Fertility and Reproductive Outcomes in Different Forms of Congenital Adrenal Hyperplasia

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Editor
M. Eda Ertorer
Department of Endocrinology
Başkent University, Faculty of Medicine
Adana Dr. Turgut Noyan Application and Research Center
Adana
Turkey

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Preface

"Congenital Adrenal Hyperplasia" represents a group of genetically inherited enzymatic deficiencies that exhibit a wide spectrum of clinical features. Beginning from intrauterine life till adulthood, these disorders may impact negatively on the well-being of the affected individuals that make a holistic professional approach fundamental.

In *Fertility and Reproductive Outcomes in Different Forms of Congenital Adrenal Hyperplasia*, an assembly of outstanding authors with great expertise on this topic has shared their knowledge and has interpreted the relevant latest medical literature. Being concise and comprehensive and didactic has been the challenge. I am confident that our combined efforts will succeed in providing better care for our patients.

Adana, Turkey M. Eda Ertorer

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Congenital Adrenal Hyperplasia (CAH): Definition and Enzymatic Defects in Various Forms

Fahrettin Kelestimur and Kursad Unluhizarci

Introduction

The adrenals are endocrine glands where catecholamine and steroid hormone production take place. Steroid hormones are mainly synthesized and secreted from adrenal glands, ovaries, testes, and also from some peripheral tissues such as adipose tissue. In contrast to other secreting glands such as pancreas, adrenal glands do not store hormones, instead, the adrenal cortex hormones are mainly secreted as de novo synthesis. From anatomical point of view, adrenal cortex consists of three layers:

1. Zona glomerulosa is responsible for the synthesis of mineralocorticoids and represents 15% of the adrenal cortex.
2. Zona fasciculata is responsible for the synthesis of glucocorticoids and represents 75% of the adrenal cortex.
3. Zona reticularis is responsible for the synthesis of adrenal androgens and represents 10% of the adrenal cortex.

Cholesterol is the main substrate for all steroid hormone biosynthesis. It is mostly derived from dietary cholesterol, transported to the cell via LDL-cholesterol and HDL-cholesterol; however, it can be synthesized de novo from acetate, as well [1]. Due to their common origin and similar structures, steroid hormones constitute a heterogeneous group with a wide range of physiological functions. The organs and tissues having the ability to produce steroid hormones express different

F. Kelestimur
Department of Endocrinology, Yeditepe University Medical School, Istanbul, Turkey

K. Unluhizarci (✉)
Department of Endocrinology, Erciyes University Medical School, Kayseri, Turkey
e-mail: kursad@erciyes.edu.tr

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steroidogenic enzymes and also cofactors, leading to site-specific pathways of steroid hormone synthesis. In this manner, a "steroidogenic cell" definition ensues a highly specific cell which has the capability to produce steroids de novo using cholesterol as substrate, a process that requires the expression of P450 side chain cleavage (scc) enzyme [2].

Steroidogenic enzymes can be mainly divided into two groups: cytochrome P450 (CYP) and hydroxysteroid dehydrogenases/ketosteroid reductase (HSDs/KSR) [3]. Most steroidogenic enzymes are cytochrome P450 enzymes, and this definition comes from the basis of light absorbance at the 450 nm wavelength when reduced with carbon monoxide. Cytochrome P450 enzymes have the ability to activate molecular oxygen in order to catalyze oxidative reactions. These enzymes have unidirectional function, catalyzing irreversibly the reactions they involved and have no feedback from downstream precursors [3, 4].

The CYP enzymes are named as type 1 enzymes which are located in mitochondria and type 2 enzymes which are located in endoplasmic reticulum. There are different cofactors for CYP enzymes: ferrodoxin and ferrodoxin reductase for type 1 and P450 oxydoreductase for type 2 enzymes. Six CYP enzymes involved in steroidogenesis are: cholesterol side chain cleavage enzyme (P450scc, CYP11A1), 11-beta hydroxylase (P450c11, CYP11B1), and aldosterone synthase (P450c11AS, CYP11B2) belong to CYP type 1 enzymes, while 17-hydroxylase (P450c17, CYP17A1), 21-hydroxylase (P450c21, CYP21A2), and P450 aromatase (P450 aro, CYP19A1) are CYP type 2 enzymes. It is well-known that each CYP enzyme can metabolize more than one substrate and is involved in multiple steps of steroidogenesis [2].

The HSD enzymes catalyze reversible reactions, they modulate the steroid flux in reductive or oxidative mode [3]. Based on their activity, they are classified into dehydrogenases which use nicotinamide adenine dinucleotide^{+} (NAD^{+}) to oxidate hydroxysteroids to ketosteroids, and reductases which use nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) to reduce ketosteroids to hydroxysteroids. Relevant HSDs to human diseases are mainly 3-beta hydroxysteroid dehydrogenase (3-HSD) type 2 (HSD3B2), 11 HSD type 1 and type 2 (HSD11B1 and B2), and a series of 17 HSDs [3].

Among several steps in steroidogenesis, the first step is transport of cholesterol across the membrane via steroidogenic acute regulatory protein (StAR), and then, it is converted to pregnenolone by P450scc [5]. In zona fasciculata, CYP17A1 hydroxylates pregnenolone to form 17-hydroxyprogesterone (17-OHP), which is converted to 11-deoxycortisol, and then cortisol is produced via several enzymes (including CYP11B1 and HSD3B2). In zona glomerulosa, HSD3B2 converts pregnenolone to progesterone which is converted to aldosterone by a series of enzyme activities including 21-hydroxylase and aldosterone synthase. In zona reticularis, 17-hydroxypregnanelone is converted to DHEA via 17-alpha hydroxylase which is then converted to androstenedione by the enzyme effect of HSD3B2 [3, 6]. Pathways of steroid hormone synthesis are shown in Fig. 1.

Deficiencies in main pathways of steroid biosynthesis lead to congenital adrenal hyperplasia (CAH) which is a group of disorders characterized by enzymatic defects

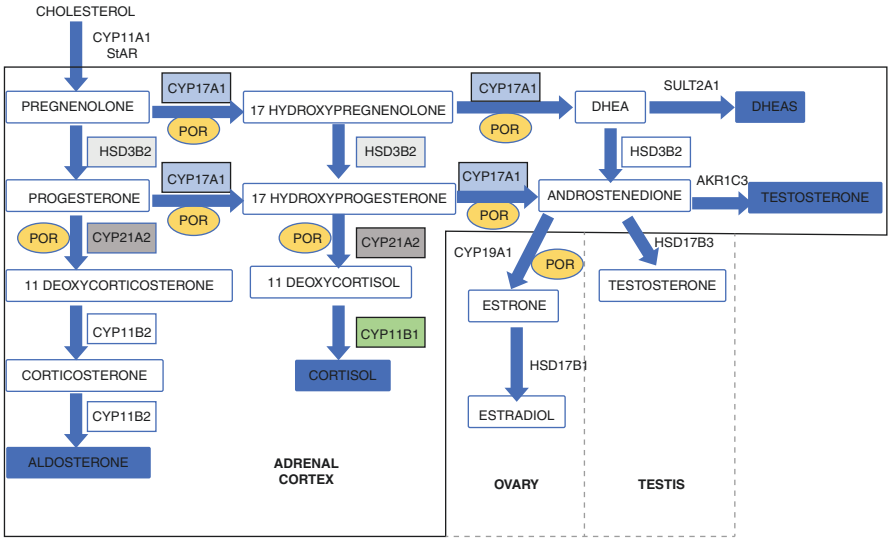

Fig. 1 Pathway of adrenal steroid hormone biosynthesis. *CYP11A1* cholesterol desmolase, *CYP17A1* 17α-hydroxylase, *CYP21A2* 21-hydroxylase, *CYP11B1* 11β-hydroxylase, *CYP11B2* aldosterone synthase, *HSD3B2* 3β-hydroxysteroid dehydrogenase, *CYP19A1* P450 aromatase, *HSD17B1* 17β-hydroxysteroid dehydrogenase type1, *HSD17B3* 17β-hydroxysteroid dehydrogenase type 3, *StAR* steroidogeneic acute regulatory protein, *POR* P450 oxidoreductase, *SULT2A1* steroid sulfotransferase 2A1, *AKR1C3* 17β-hydroxysteroid dehydrogenase type 5, *DHEA* dehydroepiandrosterone, *DHEAS* dehydroepiandrosterone sulfate

in cortisol biosynthesis. The most probable first case had been described more than 150 years ago, her autopsy had showed ambiguous genitalia associated with female internal reproductive organs and large adrenal glands [7]. The disease can appear in childhood, adolescence, or adulthood depending on clinical features. Due to the mutations, one or more of the glucocorticoid, mineralocorticoid, and androgen biosynthesis pathways can be affected, and the patients may present with diverse clinical and laboratory abnormalities (Table 1). All types of the CAH are monogenetic, and most of the patients are compound heterozygotes, indicating that they have different mutations on two alleles of a particular gene [8]. In this case, the clinical spectrum and the severity of the disease are dependent on more functional allele [9, 10]. In all types of CAH, low cortisol production stimulate secretion of corticotrophin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) which leads to adrenal hyperplasia and eventually accumulation of steroid hormone precursors and usually hyperandrogenemia.

The disorder is inherited as an autosomal recessive manner and the affected enzymes are: 21-hydroxylase, 11-beta hydroxylase, 17-alpha hydroxylase, HSD3B2 type 2, StAR, P450 cholesterol side-chain cleavage enzyme (SCC), and P450 oxydoreductase (POR). Adrenal steroid biosynthesis is modulated by zone-specific enzymes where the mutations on these enzymes lead to different phenotypes of CAH. Eventually, the disease manifests with a broad range of clinical and

Table 1 Clinical characteristics of various forms of congenital adrenal hyperlasia

	21-Hydroxylase deficiency	11-Beta hydroxylase deficiency	17-Alpha hydroxylase deficiency	3-Beta hydroxy-steroid dehydrogenase deficiency	POR deficiency	Lupoid hyperplasia	Side chain cleavage enzyme deficiency
Affected gene	*CYP21A2*	*CYP11B1*	*CYP17A1*	*HSD3B2*	*POR*	*StAR*	*CYP11A1*
Salt wasting	Yes in classical form	No	No	Yes	No	Yes in classical form	Yes in classical form
Enzymatic defect in gonads	No	No	Yes	Yes	Yes	Yes	Yes
Sex steroid deficiency	No	No	Yes	Yes in classical form	Yes	Yes	Yes
Hypertension	No	Yes in classical form	Yes	No	Yes	No	No

POR P450 oxidoreductase, *StAR* steroidogenic acute regulatory protein

biochemical phenotype with or without derangements in glucocorticoid, mineralocorticoid, and sex steroid production.

Congenital adrenal hyperplasia is mainly divided into two groups: more severe classical forms and milder non-classical forms. When any mutation/mutations cause complete or near complete deficiency of the enzymes, classical forms of CAH ensue with severe clinical manifestations such as virilization of the females or salt wasting in both sexes [11, 12]. Milder forms, called non-classical forms, are typically asymptomatic at birth, and they are usually not distinguishable from other hyperandrogenic disorders, such as polycystic ovary syndrome (PCOS) in adult ages. The current classification system of the disease is considered artificial since the disease severity is better represented by a continuum on the basis of residual enzyme activity.

