11B2*. Aldosterone secretion is regulated by the renin-angiotensin system and serum potassium concentrations.

In the zona fasciculata, pregnenolone is hydroxylated by the enzyme 17α-hydroxylase/17,20-lyase encoded by *CYP17A1* to 17-hydroxypregnenolone, which is converted to 17-hydroxyprogesterone (17-OHP) by 3β-hydroxysteroid dehydrogenase type 2. Subsequently, 17-OHP is converted by 21-hydroxylase to 11-deoxycortisol, which is then converted by 11β-hydroxylase to cortisol. In the zona reticularis, the enzyme 17α-hydroxylase/17,20-lyase converts 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA), which is subsequently converted to androstenedione by 3β-hydroxysteroid dehydrogenase type 2. DHEA can undergo sulfation by steroid sulfotransferase, SULT2A1, to form DHEAS.

Thus, the substrates immediately proximal to 21-hydroxylase, progesterone and 17-OHP, are elevated in patients with 21-OHD. Unfortunately, the pathophysiology of CAH is more complex than would be predicted for an autosomal recessive disorder in

which the expression of the defective protein is limited to the adrenal cortex. This complexity is likely due to genetic variants at other loci which influence steroid metabolism and steroid responsiveness. More recently described alternative pathways affecting steroid hormone metabolism may also influence the clinical manifestations.

In the alternative "backdoor" pathway, 17-OHP is sequentially converted by 5α-reductase and the 3α-reductase activities of AKR1C2/4 to generate 5α-pregnane-3α,17α-diol-20-one (pdiol) that is subsequently converted to dihydrotestosterone (DHT) [5]. This alternative pathway bypasses testosterone as an intermediate. Urinary concentrations of metabolites indicative of increased flux through the alternative pathway are higher in affected individuals, particularly infants [6]. This pathway may contribute to the androgen excess responsible for prenatal virilization of affected female fetuses [7].

Under normal circumstances, the direct conversion of 17-OHP to androstenedione is not significant in humans. Yet, when 17-OHP accumulates in 21-OHD, it is metabolized by this alternative pathway [8]. Defective 21-OHD also promotes accumulation of other steroid hormone intermediates such as 21-deoxycortisol, 16α-hydroxyprogesterone, 11-ketoandrostenedione, and 11-ketotestosterone [9]. The enzyme 17β-hydroxysteroid dehydrogenase type 5 also known as aldo-keto reductase 1C3 (AKR1C3) can convert DHEA and androstenedione to androstanediol and testosterone, respectively [10]. It has been suggested that 11β-hydroxyandrostenedione, 11-ketoandrostenedione, 11β-hydroxytestosterone, and 11-ketotestosterone (11KT) are specific markers for adrenal-derived C-19 androgen hormones [11]. Regarding androgenic potency, 11β-hydroxytestosterone and 11-ketotestosterone have similar but slightly lower androgenic activity than testosterone using an in vitro cell-based luciferase reporter assay [12].

Clinical Features

Consequences of cortisol deficiency include poor cardiac function, poor vascular response to catecholamines, and increased secretion of antidiuretic hormone [13]. For 21-OHD, complete loss of function mutations abrogate aldosterone synthesis leading to hyponatremia due to impaired urinary sodium reabsorption. The hyponatremia leads to hypovolemia, elevated plasma renin levels, and, eventually, shock if not promptly recognized and treated. In the absence of aldosterone, potassium cannot be excreted efficiently resulting in hyperkalemia [14]. In 21-OHD, the elevated 17-OHP and progesterone concentrations exacerbate the mineralocorticoid deficiency because both hormones have antimineralocorticoid effects and, in vitro, interfere with aldosterone-mediated mineralocorticoid receptor transactivation [15]. In addition, the lack of prenatal cortisol exposure disrupts adrenomedullary development and can be associated with epinephrine deficiency and hypoglycemia [16].

Female infants with classical 21-OHD, either salt-losing or simple virilizing, generally present in the neonatal period with ambiguous genitalia. In some instances, the diagnosis of genital ambiguity has been suspected based on prenatal ultrasound

findings. For affected female infants, the external genital findings can range from a nearly male appearance with penile urethra and bilateral undescended testes to minimal clitoromegaly. The most common physical findings in affected girls include clitoromegaly, fused rugated labia majora, and a single perineal orifice. The extent of prenatal virilization can lead to misassignment of gender at birth. Occasionally, the minimally virilized girl may not be identified until progressive clitoromegaly prompts a medical evaluation.

Affected 46,XX female infants with 21-OHD have normal female internal genitalia. The uterus can be identified on ultrasound. The ovaries may be too small to be visualized on ultrasound. Despite excessive prenatal androgen exposure, ovarian position is normal, Mullerian structures persist, and the Wolffian ducts regress. The Mullerian structures develop normally to form the fallopian tubes, uterus, and upper vagina. Virilized girls may have incomplete separation of the urethra and vagina resulting in a urogenital sinus and a single perineal orifice.

Apart from hyperpigmentation, external genital development is normal in boys with 21-OHD. Whereas girls are usually detected due to genital ambiguity, boys with salt-losing CAH appear well in the immediate newborn period. Infants with CAH tend to feed poorly and fail to regain their birth weight. Typically, they develop vomiting, hypotension, hyponatremia, and hyperkalemia in the first 10–14 days of life. Prior to implementation of newborn screening, affected boys typically presented with hyponatremic dehydration, hyperkalemia, and shock with the potential for a fatal outcome.