Endocrinology and its development depend on hormone assays. In daily practice, androgens and other steroid hormones have been determined by immunoassays. In those assays, small quantities of serum samples have been used; however, their standards and limitations are highly debated during the last years [13]. The Endocrine Society has suggested using liquid chromatography/tandem mass spectrometry (LC-MS/MS) or immunoassays after extraction and chromatography for measuring steroid hormones, particularly testosterone in women and children [13]. The diagnosis of CAH relies on measuring the increased intermediate hormones which are different in various enzyme deficiencies biochemically and eventually on genetic confirmation of the disease. Mass spectrometry-based steroid metabolome profiling is gaining great attention which provide identification of several metabolomes/intermediate hormones associated with the disorders of steroid biosynthesis. Particularly 24-h urinary steroid metabolome analysis has revealed many steroidogenic disorders and is becoming a novel diagnostic strategy in clinical practice [14].

Enzymatic Defects in Various Forms

21-Hydroxylase Deficiency

By far, 21-hydroxylase deficiency (21-OHd) is the most common form of CAH, accounting for approximately 95% of the cases. The incidence of classical form of the disease is reported to be in 1/7000 to 1/18,000 live births depending on ethnicity and the location [15, 16]. In physiological conditions, 21-hydroxylase enzyme leads the conversion of 17-OHP to 11-deoxycortisol and progesterone to 11-deoxycorticosterone, which are the key precursors in the synthesis of cortisol and aldosterone, respectively. Various clinical manifestations may be seen with any kind of mutations on *21-hydroxylase* gene. To date, more than 300 CYP21A2 mutations have been discovered, but 10–12 common mutations account for 90% of 21-OHd cases [17, 18]. The de novo mutation rate is reported as very low and is approximately 1% [5].

The *CYP21A2* gene is a 10-exon, 3.1 kb gene that is mapped on the short arm of chromosome 6 within the major histocompatibility complex region. The active gene

encoding 21-hydroxylase enzyme; CYP21A2 and the inactive gene; CYP21A1 (pseudogene) have 98% homology. Exchange of genetic material between CYP21A1 and CYP21A2 results in transfer of pseudogene mutations to the *CYP21A2* gene. There are 15 mutations that cause CYP21A1 to be inactive [15, 19]. Pseudogene-derived CYP21A2 mutations constitute for more than 90% of mutations in the *CYP21A2* gene. Due to the mutation severity, glucocorticoid and mineralocorticoid deficiencies may occur, and the disease is characterized by elevated precursors of steroid biosynthesis, mainly 17-OHP, which is used for the biochemical diagnosis of 21-OHd. In accordance with the blockade of cortisol biosynthesis, adrenal androgen overproduction is driven via 17-OHP pathway.

The classical form of the disease is called as "salt wasting form" in case of completely inactivated *CYP21A2* gene or it is called as "simple virilizing form" where the mutated gene retains 1–2% of 21-hydroxylase activity which prevents neonatal crisis [15, 20]. In a recent survey, 126 (54.1%) out of 233 CYP21A2 mutations are found to associate with the salt wasting form of the disease, where 38 (16.3%) are with the simple virilizing form [21]. In both situations, exposure to excess fetal androgens leads to virilization of external genitalia of 46,XX fetuses. The diagnosis of classical CAH may be suspected after careful examination of external genitalia of new-borns although classical CAH is not the only cause in this situation [22, 23]. In countries where CAH screening is introduced, time required for the diagnosis is markedly reduced.

Patients with salt wasting form of the disease have life-threatening impairment in the synthesis of cortisol and aldosterone, and if those patients are not treated appropriately, low levels of aldosterone leads to hyperkalemia, hyponatremia, hypovolemia, and occasionally convulsions [24]. Salt wasting form is the most severe type and accounts for 75% of all classical CAH cases [11]. In addition to aldosterone deficiency, elevated 17-OHP and progesterone concentrations exacerbate the mineralocorticoid deficiency, since both hormones have anti-mineralocorticoid effects. Even though patients with simple virilizing form of CAH do not present with overt mineralocorticoid deficiency, partial deficiency in mineralocorticoid production has also been found in this form. Affected males are particularly prone to salt wasting crisis and possibly death due to delayed diagnosis in the absence of ambiguous genitalia which is a warning sign in females. Their only sign may be genital hyperpigmentation and sometimes penis enlargement. Impaired cortisol production leads to excess androgen production which causes antenatal virilization of genetic females, precocious puberty in boys and rapid skeletal growth in both sexes. Another issue which is related to inappropriate therapy in males is testicular adrenal rest tissues (TARTs). These are benign testicular masses and have close resemblance to adrenocortical tissue. ACTH and angiotensin II receptors have been shown in TART tissue indicating that they are hormone responsive tissues [25, 26].

In healthy females, Mullerian structures develop normally and the absence of testicular tissue and androgens lead to regression of Wolfian structures. However, in patients with CAH, due to the increased production of testosterone from the adrenal glands, female infants may have ambiguous genitalia since external genitalia development is sensitive to androgens. In the absence of appropriate and timely

treatment, premature adrenarche, advanced skeletal age, centrally induced precocious puberty are seen in those patients. Simple virilizing form of the disease is quiet similar to the salt wasting form, except aldosterone production is sufficient to prevent salt loss and mineralocorticoid replacement is not essential.

Physiologically replacing glucocorticoid orally in a circadian manner is not easy, and many patients still exhibit androgen excess symptoms under glucocorticoid treatment. Moreover, an alternative pathway contributes to hyperandrogenemia which is not easily controlled by classical glucocorticoid replacement therapies. This is also called as the "backdoor pathway," and dihydrotestosterone is produced from excess 17-OHP via a chain of enzymatic reactions (Fig. 2). In this pathway, dihydrotestosterone is synthesized from androsterone without testosterone as an intermediate hormone [27]. It contributes to hyperandrogenemia of the patients with 21-OHd. Recently, a new form of androgens, called 11-oxygenated androgens, derived from 11-beta hydroxylation of androstenedione and testosterone by CYP11B1 has been found [28]. The adrenal glands are the only source of these hormones since CYP11B1 is exclusively expressed in the adrenals (Fig. 3). The final products of this pathway are 11-keto-androstenedione and 11-keto-testosterone which activates the androgen receptors similar to dihydrotestosterone, and they are found to be increased in patients with 21-OHd [29, 30].

Non-classical form of the disease is one of the most common autosomal recessive disorders in population. These patients exhibit at least 20–50% enzyme activity and do not require glucocorticoid replacement in daily life and female patients have normal appearing external genitalia [31, 32]. In a recent survey, 61 (26.2%) out of 233 CYP21A2 mutations are associated with non-classical form [21]. In patients

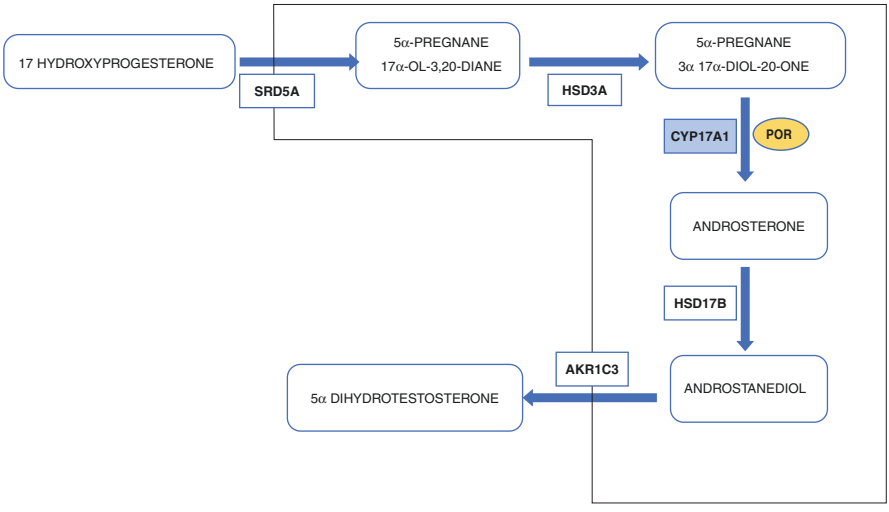

Fig. 2 Alternative pathway in patients with 21 hydroxylase deficiency. *SRD5A* steroid 5α-reductase, *HSD3A* 3α-hydroxysteroid dehydrogenase, *CYP17A1* 17α-hydroxylase, *HSD17B* 3β-hydroxysteroid dehydrogenase, *AKR1C3* aldo-keto-reductase C3, *POR* P450 oxidoreductase

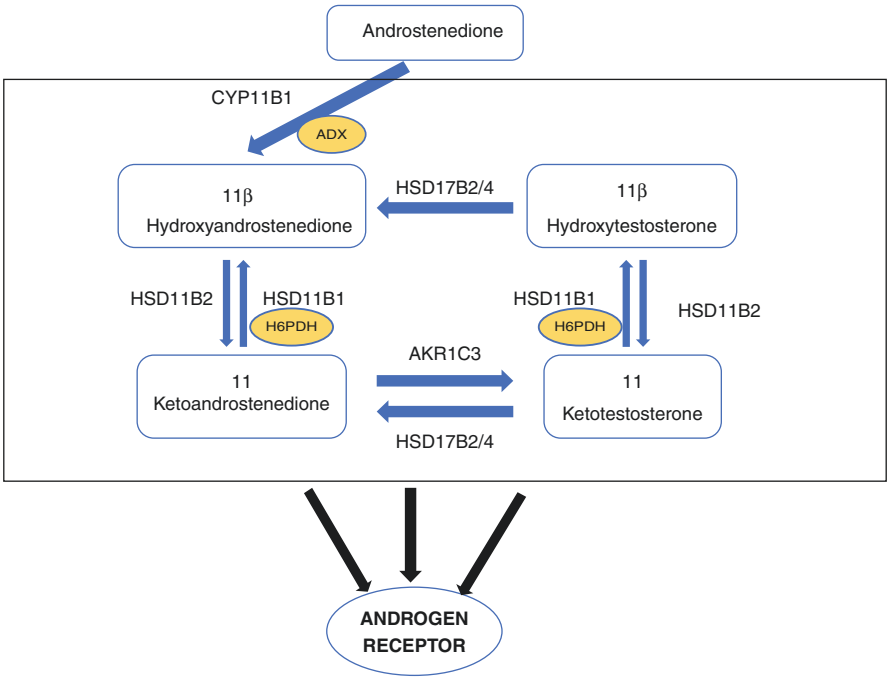

Fig. 3 11-Oxygenated pathway in patients with 21-hydroxylase deficiency. *CYP11B1* 11β-hydroxylase, *HSD11B1* cortisone reductase, *HSD11B2* 11β-hydroxysteroid dehydrogenase type 2, *AKR1C3* aldo-keto-reductase C3, *ADX* adrenodoxin, *H6PDH* glucose 6-phosphate dehydrogenase, *HSD17B2* 17β-hydroxysteroid dehydrogenase type 2

with NCAH, children do not have ambiguous genitalia, and diagnosis is often made mainly during the differential diagnosis of PCOS in adolescents or adults. Most of the patients present with clinical signs and symptoms indistinguishable from PCOS, such as hirsutism, menstrual disorders, acne, and decreased fertility [33–35]. Patients with NCAH usually do not have elevated ACTH concentrations. The diagnosis of NCAH depends on measurement of basal and/or stimulated serum 17-OHP level. If follicular phase serum 17-OHP level is higher than 2 ng/ml, the patient should go to ACTH stimulation test [33, 36, 37]. For ACTH stimulation test, after a basal blood sampling for cortisol and 17-OHP levels, 0.25 mg ACTH is administered via intravenous route and samples are obtained at 30 and 60 minutes. Basal or ACTH stimulated serum 17-OHP level greater than 10 ng/ml is considered as the criteria for diagnosing NCAH due to 21-hydroxylase deficiency [38–40]. Patients with classical CAH have very high basal serum 17-OHP level, and most cases do not require ACTH stimulation test for the biochemical diagnosis of the disease.