Pubarche refers to the development of pubic hair, axillary hair, apocrine body odor, and acne. Pubarche is the physical manifestation of adrenarche which reflects adrenal pubertal maturation and increased production of adrenal C19 steroids. Children with simple virilizing or NCAH often present with premature development of pubic hair (premature pubarche). Premature pubarche is defined as the presence of pubic hair, axillary hair, or apocrine odor developing before 8 years in girls and 9 years in boys. Additional features in children include tall stature, accelerated linear growth velocity, and advanced skeletal maturation. Clitoromegaly may develop in girls. Boys manifest phallic enlargement with prepubertal-sized testes. In a multicenter study, children less than 10 years of age most often presented with premature pubarche [17]. Among children with premature pubarche, the diagnosis of CAH should be considered when basal 17-OHP, androstenedione, and testosterone concentrations are elevated and/or bone age is advanced [18]. Nevertheless, CAH is an uncommon cause of premature adrenarche [19].

Symptoms of milder, late-onset, or nonclassic 21-OHD (NC-21-OHD) include hirsutism, irregular menses, chronic anovulation, acne, and infertility. Hirsutism, defined as excessive growth of coarse terminal hairs in androgen-dependent areas in women, has been reported to be the most common presenting feature among women [20, 21]. Hirsutism reflects the apparent sensitivity of the pilosebaceous unit/hair follicle to both circulating androgen and local androgen concentrations. Importantly, the extent of the hirsutism correlates poorly with circulating androgen concentrations [22].

Acne can occur among patients with NC-21-OHD but is rarely the primary clinical manifestation. Consideration should be given to further evaluation for patients with severe cystic acne refractory to oral antibiotics and retinoic acid treatment.

Severe androgenic alopecia accompanied by marked virilization in older previously undiagnosed women has been described [23].

The nature of the symptoms leads to an ascertainment bias favoring diagnosis in affected women. Men with NC-21-OHD are typically identified through family studies. Individuals with NC-21-OHD usually do not have elevated ACTH concentrations. Some have an overresponsive ACTH-stimulated glucocorticoid response, possibly reflective of subtle adrenal hyperplasia [24].

Due to the similar clinical features, it may be difficult to distinguish women with NC-21-OHD from those with polycystic ovary syndrome (PCOS) [25, 26]. Women with NC-21-OHD tend to have higher 17-OHP and progesterone concentrations than women with PCOS [27]. Insulin resistance, obesity, polycystic ovary morphology, and elevated LH/FSH ratios tend to be more common among women with PCOS. However, none of these features clearly differentiate women with NCAH from those with PCOS [28]. Anti-Mullerian hormone concentrations do not discriminate women with NCAH from those with PCOS [29].

Family studies have demonstrated that not all individuals with genotypes consistent with NC-21-OHD develop symptoms of androgen excess. Curiously, in a study of 145 probands with 21-OHD, 4% of parents were identified to have undiagnosed or cryptic NC-21-OHD [30]. Apart from infertility among the women, these individuals had achieved normal adult heights and did not report episodes of adrenal insufficiency [30].

One uncommon feature is an adrenal myelolipoma. These adrenal mass lesions consist of myeloid, erythroid, and megakaryocytic cell lines and appear as hyperechoic masses on ultrasound and fat-containing masses on CT scan. Typically, these lesions are benign, but larger lesions are at risk for hemorrhage or rupture. MRI signal characteristics depend on the composition of the lesion.

Hypothalamic-Pituitary-Gonadal (HPG) Axis and Reproductive Concerns

Oligo-amenorrhea, chronic anovulation, and infertility are common presenting complaints for women with NC-21-OHD and can occur in women with classic 21-OHD despite adequate hormone replacement therapy. Hence, women with 21-OHD can develop a secondary “PCOS” phenotype [31]. The specific molecular mechanisms responsible for the altered hypothalamic-pituitary-adrenal (HPO) axis function accompanied by apparent ovarian androgen excess are unclear. Increased circulating concentrations of adrenal androgens and progestins likely influence HPO axis function.

Additional features affecting female reproduction include vaginal stenosis with dyspareunia, impaired sensation, changes in cervical mucous, poor self-esteem, and disinterest in having children. Impaired quality of life and risk for depression have been reported to be higher in women with 21-OHD [32]. Potential contributing factors include engaging in high-risk behaviors, perception of being different from

other women, and perceived lack of autonomy [33]. Reproductive outcomes for women with CAH can be greatly improved by adequate suppression of progesterone and 17-OHP to promote ovulation and implantation of the fertilized ovum; this may require optimizing both glucocorticoid and mineralocorticoid therapies [34]. Occurrence of miscarriages is higher among untreated women with NC-21-OHD [20, 21]. The consequences of prenatal androgen exposure on the developing female brain are being explored [35].

Quality of life (QoL) for women with CAH has been a long-standing concern for women with CAH. One series reported later sexual debut, fewer pregnancies and children, and increased incidence of homosexuality; these outcome measures were related to type of surgical correction and the severity of their mutations [36]. Girls with CAH are reported to prefer more masculine toys, more male-dominant occupations, rougher sports, and non-heterosexual orientation [37]. Another series of 24 women, who answered a questionnaire, reported that 87.5% of women indicated that CAH had not interfered with their social relationships [38]. Regarding gender identity and sexual orientation, 25% of women indicated that they had occasionally wished to be a man, and 62% reported having heterosexual orientation at all times [38]. This area of investigation has been confounded with several factors including small numbers of subjects, lack of control subjects, changes in surgical techniques over time, and variability of age at the time of surgical correction.

Careful consideration regarding surgery is urged for girls with genital ambiguity. Only experienced surgeons/urologists should perform feminizing genitoplasty and vaginal reconstruction [39]. During adolescence, the adequacy of the vaginal introitus for the use of tampons and sexual intercourse should be assessed. For girls with vaginal stenosis, dilatation is often helpful.

Gonadal adrenal rest tumors, predominantly testicular adrenal rests (TARTs), occur in up to half of men with 21-OHD. These tumors arise from adrenal cells that descend with the testes during testicular development. TARTs are not malignant but can compress the rete testis and seminiferous tubules culminating in testicular atrophy and obstructive azoospermia. Ultrasound and MRI are helpful to detect TARTs less than 2 cm because small lesions are generally not palpable. TARTs may be present in childhood and adolescence and have rarely been described in men with NC-21-OHD [40, 41]. Although TARTs have been attributed to poor adherence with hormone replacement therapy, pathogenesis of TARTS may be more complicated [42]. In affected males, the elevated adrenal C19 steroid secretion can suppress gonadotropin secretion resulting in hypogonadotropic hypogonadism and subsequent oligospermia. Ovarian adrenal rest tumors (OARTs) have been infrequently reported in affected women.

Molecular Genetics

The *CYP21A2* gene is located in a complex genetic region at chromosome 6p21.3 where it lies in close proximity to a highly homologous pseudogene, *CYP21A1P*. *CYP21A2* and *CYP21A1P* are arranged in tandem repeats with the *C4A* and *C4B* genes, which encode complement component 4. The tenascin (*TNX*) and serine

threonine nuclear protein kinase (*RP*) genes are also mapped to this region. These four genes, *RP*, *C4*, *CYP21*, and *TNX*, form a unit known as RCCX. Most alleles carry two RCCX units in which one has *CYP21A2* and the other has *CYP21A1P* (Fig. 5.2).

To date, over 200 *CYP21A2* mutations have been reported (http://www.hgmd.cf.ac.uk; www.cypalleles.ki.se). Yet, despite the large number of reported mutations, approximately ten mutations account for the majority of affected alleles. Most mutations result from gene conversion events in which the functional gene acquires deleterious *CYP21A1P* sequences or from misalignment during meiosis that can give rise to duplication or deletions of the RCCX unit. Haplotypes with three or four RCCX units have been described [43]. Another example of misalignment is a *CYP21A1P*/*CYP21A2* chimera in which a portion of the *CYP21A1P* gene is fused to a portion of the *CYP21A2* gene [44]. Rarely, CAH can be associated with uniparental disomy [45]. The de novo mutation rate is approximately 1%.

Most affected individuals are compound heterozygotes with different mutations on each allele. Mutations range from complete loss of function to mild missense mutations. Estimates of in vitro 21-hydroxylase activity range from <1% for mutations associated with salt-losing CAH to 2–10% for simple virilizing CAH and to 30–50% for NCAH. Genotype, residual enzyme activity, and phenotype generally correlate such that an individual's phenotype reflects their milder mutation. Patients with classical salt-losing CAH usually carry complete loss of function mutations on both alleles. Patients with simple virilizing CAH typically have a complete loss of function mutation on one allele and the I172N or intron 2 splicing mutation on their other allele. Patients with NCAH often carry different mutations with at least one allele carrying a mild missense mutation such as V281 L. Approximately 25–50% of individuals with NCAH are reported to have mild mutations on both alleles [46–

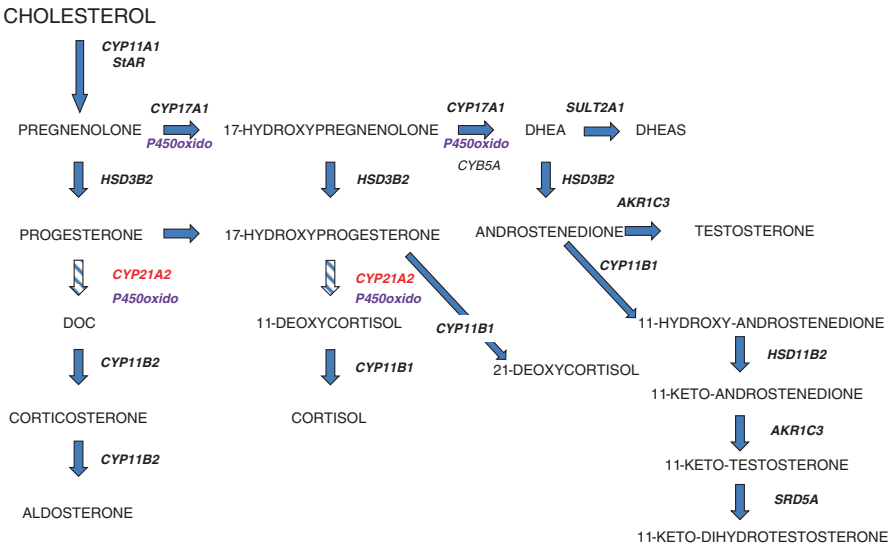

Fig. 5.2 Genetic organization of *CYP21A2* and *CYP21A1P*. This figure illustrates the location of the *C4A*, *CYP21A2*, *C4B*, and *CYP21A1P* genes on the short arm of chromosome 6

48]. Mutations associated with NCAH include V281L, P453S, and R339H. The P30L mutation is often detected in patients with NCAH but is typically associated with more severe androgen excess [49].