Serum 17-OHP levels may have variability among the patients thus, genetic confirmation is necessary to clearly confirm the diagnosis and also to determine the degree of mutation and disease severity [41]. Use of genetic testing is also useful in prenatal counseling of mothers affected by CAH. CYP21A2 and its highly homologous pseudogene are located close together in the HLA class III region on the short

arm of chromosome 6 and point mutations or deletions constitute important parts of the disease-causing mutations [42]. Compound heterozygous mutations have been seen in most of the patients where the clinical phenotype is determined by the less severe allele [43]. Moreover, those patients carry more than one mutation on either or both CYP21A2 alleles which leads to a wide spectrum of phenotypes. Molecular analysis of CYP21A2 should be extensive and performed in an authorized laboratory.

Four different groups of CYP21A2 mutations exist (Table 2). Recently, the largest European study has evaluated the genetic data from 538 CAH patients and has found that genotype–phenotype correlation is high in severe mutations (null: 97%, A: 91%) but remarkably low in B (48%) and C (58%) phenotypes [41]. As reported in several studies, although a well-established genotype–phenotype correlation exist, discordances may be seen in some specific mutations. Especially, of the pseudogene-derived mutations, a promoter region mutation in exon 1 (p.P30L) is usually associated with NCAH; however, a clinical phenotype of classical CAH has been found in about 30% of cases [19]. The reasons of these discrepancies are not well-known. Effects of alternative pathways such as androgen-producing "backdoor pathway" or instead of carrying two mild mutations, being compound heterozygous with one severe allele may play a role in heterogeneous phenotypes [44]. Accumulating evidence suggests that apart from null genotype which results in complete loss of enzyme function, the clinical presentation of CAH surges depending on the unknown confounding factors.

During last years, heterozygous carriers for 21-hydroxylase enzyme have gained a great attention which is associated with an increased risk of developing hyperandrogenism [45, 46]. Heterozygous carriers are found in most populations with a prevalence of 1 in 50 [46–48]. Recently, it has been shown that approximately 80% of heterozygous carriers show features of NCAH including oligomenorrhea, hyperandrogenism, and overweight, suggesting that monoallelic mutations of 21-hydroxylase enzyme may also affect the enzyme activity [46]. However, currently, any basal- or ACTH-stimulated level of serum 17-OHP level cannot be used for the detection of heterozygosity for 21-hydroxylase enzyme.

Table 2 Classification of most commonly detected CYP21A2 mutations in patients with congenital adrenal hyperplasia

Group	Null	A	B	C
Phenotype	Salt wasting	Salt wasting	Simple virilizing	Non-classic
In vitro activity of CYP21A2	0%	<1%	1–2%	20–50%
Mutation	30 kb deletion 8 bp deletion Exon 6 cluster[a] p.318X p.R356W p.Leu307fs	IVS2-13A/C>G	p.I172N p.I77T	p.P30L[b] p.V281L p.R339H p.P453S

Modified from Hannah-Shmouni, et al. [8]

[a]Denotes three clustered mutations in exon 6

[b]May be associated with more severe hyperandrogenism

11-Beta Hydroxylase Deficiency

It has been reported that CAH due to 11-beta hydroxylase deficiency (11B-OHd) comprises 5–8% of the patients [49, 50]. It is the second most common form of CAH; however, in some communities such as Jews from Morocco, Israel, and Turkey, the frequency of the disease is higher [51–53]. Inactivating mutations on the gene *CYP11B1* result in impaired hydroxylation of 11-deoxycortisol and deoxycorticosterone, which then lead to deficiency of cortisol and corticosterone in the zona fasciculata and glomerulosa, respectively [54]. As in patients with 21-OHd, low cortisol level activates the negative feedback mechanism of hypothalamo–pituitary–adrenal axis leading to overproduction of ACTH with subsequent adrenal cortex hyperplasia. Females present with symptoms of hyperandrogenism and varying degrees of virilization of external genitalia with intact internal gonads. Surgical reconstruction may be required depending on the severity of virilization [55]. Males usually present with accelerated somatic growth and mostly short adult stature.

More than 100 mutations have been reported in *CYP11B1* gene, and all the mutations have been detected along the whole gene. Depending on the mutation and its severity, elevated concentrations of deoxycorticosterone suppress the renin–angiotensin system, resulting in extracellular fluid expansion, hypertension, and hypokalemia [56]. Particularly, the classical form of 11B-OHd is similar to 21-OHd with signs of androgen excess, but patients have hypertension, rather than salt loss, due to mineralocorticoid receptor stimulation mediated by mineralocorticoid precursors. However, it is important to note that salt wasting can be precipitated by glucocorticoid treatment due to suppression of deoxycorticosterone and resultant low mineralocorticoid activity [57]. Non-classical form of 11B-OHd has similar characteristics of non-classical 21-OHd characterized by hirsutism, menstrual disturbances, and acne [54]. In contrast to classical form of the disease, hypertension is not a usual finding in those patients.

There is unfortunately no clear diagnostic nomogram for the diagnosis of 11B-OHd, compared to 21-OHd. Urinary tetrahydro-metabolites of steroid precursors such as tetrahydro-11-deoxycortisol and tetrahydrocorticosterone are unequivocally elevated and can be used for the diagnosis [14, 55]. Basal or ACTH stimulated 11-deoxycortisol levels three times above the 95 percentile for normal population have been used for the diagnosis of the non-classical form [52, 58, 59].

17-Alpha Hydroxylase Deficiency

17-Alpha hydroxylase deficiency is an uncommon cause of CAH, accounting for 1% of CAH cases and results from mutation in *CYP17A1* gene. This gene located on the long arm of chromosome 10 encodes an enzyme that express both 17-alpha hydroxylase and 17, 20 lyase activities. Inactivating mutations have been located along the whole gene without any distinct specific area. Since 17-alpha hydroxylase

is located on the critical steroidogenic pathway, severe mutations in the *CYP17A1* gene result in problems both in adrenal and gonadal sex steroid production [60, 61]. Patients with 17-alpha hydroxylase deficiency present with sexual infantilism, pubertal failure, and lack of adrenarche due to decreased production of DHEAS [61]. Both, 46,XX and 46,XY patients have female external genitalia, usually present as girls without secondary sex characteristics during puberty associated with hypergonadotropic hypogonadism.

CYP17A1 gene is expressed in zona fasciculata and zona reticularis but not in zona glomerulosa. Thus, ACTH-mediated steroidogenesis results in high levels of corticosterone and deoxycorticosterone. Increased deoxycorticosterone leads to sodium retention, hypokalemia, suppression of aldosterone production, and renin levels. It is remarkable that patients rarely present with adrenal failure despite being severe glucocorticoid deficient due to the affinity of mineralocorticoid precursors (particularly deoxycorticosterone) on the glucocorticoid receptors [62].

3-Beta Hydroxysteroid Dehydrogenase Deficiency

This enzyme exists in two forms: type 1 and type 2 enzymes which are encoded by HSD3B1 and HSD3B2, respectively. The gene is located in close proximity to each other on the short arm of chromosome 1. *HSD3B2* gene is mainly expressed in adrenals and gonads while *HSD3B1* gene is mainly expressed in placenta and peripheral tissues. The disease is caused by the gene encoding *HSD3B2* and characterized by aldosterone, cortisol, and androstenedione deficiencies. It is a rare (affecting <0.5% of all CAH patients) form of CAH, and the clinical spectrum varies according to the severity of the genetic mutation and may include salt wasting in both sexes, incomplete masculinization in males and virilization in females [63]. Classically, infants present with salt wasting adrenal crisis and high renin-associated hypotension similar to CYP21A2 deficiency. However, this enzyme deficiency is associated with sexual differentiation disorders in both sexes. There are no reported mutations in the human *HSD3B1* gene already [62].

Since HSD3B2 catalyzes the conversion of Δ5-steroids (DHEA, pregnenolone and 17-hydroxypregnenolone) to Δ4-steroids (testosterone, progesterone, androstenedione and 17-OHP), higher ratios of the Δ5 over Δ4 steroids are detected and they are used for hormonal diagnosis of HSD3B2 deficiency. It presents with raised 17-hydroxypregnenolone to 17-OHP and DHEA(S) to androstenedione ratios in serum samples [64]. In general, 17-hydroxypregnenolone level above 100 nmol/L, either in basal condition or after 0.25 mg ACTH stimulation is accepted as the best hormonal criterion for the diagnosis of HSD3B2 deficiency [65–67].

Non-classical HSD3B2 deficiency is considered very rare, and some adult women with mild hyperandrogenism who have elevated serum DHEAS may have partial defect in HSD3B2 activity but those patients do not exhibit any identified gene mutation [12]. Their final diagnosis is usually a form of PCOS associated with alteration in intra-adrenal HSD3B2 activity [68–70].

P450 Oxidoreductase (POR) Deficiency

Cytochrome P450 oxidoreductase (POR) is a cofactor which transfers electrons from NADPH to cytochrome P450 enzymes for drug and toxin metabolism in addition to steroidogenesis [71]. The *POR* gene is located on long arm of chromosome 7. The enzymes 17-alpha hydroxylase, 21-hydroxylase, and aromatase are dependent on POR as an electron donor enzyme for their catalytic activity. The clinical picture is combination of 17 alpha-hydroxylase and 21-hydroxylase deficiency [72]. Previously, this clinical picture has been named as "apparent combined 17-alpha hydroxylase and 21-hydroxylase deficiency" [69]. Most patients with POR deficiency present with ambiguous genitalia. 46,XX infants are partially virilized, and 46,XY infants are partially undermasculinized, and in both sexes, delayed developmental of sexual characteristics occur in pubertal period, particularly females often develop hypergonadotropic hypogonadism [73, 74]. The pathogenetic mechanisms of manifestations are associated with impaired synthesis of testosterone, dihydrotestosterone and conversion of the androgens to estrogens. All these activities depend on CYP17A1, CYP21A2, CYP19A1 which require POR [71].