As noted above, the *CYP21A2* locus is quite complex which precludes molecular genetic analysis as the first-line diagnostic test. Molecular genetic testing is also confounded by the possibility of multiple mutations on a single allele and the presence of different *CYP21A2* mutations in one family. Multiple genetic testing strategies such as PCR-based mutation detection methods, sequencing, and multiplex ligation-dependent probe amplification may be needed to accurately interrogate and segregate the mutations in an affected individual. In some instances, it may be necessary to perform genetic analyses on the parents to segregate the specific maternal and paternal mutations and confirm that mutations are on opposite alleles. Despite these potential obstacles, genetic analysis can be a useful adjunct to newborn screening [50].

As noted above, one RCCX unit contains *CYP21* and *TNX* genes (Fig. 5.2). TNXB encodes tenascin-X, which is an extracellular matrix glycoprotein involved in collagen organization and matrix integrity. Mutations in *TNXB* are associated with Ehlers-Danlos syndrome. Several distinct alleles have been characterized with loss of *CYP21A2* and specific *TNXB* alleles. Patients have been described to have monoallelic or biallelic *TNXB* variants. The severity of the Ehlers-Danlos syndrome is dependent on the *TNXB* genotype. Patients with CAH will benefit from evaluation for features associated with Ehlers-Danlos syndrome such as hypermobile joints and skin laxity [51].

Diagnosis

An elevated 17-OHP concentration provides confirmation of the diagnosis of 21-OHD deficiency. Most affected infants have random 17-OHP values >5000 ng/dl (150 nmol/L) [52]. For infants, additional laboratory evaluation can include electrolytes, plasma renin activity, progesterone, and androstenedione concentrations. Pelvic ultrasound imaging and chromosome analyses are recommended for virilized female infants.

For individuals with symptoms suggestive of NC-21-OHD, an early morning basal 17-OHP has been suggested as an effective screening test. Armengaud et al. reported 100% sensitivity and 99% specificity with a threshold value of 200ng/dl (6 nmol/L) to diagnose NC-21-OHD in children with premature pubarche [19]. A bone age X-ray should be obtained to assess for acceleration of skeletal maturation.

Blood samples for 17-OHP determinations should be obtained in the follicular phase for reproductive-aged cycling women because the 17-OHP concentration may be elevated during the luteal phase. In this situation, Escobar-Morreale et al. recommended using a basal 17-OHP of 170 ng/dl (5.1 nmol/L) as the "cut point" for women [53]. Nevertheless, for any age group, an ACTH stimulation test may be warranted to complete the evaluation for 21-OHD. For an ACTH stimulation test, following collection of a basal blood sample, 0.25 mg synthetic ACTH (Cortrosyn) is administered by intravenous or intramuscular routes; a second blood sample is

collected at 30 and/or 60 min. In addition to 17-OHP, cortisol should be measured especially among individuals with NC-21-OHD to assess the adequacy of cortisol secretion. To differentiate 21-OHD from other disorders of steroidogenesis, determination of progesterone, 17-hydroxypregnenolone, 11-deoxycortisol, DHEA, deoxycorticosterone, and androstenedione may be warranted [54].

In general, *CYP21A2* mutations on both alleles will be identified when ACTH-stimulated 17-OHP concentrations are greater than 1500 ng/dl (45 nmol/L). However, some individuals with diagnostic genotypes have ACTH-stimulated 17-OHP values between 1000 and 1400 ng/dl (30–45 nmol/L). Individuals with 21-OHD have elevated 21-deoxycortisol concentrations, but commercial availability of this hormone assay is limited. Liquid chromatography-tandem mass spectroscopy (LC-MS/MS) has demonstrated elevated 17-OHP, 21-deoxycortisol, 16α-hydroxyprogesterone, and progesterone; these steroids comprise sensitive and specific biomarkers to accurately identify patients with CAH due to 21OHD [9]. As noted above, the 11oxo-C19 steroids, 11β-hydroxyandrostenedione, 11-ketoandrostenedione, 11β-hydroxytestosterone, and 11-ketotestosterone (11KT), are elevated in 21-OHD [11].

Newborn Screening

Newborn screening (NBS) for 21-OHD was initiated in the late 1970s using filter paper whole-blood 17-OHP measurements of whole-blood 17-OHP [55]. All 50 states and many countries have developed NBS programs. To minimize false-positive results, blood samples should be collected after 48 h of life. Automated time-resolved dissociation-enhanced lanthanide fluoroimmunoassays (DELFIA) are often used for 17-OHP determinations. The major goals of NBS are to identify infants with salt-losing 21-OHD, to prevent misidentification of affected females, and to decrease the morbidity and mortality associated with acute adrenal insufficiency [56]. Nevertheless, false-positive screening results occur among preterm, stressed, or heterozygous infants. Cross-reactivity with sulfated steroids and 16α-hydroxyprogesterone is another reason for false-positive results. Decreased 11β-hydroxylase activity in the neonate may be another confounder contributing to false-positive testing [57]. Birth weight and gestational age cut points have been developed to minimize recalls for false-positive tests. False-negative 17-OHP results leading to delayed diagnoses have been reported for both newborn girls and boys [58].

Treatment

Treatment needs to be focused on the individual's symptoms. In other words, treatment should not be initiated merely to decrease abnormally elevated hormone concentrations. For children and adolescents, treatment goals include normal linear

growth velocity, normal rate of skeletal maturation, appropriately timed spontaneous pubertal development, and positive self-esteem. Treatment goals for adolescent and adult women include normal menstrual cyclicity, fertility, and prevention of further hirsutism and acne. Maintenance of fertility is also a concern for adult males with classic CAH. Healthcare should ideally be provided in a multidisciplinary setting with endocrinologists, pediatricians/internists, surgeons/urologists/gynecologists, behavioral health specialists, and nurse educators [59].

Laboratory goals include androstenedione and testosterone concentrations that are appropriate for age, gender, and stage of puberty. Normalization of 17-OHP and progesterone concentrations generally indicates excessive hormone replacement therapy.

Hydrocortisone (Cortef®) is the preferred glucocorticoid replacement in infants, children, and adolescents. The usual dosage ranges from 6 to 15 mg/m^2/day generally administered three times per day (for a 1.75 m^2 individual, 7.5 mg in the morning, 5 mg in the afternoon, and 10 mg before bed are equivalent to 12.8 mg/m2/day). Some clinicians advocate reverse circadian dosing with the highest dose in the evening. However, the larger bedtime dose may not adequately suppress the early morning ACTH rise, and some individuals complain of insomnia with a higher bedtime dose. Hydrocortisone dose equivalence greater than 17 mg/m^2/day during childhood (> 30 mg per day for a 1.75 m^2 individual) was associated with greater compromise of adult height [60]. Prednisone and dexamethasone have longer half-lives such that less frequent dosing is needed; these medications may be considered for use in the adult patient. Some adult patients with classic CAH are well controlled on combinations of hydrocortisone and small doses of prednisone or dexamethasone at bedtime [61]. Some women with CAH experience persistent hyperandrogenic anovulation and benefit from taking oral contraceptives. Cosmetic hair removal including shaving, waxing, electrolysis, laser therapies, and topical eflornithine cream may be helpful.

Several factors should be contemplated regarding the use of glucocorticoid replacement therapy for patients with NC-21-OHD. Many patients with NC-21-OHD will not require daily glucocorticoid replacement to maintain their health. Indeed, the vast majority of men with NC-21-OHD are generally asymptomatic and do not benefit from treatment. Older adolescent and adult women can be treated with oral contraceptives to decrease the ovarian contribution to androgen excess. Children and adolescents with NC-21-OHD may have extremely advanced skeletal maturation and may benefit from glucocorticoid replacement therapy. For patients with NC-21-OHD, daily or stress-dose glucocorticoid treatment may be indicated only when ACTH-stimulated cortisol is less than 18 mg/dl (500 nmol/L). Some women and men with NC-21-OHD may benefit from short-term hydrocortisone or prednisolone therapy to treat infertility [62]. As noted above, suppression of progesterone can improve fecundity in women with CAH [34]. Hence, therapy for patients with NC-21-OHD needs to be individualized and may vary according to the patient's specific current needs.

The synthetic hormone, 9α-fludrocortisone acetate, is used for mineralocorticoid replacement with the goal of achieving a plasma renin activity that is within normal limits for age. Due to their salt-poor diet, transient pseudohypoaldosteronism, and

immature kidneys, infants typically require higher mineralocorticoid replacement during the first few months of life. Some infants may require additional salt supplementation.

Stress dosing is necessary for significant illnesses, surgery, or life-threatening stress. Tripling the usual daily dose is the semi-arbitrary guideline for stress dosing. If the individual is unable to take or tolerate oral medications, parental hydrocortisone should be administered as follows: < 12 months of age, 25 mg; 1–4 years of age, 50 mg; and > 4 years of age, 100 mg. All individuals on glucocorticoid treatment require instruction regarding oral stress doses and administration of parental hydrocortisone. All patients with CAH should wear medical alert identification badges/jewelry.

Treatment of CAH is often challenging because of the difficulty inherent in balancing overtreatment and undertreatment. Parameters that influence optimal dosing include variation in absorption from the gastrointestinal tract, CBG concentrations, and cortisol half-life in the circulation [63]. For this reason, novel therapies are being explored. One approach has been the development of a time-released glucocorticoid preparation, Chronocort® [64]. Continuous glucocorticoid replacement using a subcutaneous pump has been tried [65]. In a short clinical trial, abiraterone acetate, which inhibits *CYP17A1*, has been used in conjunction with replacement hydrocortisone treatment [66].

Prenatal Treatment

To prevent prenatal virilization of the external genitalia of affected females, prenatal dexamethasone treatment was explored starting in the 1980s [67, 68]. Dexamethasone has been used because it is not inactivated by 11β-hydroxysteroid dehydrogenase type 2 and can cross the placenta. Whereas this treatment appears to be efficacious to decrease virilization of the external genitalia, numerous safety concerns have arisen. To be effective, dexamethasone treatment must be started within 6–7 weeks of conception. Yet, genetic diagnosis by chorionic villus biopsy cannot be safely done until 10–12 weeks. Thus, all at-risk pregnancies are treated even though only one in eight fetuses is predicted to be an affected female and seven of eight fetuses are unnecessarily exposed to prenatal dexamethasone treatment.