The POR deficiency is not only a disorder of steroidogenesis, it also affects all microsomal CYP enzymes that require POR for appropriate function. P450 liver enzymes and heme oxygenase that are involved in drug metabolism, need POR to provide electrons, thus, mutation of *POR* gene may affect drug metabolism. Moreover, estrogen and glucocorticoids are the drugs affected by POR deficiency, indicating that metabolic clearance of exogeneous hormone replacement is expected to be reduced. This is important that patients may be exposed to higher drug doses than considered [75]. Those patients also have skeletal abnormalities which have been described as a part of the Antley–Bixler syndrome phenotype. Typical malformations of this syndrome are large joint synostosis, hand and foot malformations, craniosynostosis [76]. The POR deficiency is the most complicated form of CAH, and some mothers may also exhibit signs of virilization due to insufficient aromatization of fetal androgens in pregnancies complicated by POR deficiency.

Most patients have normal baseline cortisol but give an inadequate response to ACTH test, indicating partial glucocorticoid deficiency. Thus, those patients may require stress doses of glucocorticoids in stressful clinical situations. Pregnenolone and progesterone are characteristically increased, 17-OHP is only mildly increased. The diagnosis is usually established by urinary steroid profiling by gas chromatography-mass spectrometry, which reveals the combined impairment of CYP21A2 and CYP17A1 activities. Confirmation of the disease requires genetic testing.

Lipoid Congenital Adrenal Hyperplasia (StAR Deficiency)

Steroidogenic acute regulatory protein (StAR) regulates the transfer of cholesterol from the outer to inner mitochondrial membrane which is a key step in steroid biosynthesis except placenta. StAR deficiency leads to this rare form of CAH, and its

classical form is characterized by deficiency of all steroid hormones [77, 78]. Patients have high basal concentrations of ACTH, elevated plasma renin activity, and hypergonadotropic hypogonadism. It is named as "lipoid" due to the accumulation of lipid droplets in the adrenal glands. In addition to the loss of StAR-mediated steroidogenesis, accumulation of cholesterol in the adrenal cells further deteriorates steroid hormone production, and it is called as "second hit." In its severe form, the clinical picture is composed of neonatal crisis and female external genitalia in both sexes [79]. Genetic 46,XY males are mostly diagnosed because of the absence of Mullerian structures at ultrasonography which leads to consideration of disorders of sexual differentiation in differential diagnosis [79]. In addition to classical form of the disease, non-classical form retains 20–30% of StAR activity and presents as compensated primary adrenal insufficiency and various degrees of gonadal function ranging from hypergonadotropic hypogonadism to normal gonadal function [79, 80].

Side Chain Cleavage Enzyme Deficiency (P450scc)

This enzyme is encoded by CYP11A1 and is involved in the first step of steroidogenesis, and it catalyzes the conversion of cholesterol to pregnenolone in steroidogenic cells. A cell is defined as steroidogenic if it expresses P450scc which is the only enzyme that can catalyze the conversion of cholesterol to pregnenolone [79]. Clinical and laboratory characteristics are almost identical to lipoid CAH. Those patients exhibit adrenal insufficiency and 46,XY sex reversal due to disruption of steroidogenesis. Both deficiencies can be distinguished by DNA testing and in contrast to StAR deficiency, patients with side chain cleavage enzyme deficiency have atrophic adrenal and gonads [78, 79]. As StAR mutations are more common, it has been suggested that sequencing StAR first and if mutations are not found, then sequencing P450scc [79]. Although some P450scc-deficient children have early onset adrenal insufficiency, the age of clinical onset mostly correlate with the enzyme activity, with late-onset patients having substantial residual activity [81].

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Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency: Genetic Characterization and the Genotype–Phenotype Correlation

Duarte Pignatelli and Sofia S. Pereira

Introduction

Congenital adrenal hyperplasia (CAH) results from the insufficient synthesis of cortisol occurring as a consequence of a deficiency of one of the steroidogenic enzymes being inherited as an autosomal recessive disorder. Approximately 95% of the cases are due to 21-hydroxylase deficiency (21-OHd), an enzyme that is expressed mostly in the adrenal cortex [1]. It is one of the most common autosomal recessive diseases, but in spite of the progress in its genetic diagnosis, this is a complex process with significant risk of attaining incomplete results and consequently misunderstanding of the real situation [2].

D. Pignatelli (✉)
Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, Porto, Portugal

Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal

Department of Endocrinology, Hospital S. João, Porto, Portugal

Department of Biomedicine, Faculty of Medicine of the University of Porto, Porto, Portugal
e-mail: dpignatelli@ipatimup.pt

S. S. Pereira
Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, Porto, Portugal

Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal

Department of Anatomy, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal

Clinical and Experimental Endocrinology, Multidisciplinary Unit for Biomedical Research (UMIB), Porto, Portugal
e-mail: sspereira@icbas.up.pt

M. E. Ertorer (ed.), *Fertility and Reproductive Outcomes in Different Forms of Congenital Adrenal Hyperplasia*, https://doi.org/10.1007/978-3-030-82591-1_2

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21-OHd) encompasses very important disorders with high morbidity (the classical forms) or high prevalence (the non-classical forms). They affect patients' life in many ways, ranging from salt-wasting and life-threatening crises to virilization with variable degrees of genital ambiguity and with all its consequences of gender determination and reconstructive surgeries, ultimately affecting normal sexuality and reproduction. Some researchers believe that this division of the classical forms is artificial as the simple virilizing forms may also have variable degrees of salt wasting [3–5]. Finally, there are non-classical forms (NCAH) in which the enzymatic activity is significantly retained, between 20% and 60%, and in consequence, there is normal production of mineralocorticoids and cortisol. The resulting phenotype is characterized mainly by androgen excess that is less severe or occurring later in life; nevertheless, it may have an impact on sexuality and reproduction or simply affect the patients' self-esteem and quality of life. One of the important features of NCAH forms is their much higher prevalence in comparison to the classical forms.

Genetic alterations of classical salt-wasting forms lead to complete absence of 21-OH enzyme and consequently to impairment of cortisol and aldosterone biosynthesis. In classical forms, there is also highly increased secretion of androgens, clinically translated in female virilization and precocious puberty in males. This is the result of increase in ACTH production (or action) due to enzymatic blockage causing loss of feedback control upon hypothalamus and pituitary, leading to diversion of steroidogenic precursors to the androgenic pathways.

Other pathogenic genetic variants cause less severe alterations that are compatible with the synthesis of 21-hydroxylase protein with some activity, some cortisol and aldosterone synthesis, in amounts that are compatible with life, without the need for their replacement. In those cases, the disease manifests itself later in life, mostly by excessive production of androgens. In these "non-classical," less severe forms, hyperandrogenemia also results from diversion of 17-hydroxyprogesterone and other precursors to alternative androgenic pathways in spite of the fact that cortisol is not significantly reduced, and ACTH not significantly elevated (altered enzymatic kinetics).

The Structure of the *CYP21A2* Gene

CYP21A2 is located in chromosome 6, in the area of the human histocompatibility complex (HLA), a region with a complex organization of genes with a great variability in their size and copy numbers [6–9].

At a distance of 30 kb from *CYP21A2*, there is a pseudogene (e.g., a nonfunctional gene) named *CYP21A1P*. Both the functional gene and the pseudogene have ten exons and share a high level of genetic homology (98% in their exons and 96% in their introns) [10, 11]. The pseudogene is inactive because it accumulates a great number of mutations along human development.

The neighbor localization and high homology of these two genes frequently lead to misalignments during meiosis that result in recombinations between the gene and the pseudogene (gene conversions) that are responsible for the majority of point mutations in the *CYP21A2* gene.

In close proximity to these two genes, there are other genes in the same region of chromosome 6 forming genetic units called RCCX. These genes are *RP1*, *C4*, *TNXB*, and two truncated pseudogenes, *RP2* and *TNXA* [12]. The genes *C4B* and *C4A* are translated into the fourth component of complement [13, 14], while *TNXB* encodes for an extracellular matrix protein called tenascin-X23 and the *RP1* gene gives origin to a serine/threonine nuclear protein kinase [12]. The proximity of *CYP21A2* and *TNXB* genes explains the existence of a syndrome with simultaneous characteristics of CAH and of the hypermobility type of Ehlers–Danlos syndrome, resulting from the simultaneous deletion of both genes the CAH-X syndrome [15–17].

The most common organization of this region has two RCCX modules, one with the *CYP21A1P* pseudogene and the other with the *CYP21A2* active gene. The orientation of the genes from telomere to centromere is: *RP1-C4A-CYP21A1P-TNXARP2-C4B-CYP21A2-TNXB*. The haplotype, bearing two RCCX modules, is present in about 69% of the Caucasian population [18]. A mono-modular haplotype may also occur (in 17% of the population) while a "three modular" haplotype has been reported to be present in 14% [12, 19]. Most haplotypes with three modules have two copies of the *CYP21A1P* pseudogene and one copy of the *CYP21A2* gene. Two copies of the *CYP21A2* gene and only one of the *CYP21A1P* can also occur, and this situation has been described particularly in cases with the p.(Gln319∗) pathogenic variant and of chimeric *CYP21A1P*/*CYP21A2* genes [19–21].

Another important aspect of the structure of *CYP21A2* gene is the high prevalence of copy number variations, which in conjunction with the enormous amount of possible genetic variants makes the characterization of *CYP21A2* alleles a difficult task. Genetic variants have been identified in both the coding and non-coding regions of the gene inclusively in the 5′UTR and the 3′UTR regions. Consequently, it is recognized that the characterization of the gene must include the sequencing of every exon, and the intron–exon boundaries [2].

The Origin of *CYP21A2* Alterations

Due to gene and the pseudogene localization and genetic complexity of the region, recombination events are generally the cause of *CYP21A2* pathogenic modifications.

In fact, intergenic recombinations between the inactive and active genes (gene conversions) are responsible for more than 95% of the pathogenic variants causing 21-OHd [22, 23].

About 75% of the pathogenic variants are transferred by small conversions from the pseudogene during meiosis. These conversions can involve one or more pseudogene variants. They are called "microconversions."

In the other 20–25% of the cases, 21-OHd CAH results from gross misalignment (unequal crossing over) during meiosis. Gene deletions, gene duplications, and deletions involving CYP21A2 and other contiguous genes usually ensue [24, 25]. Rarely, 21-OHd CAH can also be the result of uniparental isodisomy [26].

More than 250 genetic variants of CYP21A2 are capable of causing human disease. The majority of these will result in classical forms of 21-OHd [27]. One hundred and fifty-three genetic variants have been demonstrated to be missense mutations and have been shown to be able to give rise to all forms of the disease. On the contrary, nonsense and frameshift mutations always result in classical 21-OHd forms [27].

Genetic variants have also been observed in non-translated regions of *CYP21A2* gene. Some of these affect the promoter, resulting in promoter conversion [28].

Sequencing of *CYP21A2* gene in suspected cases should be considered essential in modern medical practice. Only a complete genetic analysis can accurately determine the genotype as pathogenic variants are frequently complex [2].