Clinical outcome studies have demonstrated increased social anxiety, low birth weight (LBW), failure to thrive, developmental delay, mood disturbance, and poor school performance. Hirvikoski et al. reported a significant negative effect on short-term memory/verbal working memory in children unaffected with CAH who had been treated with dexamethasone during the first trimester of fetal life; however, long-term memory and learning, as well as full-scale IQ, were comparable to untreated controls [69]. Early prenatal dexamethasone exposure has been reported to affect cognitive functions in healthy unaffected girls [70].

In another treatment paradigm, antenatal GCs are used in infants at risk for preterm delivery. In this situation, structural changes in the brain characterized by cortical thinning specifically in the rostral anterior cingulate cortex in children 6–10 years of age were reported in term infants. This area of the brain is important for emotional regulation [71].

Data available in additional clinical outcome studies and using animal models raise significant concern about the use of prenatal dexamethasone and urge that it not be used except in research studies under the guidance of the appropriate Institutional Review Board [72]. One novel approach has utilized cell-free DNA that is found in the maternal circulation. Identification of the *SRY* gene accompanied by sequencing of the *CYP21A2* gene has been used to identify affected females who might benefit from treatment [73]. The drawback of this approach is that the results must be quickly obtained to guide treatment decisions. Another option is preimplantation genetic diagnosis, which allows selection of unaffected embryos for reimplantation [74].

Outcome

Data reporting outcome on older populations of patients with CAH are disappointing. Treatment regimens vary widely with use of diverse glucocorticoid preparations, differing dosages, and dissimilar regimens regarding diurnal dosing. Medical issues identified in adults in the CaHASE study from the United Kingdom included osteopenia, osteoporosis, short stature, obesity, hypertension, and infertility [75, 76]. Data accrued through the NIH Natural History Study showed poor outcomes associated with highly variable treatment attributed to generally poor adherence to medical management [77]. Bachelot et al. reported their outcome experience regarding adult patients followed at a single medical center; they found obesity, abnormal bone mineral density, adrenal tumors, TARTs, and menstrual irregularity were common [78]. Analysis of patients with CAH enrolled in the Swedish CAH register revealed increased cardiovascular and metabolic morbidity especially obesity [79]. The common theme for these disappointing outcome studies is poor medical supervision and suboptimal management in adult patients. Patients seem to be lost for follow-up after transitioning from pediatric to adult healthcare.

Future Directions

In the interval of time since the initial description of CAH by Luigi de Crecchio, much has been learned about the pathophysiology and molecular genetics of this common autosomal recessive disorder [80]. Nevertheless, better diagnostic tools and improved hormone replacement regimens would greatly benefit our patients [81]. Finally, as more 21OHD patients are adults than children, the focus of research needs to shift to transition of care, long-term complications, and reproductive health.

References

1. Thil'en A, Nordenström A, Hagenfeldt L, von Döbeln U, Guthenberg C, Larsson A. Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. Pediatrics. 1998;101:E11.
2. 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):583–90.
3. Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI. High frequency of non-classical steroid 21-hydroxylase deficiency. Am J Hum Genet. 1985;37:650–67.
4. Miller WL. Steroid hormone synthesis in mitochondria. Mol Cell Endocrinol. 2013;379(1–2):62–73.
5. 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;144:575–80.
6. 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:E367–75.
7. Auchus RJ. The backdoor pathway to dihydrotestosterone. Trends Endocrinol Metab. 2004;15:432–8.
8. Kamrath C, Hartmann MF, Wudy SA. Androgen synthesis in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Horm Metab Res. 2013;45:86–91.
9. 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;100:2283–90.
10. Pretorius E, Arlt W, Storbeck KH. A new Dawn for androgens: novel lessons from 11-oxygenated C19 steroids. Mol Cell Endocrinol. 2016; doi:10.1016/j.mce.2016.08.014.
11. Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano T, Shields JJ, Merke DP, Rainey W, Auchus R. Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. Eur J Endocrinol. 2016;174:601–9.
12. 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;98:1182–8.
13. Arlt W, Allolio B. Adrenal insufficiency. Lancet. 2003;361:1881–93.
14. White PC, Bachega TA. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: from birth to adulthood. Semin Reprod Med. 2012;30:400–9.
15. 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. Horm Res Paediatr. 2015;
16. 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;343:1362–8.
17. Moran C, Azziz R, Carmina E, et al. 21-hydroxylase-deficient nonclassic adrenal hyperplasia is a progressive disorder: a multicenter study. Am J Obstet Gynecol. 2000;183:1468.
18. Armengaud JB, Charkaluk ML, Trivin C, Tardy V, Bréart G, Brauner R, Chalumeau M. Precocious pubarche: distinguishing late-onset congenital adrenal hyperplasia from premature adrenarche. J Clin Endocrinol Metab. 2009;94:2835–40.
19. Binay C, Simsek E, Cilingir O, Yuksel Z, Kutlay O, Artan S. Prevalence of nonclassic congenital adrenal hyperplasia in Turkish children presenting with premature pubarche, hirsutism, or oligomenorrhoea. Int J Endocrinol. 2014;2014:768506.