The effort to genotype a patient with 21-OHd used to be simplified by focusing on a group of 10 pathogenic variants that account for the majority of affected alleles. However, a recent study has demonstrated that this targeted *CYP21A2* mutation analysis may fail to identify mutations on one allele in 10.4% of the cases [29].

In light of the present knowledge, familial segregation studies should always be done, as this is the only way to be sure if two detected pathogenic variants affect the two alleles (trans configuration) or are located in the same allele with the other one being normal (cis configuration). For this purpose, both parents have to be studied together with the proband.

CYP21A2 Genetic Modifications

There are large rearrangements and small conversions. Large recombinations result from unequal crossing over during meiosis. The other type of conversion occurs when a small segment of the functional *CYP21A2* gene is replaced by a segment from the *CYP21A1P* pseudogene (microconversions). The altered *CYP21A2* gene will carry a point mutation or a short sequence that may involve one or more exons [10, 22, 30, 31]. As these variants are pathologic, they will give rise to an inactive or at least significantly modified 21-hydroxylase protein.

Large Deletions and Conversions

Approximately 25% of alleles carry a deletion, a large gene conversion, or the formation of an inactive chimeric gene which is the result from a meiotic recombination in which the final product is a non-functional chimeric gene with its 5′ end belonging to *CYP21A1P* and the 3′ end to *CYP21A2* [32–34].

Several different chimeric *CYP21A1P/CYP21A2* genes have been identified as a result of variable length deletions [35–38].

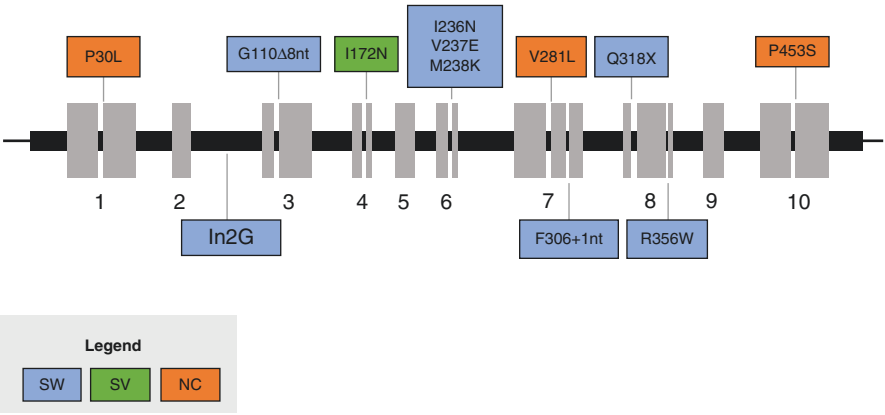

Fig. 1 Location of the most common *CYP21A2* mutations that are transferred by gene conversion and their association with the clinical outcome. Numbered boxes represent *CYP21A2* exons. *SW* salt wasting, *SV* simple virilizing, *NC* non-classical

Point Mutations and Small Deletions/Insertions

Most of the intergenic recombinations (75%) correspond to abnormal nucleotides normally present in the pseudogene that are transferred to the functional gene by microconversion [30] (Fig. 1). Other rearrangements have been reported including a deletion of 10 nucleotides in exon 8 or a duplication of 16 nucleotides in exon 9.

Other Pathogenic Variants

Many different pathogenic variants have been described, and these will certainly increase with the widespread utilization of molecular diagnosis. Most of these are rare, having been described in only one case or one family (see http://www.cypalleles.ki.se/cyp21.htm and http://www.hgmd.cf.ac.uk). Less than 5% of the pathogenic variants are not caused by gene conversions and so possibly not being presented in the pseudogene [39, 40].

Polymorphisms

Some alterations that are found in *CYP21A2* gene do not affect 21-hydroxylase production and are considered normal polymorphisms [6]. It is possible that at least some of these variants are also present in the *CYP21A1P* gene and represent gene conversions not affecting the enzyme. However, others have been described only in the *CYP21A2* gene.

Genotyping and Pregnancy

Genotyping of *CYP21A2* gene as completely as possible is very important in couples who wish to conceive. It allows confirmation of the diagnosis, and it is the only way to do a correct genetic counseling.

The parents want to learn about the risk of having a child with salt-wasting form or with severe virilization in case of a female fetus.

It is well established that in a woman with CAH, the progeny will or will not have CAH according to the partner's genotype. If he does not have any pathogenic variant in *CYP21A2*, the children will be carriers but will not have the disease. If the woman's genotype consists of two mild pathogenic variants, for instance being homozygotic for V281L (p.(Val282Leu)) and her partner is a carrier of a *CYP21A2* pathogenic variant, whatever it may be, 50% of her children will have NCAH (50% being merely a mathematical probability). However, in general population, the probability of being a carrier for a severe pathogenic variant is 1.7% (1 in 60) [41], and consequently the risk for having a child with classical 21-OHd CAH would be expected to be 1:600 since it has been reported that the probability of a patient with NCAH having a severe pathogenic variant together with a mild one is approximately 2/3 [29, 42]. The real frequency is however closer to 2.5% [43], and this made researchers believe that the carrier frequency is probably higher than is suspected, at least in some populations.

The actual recommendation is that both parents should be genotyped whenever possible as part of the prenatal study protocol if one potential progenitor has any form of 21-OHd CAH [43].

Genotype–Phenotype Correlations

There is a good correlation between genotype and the phenotype generally reported as reaching 90% or even 95% [33, 44–47]. However, it must be acknowledged that the clinical picture resulting from 21-hydroxylase deficiency is in fact a continuum of reductions in enzyme activity, and the three degrees of severity, in which the disease is generally classified, are only a simplification aiming to facilitate the clinical practice [48]. It is also recognized that the phenotypes can change with time and consequently a perfect correlation between genotype and phenotype is virtually impossible.

There are genetic variants that are considered to cause the most severe forms of the disease (100% enzymatic deficiency), resulting in salt-wasting forms of the classical 21-OHd CAH. These variants are called *severe pathogenic variants* (Fig. 2).

The missense pathogenic variant I172N (p.(Ile173Asn)) confers around 1–2% of the normal 21-hydroxylase activity, and even this small enzymatic activity is sufficient for a near normal aldosterone production, reducing almost completely the risk of salt-wasting crises. Thus, it is associated with the simple virilizing form of 21-OHd CAH. Pathogenic variants, as this one, are called the *intermediate pathogenic variants* (Fig. 2).

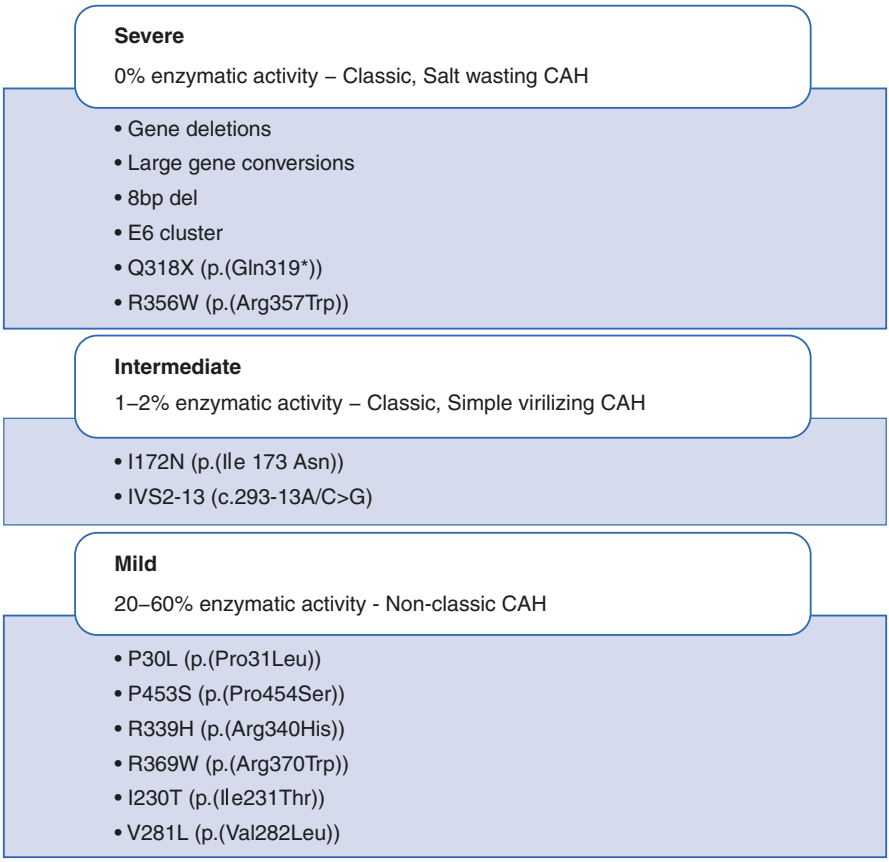

Fig. 2 *CYP21A2* pathogenic variants and their correlation with phenotype

Other pathogenic variants including P30L, P453S, R339H, R369W, I230T [48], and V281L (clearly the most frequent pathogenic variant in NCAH cases in most series) result in a conservation of enzymatic activity of 20–60% and consequently in less severe symptoms which are associated with the non-classical form (*mild pathogenic variants*) (Fig. 2).

Importantly, most of the 21-hydroxylase-deficient patients are compound heterozygotes instead of homozygotes [3, 29], and this implies that the phenotype is generally affected by the residual 21-hydroxylase activity and consequently by the less severely mutated allele [33]:

1. The most severe phenotypes (the classical forms) must have severe pathogenic variants in both alleles and none of the mild pathogenic variants.
2. The NCAH patients may have two mild mutations (a situation that occurs in approximately 35% of the cases) or one mild and one severe ones (in the other 65%).

A mild pathogenic variant present in one of the alleles allows the synthesis of 21-hydroxylase up to 50–60% of the normal activity, in spite of the fact that the severe pathogenic variant, present in the other allele, does not contribute to any synthesis.

Although the correlation between genotype and phenotype is high, it has been observed that some of these pathogenic variants confer different phenotypes depending on if they are isolated or associated with another pathogenic variant.

In fact, there is some diversity of the phenotypes in patients with less severe pathogenic variants [5, 33, 49, 50]. It has been reported that in spite of being predictable that the phenotype will correspond to the less severely affected allele, the presence of a second allele with a more severe pathogenic variant can result in a more serious clinical phenotype [51–55] with higher degrees of hirsutism and also of higher 17OHP levels than cases with two mild genetic alterations [42, 56].

The pathogenic variants IVS2-13 (c.293-13A/C>G) and I172N (p.(Ile173Asn)) can result in variable degrees of 21-hydroxylase activity (possibly through alternative splicing). Patients with these mutations are generally expected to be simple-virilizing cases but may sometimes present as salt-wasting forms, and the others may stand closer to NCAH [34, 57]. Similarly, the pathogenic variant P30L (p.(Pro31Leu)) which is considered to be a cause of NCAH has been reported to be responsible for cases of SV-CAH, as well [58, 59]. Considering about the residual 21-hydroxylase activity which is expected to present with P30L mutation, this is unexpected. The NCAH patients with P30L mutation can exhibit stronger virilization with clitoromegaly and advanced bone maturation [60]. Different mechanisms have been proposed for the increased androgenization of these patients, including the influence of other residues, accompanying promotor variability or mutations and finally variations in individual androgen sensitivity [59]. In fact, some concomitant factors capable of modifying the phenotype have been suggested such as the number of CAG repeats of the androgen receptor, other genes encoding proteins with 21-hydroxylase activity and alternative pathways of androgen biosynthesis capable of causing fetal virilization in females [59].

In a multi-national study of 1507 families with CAH, SV form of CAH was found in 17/74 patients having P30L mutation (23%) [46].

Even when it does not clearly result in a SV form, the clinical manifestations in patients with P30L will include stronger signs of virilization, earlier adrenarche, clitoromegaly, and some patients require treatment with glucocorticoids compared to other patients with NCAH form [39, 44, 61].

Moreover, genotypes P30L/I2 splice, P30L/Q318X, and P30L/8Δbp are especially associated with SV form of CAH [46]. If not treated with glucocorticoids, SV progresses steadily during childhood causing early puberty, short adult stature, and fertility problems in both genders including testicular adrenal rest tumors (TARTs) in men.

Incomplete correlation between genotype and phenotype may also result from not sequencing the whole gene and hence not having a full picture of the genetic alterations.

Genetic Sequencing

Sequencing of the entire gene by PCR mutation-detection methods together with multiplex ligation-dependent probe amplification are the golden standard for studying the *CYP 21A2* gene.

General Considerations

Specific gene amplification by PCR has dramatically improved the sensitivity of different techniques to detect *CYP21A2* pathogenic variants. Modernly, PCR conditions have been identified that allow the amplification of *CYP21A2* without amplifying the very homologous *CYP21A1P* pseudogene. These conditions result from amplifying CYP21A2 in two segments (Fig. 3).

PCR-based diagnosis may be complicated by the failure of amplifying one haplotype which may result in misdiagnosis. Examination of flanking microsatellite markers in all family members can minimize this problem.

Another important aspect is that, if only a DNA sample from the patient is analyzed, it is impossible to distinguish compound heterozygosity for different pathogenic variants occurring in *trans* and the presence of two pathogenic variants in the same allele (*cis*). Therefore, ideally both parents should also be analyzed, so as to most reliably determine the phase of different pathogenic variants (i.e., whether they lie on the same or opposite alleles). Analysis of parental alleles also permits differentiating homozygotes and hemizygotes (i.e., individuals who have a pathogenic variant on one chromosome and a deletion on the other).

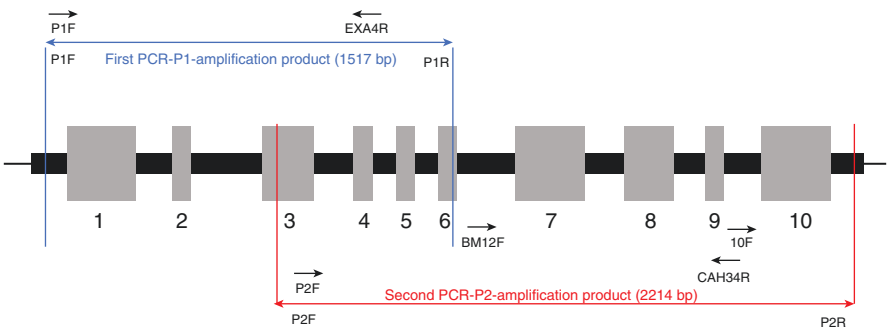

Fig. 3 Proposed strategy to whole-gene sequence of the *CYP21A2* gene. Numbered boxes represent *CYP21A2* exons and arrows represent the primers. Adapted from [62]

DNA Sanger Sequencing

The whole-gene sequencing (together with MLPA) has become the standard procedure in cases of 21-OHd. It usually covers the coding regions and the flanking intron–exon regions of the gene. This method not only detects the more common genetic variants but can also detect the novel sequence variants helping to explain some cases in which there has not been a correlation between genotype and phenotype. Main difficulty results from the homology between the gene and the pseudogene. To avoid the co-amplification of pseudogene *CYP21A2,* whole-genomic sequencing may be performed selecting the functional *CYP21A2* gene and amplifying it by PCR into two partially overlapping fragments, P1 and P2 respectively with one 517 and two 214 base-pairs (bp). After selective amplification of the targeted gene and subsequent purification, the PCR product is sequenced with internal primers that cover the entire *CYP21A2* gene [62].

MLPA

After sequencing the entire *CYP21A2* gene, one should also look for deletions and duplications [63, 64]. This is currently done using multiplex ligation-dependent probe amplification (MLPA) [65].

The MLPA assay is a technique that enables the detection of variations in the copy number of several human genes. Due to the large number of genes or genetic sequences that can be simultaneously analyzed, MLPA assay has become the gold standard for molecular analysis of all pathologies derived from the presence of gene copy number variation [66]. Besides, MLPA can be used to confirm the point mutations identified by sequencing analysis.

Detection of deletions and duplications of *CYP21A2* gene and the *CYP21A1P* pseudogene by MLPA is currently performed, using the P050-CAH Kit (MRC-Holland). This high-resolution method uses only a single pair of PCR primers, and the specificity relies on the use of progressively longer oligonucleotide probes, in order to generate locus-specific amplicons of increasing size that can be resolved electrophoretically. Comparing the peak pattern obtained to that of the reference samples, it is possible to determine which probes/locus show abnormal copy numbers [67, 68].

Final Considerations

Genotyping can also be used for disease prevention. Preimplantation genetic diagnosis is increasingly being performed to limit the transmission of several diseases, including CAH, being used in conjunction with in vitro fertilization.

Another aspect of prenatal diagnosis consists of early gender determination, by the detection of SRY in circulating fetal DNA that is present in maternal blood at very early stages of pregnancy. This allows the identification of male fetuses that do not need to be treated prenatally. In case of a female fetus, obstetricians and pediatricians may treat these cases during early stages of pregnancy to prevent genital ambiguity. It is also possible to perform sequencing methods of the *CYP21A2* gene in circulating fetal DNA, but this is complex and still carries a significant possibility of false positives or false negatives. Chorionic villus sampling and amniocentesis can still be used for prenatal treatment to prevent the masculinization of external genitalia of the female fetus with classical 21-OHd; however, they are performed rather late [2, 29, 69].

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Classical Congenital Adrenal Hyperplasia (CAH) in Childhood and Adolescence: Diagnosis and Management for Future Reproductive Life

Merih Berberoğlu

Introduction

Congenital adrenal hyperplasia (CAH) is a group of hereditary disorders with autosomal recessive inheritance, which affects both children and adults. Mutations that cause loss of function in genes encoding enzymes that play various roles in the synthesis of adrenal steroids are responsible for the presentation. The basis of these disorders include the decrease in cortisol biosynthesis and secretion, abolished negative feedback control of cortisol on hypothalamus and pituitary, leading to increased corticotropin release, which, in turn, results in hyperplasia of the adrenal cortex. The phenotypic spectrum of CAH depends on the mutated enzyme and the severity of mutation. The enzyme deficiency can be complete or partial.

In virilizing forms of CAH, increased ACTH release leads to an increase in adrenal androgen synthesis. Four enzymes that take action in cortisol synthesis and whose deficiencies lead to CAH are biosynthetic enzymes: CYP21, CYP11B1, CYP17, and 3β-HSD. Another is the StAR enzyme, a transport protein that regulates cholesterol transfer within the mitochondria in the cell [1, 2]. In particular, CYP21 and CYP11B1 deficiencies lead to an increase in metabolites and androgens behind enzyme deficiency as well as a defect in cortisol synthesis. Accordingly, significant virilization occurs in the affected female fetuses. In both genders, peripheral early puberty develops after birth. In the majority of cases, enzyme deficiency is not complete, thus sufficient amount of cortisol is made to live, but under stress, cortisol insufficiency may develop.

21-Hydroxylase (CYP21) deficiency (21-OHd) accounts for 90–95% of the cases with congenital adrenal hyperplasia while 11–β-hydroxylase deficiency

M. Berberoğlu (✉)
Department of Pediatric Endocrinology, Ankara University School of Medicine, Ankara, Turkey

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affects 5–8% [1]. These two enzymes are found only in the adrenal gland, they are not found in gonads, while the other enzymes causing CAH act in either adrenals or gonads. Accordingly, 21-Hydroxylase and 11–β-Hydroxylase enzymes are not required for sex steroid synthesis. Therefore, similar clinical manifestations occur with excessive production of androgens in both sexes with the deficiencies of the two enzymes mentioned above. StAR, P450scc, 17α-hydroxylase/17–20 lyase enzyme deficiencies, in which both adrenal glands and gonads are affected together, are rare causes of sexual development disorders in which sex steroid production is interrupted, presenting with inadequate masculinization in males and mild virilization or normal appearance in girls. In older ages, inadequate development of puberty may be observed.

In this chapter, the two enzyme deficiencies 21-hydroxylase and 11–β-hydroxylase have been emphasized as they constitute the vast majority of CAH and create similar clinical pictures with androgen excess.

21-Hydroxylase Deficiency

21-Hydroxylase deficiency (21-OHd) is the most common type accounting for 90–95% of the cases. The locus, containing the *CYP21* gene region is quite complex. There are two 21-hydroxylase loci: the functional one, CYP21A2 gene (*CYP21A2*), is located in HLA class 3 region 6p21.3 and the other, homologous non-functional pseudogene, *CYP21A1P*, is also located in 6p21.1. Meiotic recombination in this region is common due to high-sequence homology between duplicated genes [3].

The incidence of 21-hydroxylase deficiency CAH ranges from 1 in 5000 to 1 in 15,000 according to the ethnic origin. In Western countries, carrier frequency for classical mutation has been estimated to be about 1:50–60 [4]. This enzyme is involved in both mineralocorticoid and glucocorticoid synthesis by catalyzing the conversion of Δ^4 steroids; progesterone to deoxycorticosterone (DOC) in zona glomerulosa and 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol in zona fasciculata. In 21-OHd CAH, the processes mentioned above cannot be performed. Then, increased 17-OHP metabolite is converted to DHEA, DHEAS, and androstenedione (AS). As a result, increased androgens lead to virilization in the genital bridge of the affected 46, XX female fetus. The 17-OHP is also converted to dihydrotestosterone (DHT) via a different route called the "backdoor pathway" [5]. In this pathway, 17-OHP is converted to 5α-pregnane-3α,17α-diol-20-one, resulting in the formation of a large amount of androstenediol, that will act as a substrate during conversion to DHT. The CYP21A2, CYP11B2, or p450 oxidoreductase (POR) mutations that cause 17-OHP accumulation in fetal life always cause an increase in DHT levels using this route [6]. In this pathway; DHT is produced without DHEA, androstenedione, and testosterone.