20. 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;91:3451–6.
21. Bidet M, Bellanné-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;95:1182–90.
22. Yildiz BO, Bolour S, Woods K, Moore A, Azziz R. Visually scoring Hirsutism. Hum Reprod Update. 2010;16:51–64.
23. O'Driscoll JB, Anderson DC. Untreated congenital adrenal hyperplasia presenting with severe androgenic alopecia. J R Soc Med. 1993;86:229.
24. Huerta R, Dewailly D, Decanter C, Knochenhauer ES, Boots LR, Azziz R. Adrenocortical hyperresponsivity to adrenocorticotropic hormone: a mechanism favoring the normal production of cortisol in 21-hydroxylase-deficient nonclassic adrenal hyperplasia. Fertil Steril. 2000;74:329–34.
25. Lobo RA, Goebelsmann U. Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydroxylase deficiency mimicking polycystic ovarian disease. Am J Obstet Gynecol. 1980;138:720–6.
26. 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;94:684–9.
27. Escobar-Morreale HF, Sanchón R, San Millán JL. A prospective study of the prevalence of nonclassical congenital adrenal hyperplasia among women presenting with hyperandrogenic symptoms and signs. J Clin Endocrinol Metab. 2008;93:527–33.
28. Pignatelli D. Non-classic adrenal hyperplasia due to the deficiency of 21-hydroxylase and its relation to polycystic ovarian syndrome. Front Horm Res. 2013;40:158–70.
29. Oncul M, Sahmay S, Tuten A, Acikgoz AS, Gurleyen HC. May AMH levels distinguish LOCAH from PCOS among hirsute women? Eur J Obstet Gynecol Reprod Biol. 2014;178:183–7.
30. 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: 977–84.
31. Ghizzoni L, Virdis R, Vottero A, Cappa M, Street ME, Zampolli M, Ibañez L, Bernasconi S. Pituitary-ovarian responses to leuprolide acetate testing in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab. 1996;81:601–6.
32. Engberg H, Butwicka A, Nordenström A, Hirschberg AL, Falhammar H, Lichtenstein P, Nordenskjöld A, Frisén L, Landén 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.
33. Engberg H, Möller A, Hagenfeldt K, Nordenskjöld A, Frisén L. The experience of women living with congenital adrenal hyperplasia: impact of the condition and the care given. Clin Endocrinol. 2016;85:21–8.
34. Casteràs A, De Silva P, Rumsby G, Conway GS. Reassessing fecundity in women with classical congenital adrenal hyperplasia (CAH): normal pregnancy rate but reduced fertility rate. Clin Endocrinol. 2009;70:833–7.
35. 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. Arch Sex Behav. 2015;44:1363–75.
36. Nordenskjöld A, Holmdahl G, Frisén L, Falhammar H, Filipsson H, Thorén 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;93:380–6.

37. Frisén L, Nordenström A, Falhammar H, Filipsson H, Holmdahl G, Janson PO, Thorén M, Hagenfeldt K, Möller A, Nordenskjöld A. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. J Clin Endocrinol Metab. 2009;94:3432–9.
38. Kanhere M, Fuqua J, Rink R, Houk C, Mauger D, Lee PA. Psychosexual development and quality of life outcomes in females with congenital adrenal hyperplasia. Int J Pediatr Endocrinol. 2015;2015:21.
39. Lee PA, Nordenström A, Houk CP, Ahmed SF, Auchus R, Baratz A, Baratz Dalke K, Liao LM, Lin-Su K, Looijenga LH 3rd, Mazur T, Meyer-Bahlburg HF, Mouriquand P, Quigley CA, Sandberg DE, Vilain E, Witchel S, Global DSD Update Consortium. Global disorders of sex development update since 2006: perceptions, approach and care. Horm Res Paediatr. 2016;85:158–80.
40. Claahsen-van der Grinten HL, Dehzad F, Kamphuis-van Ulzen K, de Korte CL. Increased prevalence of testicular adrenal rest tumours during adolescence in congenital adrenal hyperplasia. Horm Res Paediatr. 2014;82:238–44.
41. Falhammar H, Nyström HF, Ekström U, Granberg S, Wedell A, Thorén M. Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia. Eur J Endocrinol. 2012;166:441–9.
42. 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:E1820–6.
43. Parajes S, Quinteiro C, Domínguez 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:e2138.
44. Chen W, Xu Z, Sullivan A, Finkielstain GP, Van Ryzin C, Merke DP, McDonnell NB. Junction site analysis of chimeric CYP21A1P/CYP21A2 genes in 21-hydroxylase deficiency. Clin Chem. 2012;58:421–30.
45. Parker EA, Hovanes K, Germak J, Porter F, Merke DP. Maternal 21-hydroxylase deficiency and uniparental isodisomy of chromosome 6 and X results in a child with 21-hydroxylase deficiency and Klinefelter syndrome. Am J Med Genet A. 2006;140:2236–40.
46. Livadas S, Dracopoulou M, Dastamani A, Sertedaki A, Maniati-Christidi M, Magiakou AM, Kanaka-Gantenbein C, Chrousos GP, Dacou-Voutetakis C. The spectrum of clinical, hormonal and molecular findings in 280 individuals with nonclassical congenital adrenal hyperplasia caused by mutations of the CYP21A2 gene. Clin Endocrinol. 2015;82:543–9.
47. Speiser PW, Knochenhauer ES, Dewailly D, Fruzzetti F, Marcondes JA, Azziz R. A multicenter study of women with nonclassical congenital adrenal hyperplasia: relationship between genotype and phenotype. Mol Genet Metab. 2000;71:527–34.
48. Bidet M, Bellanné-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;94:1570–8.
49. Barbaro M, Soardi FC, Östberg LJ, Persson B, de Mello MP, Wedell A, Lajic S. In vitro functional studies of rare CYP21A2 mutations and establishment of an activity gradient for nonclassic mutations improve phenotype predictions in congenital adrenal hyperplasia. Clin Endocrinol. 2015;82:37–44.
50. Nordenström A, Thilén 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. J Clin Endocrinol Metab. 1999;84:1505–9.
51. Chen W, Perritt AF, Morissette R, Dreiling JL, Bohn MF, Mallappa A, Xu Z, Quezado M, Merke DP. Ehlers-Danlos syndrome caused by Biallelic TNXB variants in patients with congenital adrenal hyperplasia. Hum Mutat. 2016;37:893–7.

52. Witchel SF, Nayak S, Suda-Hartman M, Lee PA. Newborn screening for 21-hydroxylase deficiency: results of CYP21 molecular genetic analysis. J Pediatr. 1997;131:328–31.
53. Escobar-Morreale HF, Sanchon R, San Millan JL. A prospective study of the prevalence of nonclassical congenital adrenal hyperplasia among women presenting with hyperandrogenic symptoms and signs. J Clin Endocrinol Metab. 2008;93:527–33.
54. Trapp CM, Speiser PW, Oberfield SE. Congenital adrenal hyperplasia: an update in children. Curr Opin Endocrinol Diabetes Obes. 2011;18:166–70.
55. 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;45:1003–8.
56. 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;100:1002–8.
57. Kamrath C, Hartmann MF, Boettcher C, Wudy SA. Reduced activity of 11β-hydroxylase accounts for elevated 17α-hydroxyprogesterone in preterms. J Pediatr. 2014;165:280–4.
58. Gidlöf S, Falhammar H, Thilén A, von Döbeln U, Ritzén M, Wedell A, Nordenström A. One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. Lancet Diabetes Endocrinol. 2013;1:35–42.
59. Witchel SF. The medical home concept and congenital adrenal hyperplasia: a comfortable habitat! Int J Pediatr Endocrinol. 2010;2010:561526.
60. 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;95:4161–72.
61. Auchus RJ. Management considerations for the adult with congenital adrenal hyperplasia. Mol Cell Endocrinol. 2015;408:190–7.
62. Trakakis E, Dracopoulou-Vabouli M, Dacou-Voutetakis C, Basios G, Chrelias C, Kassanos D. Infertility reversed by glucocorticoids and full-term pregnancy in a couple with previously undiagnosed nonclassic congenital adrenal hyperplasia. Fertil Steril. 2011;96:1048–50.
63. Hindmarsh PC, Charmandari E. Variation in absorption and half-life of hydrocortisone influence plasma cortisol concentrations. Clin Endocrinol (Oxf). 2015;82:557–61.
64. 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;100:1137–45.
65. Hindmarsh PC. The child with difficult to control congenital adrenal hyperplasia: is there a place for continuous subcutaneous hydrocortisone therapy. Clin Endocrinol. 2014;81:15–8.
66. Auchus RJ, Buschur EO, Chang AY, Hammer GD, Ramm C, Madrigal D, Wang G, Gonzalez M, Xu XS, Smit JW, Jiao J, Yu MK. Abiraterone acetate to lower androgens in women with classic 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2014;99:2763–70.
67. Forest MG, Morel Y, David M. Prenatal treatment of congenital adrenal hyperplasia. Trends Endocrinol Metab. 1998;9:284–9.
68. New MI, Carlson A, Obeid J, et al. Extensive personal experience: prenatal diagnosis for congenital adrenal hyperplasia in 532 pregnancies. J Clin Endocrinol Metab. 2001;86:5651–7.
69. Hirvikoski T, Nordenström A, Lindholm T, Lindblad F, Ritzén EM, Wedell A, Lajic S. Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. J Clin Endocrinol Metab. 2007;92:542–8.
70. Wallensteen L, Zimmermann M, Thomsen Sandberg M, Gezelius A, Nordenström A, Hirvikoski T, Lajic S. Sex-dimorphic effects of prenatal treatment with dexamethasone. J Clin Endocrinol Metab. 2016;101(10):3838–46.
71. Davis EP, Sandman CA, Buss C, Wing DA, Head K. Fetal glucocorticoid exposure is associated with preadolescent brain development. Biol Psychiatry. 2013;74:647–55.
72. Miller WL, Witchel SF. Prenatal treatment of congenital adrenal hyperplasia: risks outweigh benefits. Am J Obstet Gynecol. 2013;208:354–9.

73. 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:E1022–30.
74. Altarescu G. Prevention is the best therapy: the Geneticist's approach. Pediatr Endocrinol Rev. 2016 Jun;13(Suppl 1):649–54.
75. 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, United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE). Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. J Clin Endocrinol Metab. 2010;95:5110–21.
76. Han TS, Walker BR, Arlt W, Ross RJ. Treatment and health outcomes in adults with congenital adrenal hyperplasia. Nat Rev Endocrinol. 2014;10:115–24.
77. Finkielstain GP, Kim MS, Sinaii N, Nishitani M, Van Ryzin C, 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;97:4429–38.
78. 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;173:175–84.
79. Falhammar H, Frisén L, Hirschberg AL, Norrby C, Almqvist C, Nordenskjöld A, Nordenström A. Increased cardiovascular and metabolic morbidity in patients with 21-hydroxylase deficiency: a Swedish population-based National Cohort Study. J Clin Endocrinol Metab. 2015;100:3520–8.
80. De Crecchio L. Sopra un caso di apparenze virili in una donna. Il Morgagni. 1965:151–89.
81. Turcu AF, Auchus RL. The next 150 years of congenital adrenal hyperplasia. J Steroid Biochem Mol Biol. 2015;153:63–71.