There are severe mutations in both alleles of *CYP21* gene in classical 21-OHd CAH. The course of the disease is life-threatening in cases with less than 1%

residual enzymatic activity. It results in salt wasting. In patients with an enzyme activity of 1–11%, aldosterone synthesis can be achieved, which protects the patient from severe salt-wasting crisis, and this presents as simple virilizing type [1]. These cases do not exhibit signs of salt loss under normal conditions, but they are at risk of adrenal crisis under intervening stressful situations. Patients with non-classical form carry two mild mutations in both alleles or severe mutations in one allele and mild in the other. The latter conditions are defined as "compound heterozygous." The degree of enzyme deficiency in this form is between 20% and 50% [1, 7]. More than 100 mutations of 21-OH enzyme have been identified to date [8].

Extra-adrenal 21-hydroxylase activity has been defined in adult and fetal tissue and 21-hydroxylated steroids can be detected in the plasma of patients with 21-OHd which explains the decreased need for mineralocorticoids in adulthood [9].

General Features of Classical Congenital Adrenal Hyperplasia due to 21-OHd

Ambiguous genitalia is the main finding in 46,XX cases with the classical form of CAH due to 21-OHd. Prenatal exposure to excess androgens from the sixth week of gestation leads to different degrees of virilization of the genital bridge. In utero deficiency of cortisol synthesis before week 12 causes overproduction of ACTH and hence adrenal steroids. The 17-OHP turns to AS and 17-hydroxy-pregnenolone to DHEA resulting in androstenedione and testosterone overproduction, leading to varying degrees of virilization in the fetus. Normal female sexual development can be seen in Fig. 1. Clitoromegaly, fusion of labia majora, labioscrotal structure, and urogenital sinus are findings of physical examination. The appearance of the external genital organs is classified according to Prader's staging [10]. According to this, the appearance ranges from clitoromegaly in stage I to a cryptorchid male appearance in stage V. The vagina and urethra open out through a common channel as the urogenital sinus persists in severe cases. Degree of virilization shows individual variations, and there is no correlation between the severity of mutation and degree of virilization. While some patients only have clitoromegaly, others with the same mutation may have 4–5 degrees of virilization, that is, completely a male genitalia appearance. Figure 2 shows a case with Prader stage III virilized external genitalia, and Fig. 3 shows an example of the fifth degree of virilization. In latter cases, the gonads are naturally ovarian and are not palpated on physical examination. If it is not diagnosed in neonatal period and the child grows without treatment, early pubic hair growth, rapid linear growth, and advanced bone age accompany the picture due to high androgens. This leads to short stature with early closure of the epiphyses. Untreated CAH cases are tall children but become short adults. The anti-mullerian hormone will not be secreted due to lack of testicular tissue and internal genital organs will remain as female. These cases have uterus, as well. The genitalia of fourth-to-fifth degree virilized cases exhibit no doubt male external genitalia appearance, and they are raised as male. The usual reasons for visit to a physician are the

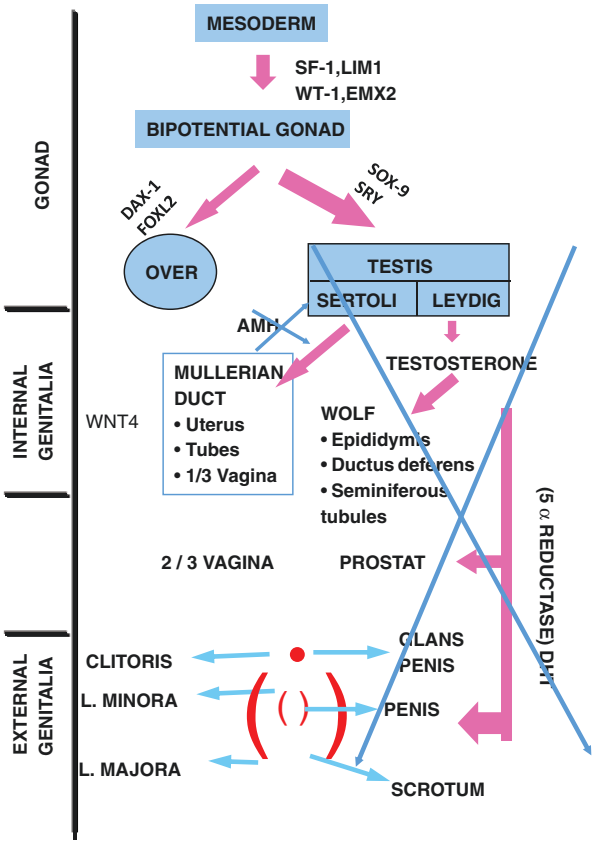

Fig. 1 Normal female sexual development

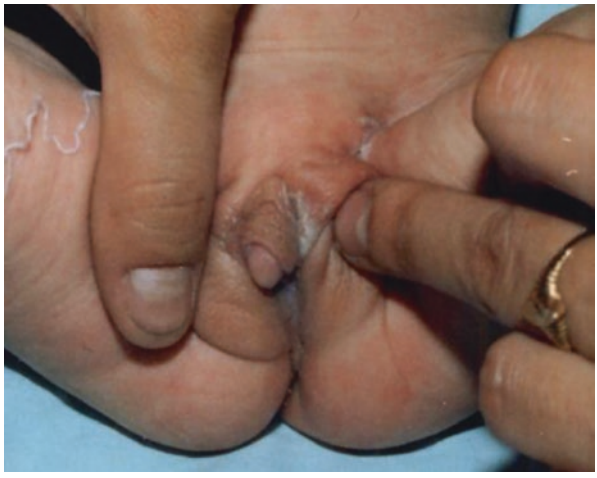

Fig. 2 Prader III virilization

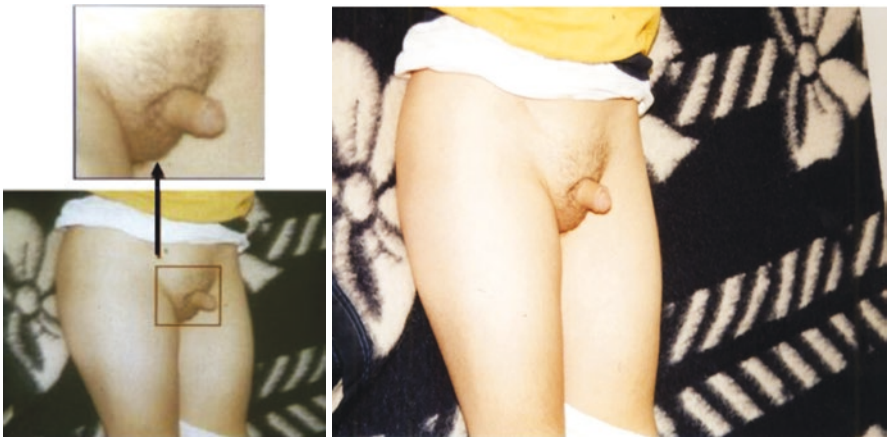

Fig. 3 Prader V virilization

absence of palpated gonads, which is perceived as bilateral cryptorchidism. There is also early pubic hair growth (Fig. 3). Despite exposure to high androgens, ovaries remain in their normal location. If performed by experienced hands, both uterus and ovaries can be demonstrated sonographically.

In severe 21-OHd CAH, aldosterone cannot be synthesized which leads to life-threatening symptoms of salt wasting. Salt loss symptoms usually present within the first 6–14 days of life. It is rarely seen beyond the second week after birth. The findings of salt-wasting include hypovolemia, hyperreninemia, hyponatremia, and hyperkalemia. Cortisol deficiency contributes to insufficient cardiac function and vascular response to catecholamines, decreased glomerular filtration rate, and increased antidiuretic hormone secretion. Cortisol and aldosterone deficiency together cause hyponatremic dehydration and shock. Patients are referred to physician with symptoms, such as difficulty in breastfeeding, lethargy, dehydration, diarrhea, and vomiting. Hyponatremia, hyperkalemia, and metabolic acidosis develop due to excessive sodium loss. In some cases, hypoglycemia accompanies to this picture. Male patients with classical 21-OHd CAH may be misdiagnosed as sepsis. Hyperkalemia in biochemical laboratory tests should alert the clinician. They can also be misdiagnosed as pyloric stenosis which may present with vomiting and metabolic alkalosis. If not noticed in time, death may occur usually within the first month after birth due to cardiovascular collapse. The tendency to salt-wasting decreases, usually, after the age of 2 or 3 years.

In classical simple virilizing types with no evidence of salt loss, girls with severe virilization are often assigned as boys by their families and are brought to the physician with complaints of failure of testicular descent, and premature pubic hair growth. If the early diagnosed cases are treated appropriately, they exhibit normal female-type pubertal development. Fertility may be possible if they maintain compliance to treatment. Excessive androgen in girls without treatment causes suppression of gonadotropins. Despite having ovaries and uterus, female pubertal

progression cannot occur. Oligomenorrhea or secondary amenorrhea is common in girls who receive treatment but do not have good compliance. Accelerated somatic growth, advanced skeletal maturation, and premature pubarche are observed in untreated cases in both sexes. External genitalia of affected males may be normal at birth or mild enlargement of penis in length and hyperpigmentation in scrotum may be seen. Although testicular size is prepubertal, macropenis, and premature pubarche can be obvious with advancing age. Peripheral precocious puberty can turn to central precocious puberty via activation of the hypothalamic–pituitary gonadal axis due to negative feedback set point change in patients who start treatment late.

In family history, there can be parental consanguineous marriages, unexplained male infant deaths, and history of early pubertal development in siblings. Congenital adrenal hyperplasia in 46,XX karyotype should be considered with high suspicion when the atypical genital structure is accompanied by nonpalpable gonads at physical examination. The diagnosis is strengthened when accompanied by genital hyperpigmentation. However, hyperpigmentation is not seen in all cases.

In laboratory evaluation, karyotype should be determined, and internal genital organs should be imaged by pelvic ultrasonography. Increased high 17-OHP levels are evidence of 21-OHd. 17-OHP level is frequently higher than 50 ng/ml in newborns, and it rises too much higher levels in preterm births [11]. Cross-reaction with fetal adrenal-induced steroids causes difficulty in interpretation of 17-OHP. It has been reported that false positivity can be decreased by 40% with measurements after diethyl extraction [12]. 21-deoxycortisol measurement by liquid/gas chromatography-tandem mass spectrometry method (LC-MS/MS), which is a new technique that has been developed over recent years, is recommended as a very supportive measurement [13].

The revised guideline in 2018 which includes a comprehensive approach to 21-OHd cases has emphasized that 17-OHP measurement with heel-prick blood samples is the first step in newborn screening [14]. The evaluation should be made according to the gestational age of the baby and false-positive results can be obtained. In the second step of CAH screening; the more reliable liquid chromatography-tandem mass spectrometry which is preferred to all other methods (e.g., genotyping) has been recommended in order to improve the positive predictive value [13]. A modified LC-MS/MS protocol using a ratio defined as the sum of 17-OHP and 21-deoxycortisol levels divided by cortisol value (17-OHP + 21-deoxycortisol/cortisol) provides a parameter that detects all affected children with 100% positive predictive value [13]. Measurement of urine metabolites, such as pregnanetriol and pregnanetriolon, by gas chromatography/mass spectrometry is another method that gives excellent specificity even in preterm babies [15]. Today, it is recommended to perform community screening via hormonal laboratory tests, since the cost of newborn screening with mutation analysis is very high. In individuals with a borderline 17-OHP, a complete adrenocortical profile assessment is recommended after ACTH stimulation test to differentiate 21-OHd from other enzyme defects [14]. Androstenedione, testosterone, and progesterone levels are typically high in 21-OHd. The hook effect should be considered when these levels are lower than expected in both sexes. Plasma renin activity may be high, as well as random

17-OHP levels in simple virilizing form of CAH infants, and is an indicator of subclinical salt loss. Hyponatremia, hyperkalemia, hyperreninemia, and low aldosterone are markedly evident in salt-wasting cases. Cortisol levels may not be detected as low in the blood, due to the interference of high levels of metabolites.

Another androgen synthesis pathway involves the production of 11-oxygentated C19 steroids through CYP11B1 activity in adrenal cortex. New biochemical markers have been identified recently through 11-oxygenated steroid measurements by LC-MS method. 11-ketotestosterone and 5α-dihydrotestosterone are potent and efficacious 11 oxy-androgens that are capable of binding and activating the androgen receptor. Circulating levels of 11-oxygenated C19 steroids in patients with 21-OHd is high, and even if the classical androgen pathway activity is downregulated, the exaggerated activity of both the backdoor pathway and 11-oxygenated C19 pathways continues routinely. Information about new steroid markers will help to improve the monitoring of metabolite concentrations in patients receiving treatment and to determine the therapeutic goals [16].

Adrenal glucocorticoids stimulate phenylethanolamine N-methyltransferase (PNMT) to synthesize epinephrine from norepinephrine in the adrenal medulla. Therefore, epinephrine deficiency has also been described in severe mutations with severe glucocorticoid deficiency. This deficiency also contributes to the development of hypoglycemia and hypotension in acute adrenal crisis [17].

Adrenal ultrasonography in newborns is also quite informative when performed by experienced radiologists. Congenital adrenal hyperplasia is associated with enlarged, lobulated adrenals with abnormal echogenicity, adrenal tail width is larger than 4 mm, and the central echogenic line is lost (cerebriform pattern) [18].

Nowadays, CAH screening programs have become widespread due to the fact that salt-wasting in neonates is life threatening soon after birth and 50% of 21-OHd CAH patients cannot be diagnosed clinically early enough [19]. Screening is usually carried out in accordance with European Society for Pediatric Endocrinology (ESPE) recommendations [20]. Molecular genetic studies are the gold standard for confirming the diagnosis in infants at risk, but it cannot be performed in all cases.

Diagnosis

Congenital adrenal hyperplasia should be considered primarily when there are signs of poor weight gain and salt-wasting in an infant especially in the presence of an ambiguous genital structure. Salt-wasting is manifested by hyponatremia, hypochloremia, hyperkalemia, metabolic acidosis, increased plasma renin activity (PRA) and urinary sodium levels, and decreased serum aldosterone levels. In simple virilizing cases, salt-wasting and increased PRA may occur due to increased metabolites that are accompanied by an increment in aldosterone levels indicating compensated salt wasting. It should be kept in mind that PRA is higher in normal newborns than older children.

Baseline 17-OHP levels in classical CAH infants are generally above 50 ng/ml. However, they can be found normal on the first postnatal day even in severe cases. Therefore, the first sample in a newborn should be taken between postnatal 48 and 72 h. The 17-OHP levels of preterm, small for gestational age (SGA) or sick newborns are also higher than normal. Therefore, tests should be repeated in suspicious cases before 08:00 AM, if required, the ACTH stimulation test should be performed by 17-OHP extraction method. At the 60th minute of ACTH stimulation test, it generally rises above 15 ng/ml in classical 21-OHd CAH [21]. Genotyping is required for definitive diagnosis of CAH. Other causes of virilization (e.g., adrenocortical tumor) should also be considered in the differential diagnosis. If high steroid levels are not suppressed by glucocorticoid therapy, a tumor should be suspected. Pseudohypoaldosteronism is the most frequently seen condition that may cause confusion in patients with simple virilizing form but is accompanied by evidence of salt loss. High levels of testosterone are important for the diagnosis in girls at any age, but in boys, postnatal elevations after months 5 or 6 may be associated with mini-puberty.

The imaging of the ovaries and uterus with pelvic ultrasonography in 46,XX cases with ambiguous genitalia will lead to diagnosis before karyotype analysis. Y chromosome analysis can be performed by in situ hybridization (FISH) to accelerate differential diagnosis. In classical CAH cases, genetic testing should be recommended for the patient and family. Neonatal CAH screening programs are becoming widespread nowadays and are especially important for the early diagnosis of newborns with salt-wasting, of males without salt-wasting, and of severe virilized female newborns (Prader 4–5) assigned male sex by mistake. Screening tests for measuring 17-OHP levels are performed most commonly by the RIA method and the most important problem is false positivity. If the blood sample is drawn after 48 hours of birth, false positivity may decrease slightly. The birth weight of some neonates with CAH may be high according to their gestational age, and this should be taken into consideration at interpretation of tests. In screening, patients with 17-OHP levels below 10 ng/ml at capillary blood samples are considered as normal, 10–30 ng/ml as suspicious, and over 30 ng/ml are considered as CAH. For confirmation, the second blood sample should be evaluated by using LC-MS method [20].

Genetic

To date, more than 100 CYP21A2 mutations have been reported. Large gene deletions, gene conversions, and point mutations have been identified [8]. According to the studies, there is a genotype–phenotype relationship. Among these mutations, the IVS2-13C>G mutation is frequently seen as salt-wasting type, whereas p.I172N and 8-bp deletion are detected in simple virilizing type. Genetic diagnosis can be made by searching for 10 mutations that are common in 95% of the cases [8]. Androgenic effects are also thought to play a role in phenotypic variability in 21-hydroxylase cases apart from CYP21A2 mutations. The CAG repeat length of the androgen

receptor gene regulating the androgenic effects has been reported to have an impact on phenotype [22]. Another contributing factor is the electron-providing enzyme P450 oxidoreductase (POR) [23].

Treatment

Steroid Treatment

Hormone replacement therapy is applied according to the clinical and laboratory findings of the CAH patient. The aims of treatment are to suppress excessive androgen production by providing sufficient glucocorticoids and, if necessary, mineralocorticoids, in order to prevent salt-wasting and achieve normal growth and development. While undertreatment with glucocorticoids causes progressive virilization, acute adrenal crisis and bone age acceleration, overtreatment leads to short stature, and cushingoid appearance. Also, long-term inadequate treatment may lead to precocious puberty, adrenal rest tumors, and ovarian hyperandrogenism in girls.

The dose of glucocorticoids should be adjusted to childhood stage and age. Cortisol requirements, which will suppress androgen production and allow normal growth and development, vary from person to person. The recommended maintenance amount is 7–18 mg/m^2/day for hydrocortisone. It has been reported that patients who receive treatment above 20 mg/kg/day during infancy have achieved reduced adult final height [24]. However, higher amounts of cortisol than maintenance doses are usually required for reaching the desired therapeutic goals. Depending on the condition of the patient, initial treatment dose can be higher. Total amount is usually divided into three doses per day. Some researchers suggest that higher doses should be given at night, while some others to be given very early in the morning. However, it has been demonstrated that giving the large portion in the morning or evening does not make any difference in terms of biochemical parameters [25].

The dose should be increased two- or threefold in fever, stress, acute-onset gastrointestinal problems, and surgical interventions. Serum 17-OHP level measurement in the early morning can be used as an indicator of ACTH suppression for monitoring treatment. Androstenedione and its derivatives reflect the suppression of adrenal androgens. The LC-MS/MS is considered as a useful method for the measurement of adrenal androgens because it reduces cross-reaction and specificity problems [26]. Since plasma testosterone, 17-OHP, and androstenedione levels fluctuate throughout the day, they should be interpreted together with clinical features.

Growth suppressive effects of glucocorticoids lose their importance after puberty. Therefore, short-acting glucocorticoids may be switched to long-acting ones. The latter suppress adrenal androgens better than the short-acting ones. They have an enhancing effect on fertility in both girls and boys. However, they can disrupt metabolic health and cause cushingoid appearance. They can be preferred in adolescent patients with drug compliance problems. Sustained-release glucocorticoids are

essential for establishing the imitation of normal circadian rhythm and are the promising treatment agents that are not yet widespread [27].

Aldosterone deficiency is evident mainly in salt-wasting form and although not clinically evident, all types of 21-OHd CAH have varying degrees of hidden aldosterone deficiency. Therefore, patients may benefit from mineralocorticoid therapy under appropriate conditions. Mineralocorticoid requirement is higher during infancy due to pseudohypoaldosteronism. The recommended daily dose, divided into two parts is 0.1–0.2 mg. Since the salt in mother's milk is insufficient for the first 6–8 months, salt can be added to the treatment until adult-type nutrition is provided. The required sodium intake is 2 mEq/kg per day. Since sensitivity increases with time in childhood and adolescence, mineralocorticoid requirement drops to the range of 0.05–0.1 mg/day. If it is not adequately supplied in salt-wasting form, it leads to hypovolemia, hyponatremia, and hyperkalemia. Plasma renin activity should be measured at regular intervals. Excessive mineralocorticoid and salt administration may cause hypertension and congestive heart failure, as well.

For emergencies, families must have injectable hydrocortisone. It is important for all patients to carry an emergency treatment ID card. In the long-term follow-up of patients, complications such as short stature, learning disorders (especially in salt-wasting type), polycystic ovary syndrome, menstrual irregularities, problems with sexual life, and infertility in adulthood are important problems. In girls of reproductive age, the probability of normal pregnancy increases if serum progesterone is lower than 0.6 ng/ml (2 nmol/L) in the follicular phase [28].

Additional Treatment Regimen to Glucocorticoids

Antiandrogens can be used with hydrocortisone when insufficient suppression of adrenal androgens is observed. In particular, flutamide, a nonsteroidal anti-androgen, has been demonstrated to suppress androgens sufficiently at a dose of 10 mg/kg/day (max 500 mg) [29]. There is no study about the effectiveness of other antiandrogens in children. Spironolactone is also contraindicated in children as an antiandrogen.

Aromatase inhibitors slow down bone maturation and keep epiphyses open longer by reducing estrogen synthesis. Their positive effects on height have been shown when combined with androgen receptor antagonists [30]. However, routine use is controversial due to the short follow-up time of cases in literature and lack of controlled studies.

11-Beta Hydroxylase (CYP11B1) Deficiency

11-Beta Hydroxylase (CYP11B1) gene is localized on 8q22 chromosome and its many mutations have been identified. This enzyme is expressed in the zona fasciculate and converts 11-deoxycortisol to cortisol. Its deficiency constitutes 5% of cases with CAH. The disorder has been seen in 1:100.000 in Caucasians and

1:6000–7000 in Jews [31]. The clinical picture consists of glucocorticoid deficiency, androgen excess, and hypertension. Either the degree of virilization or hypertension show phenotypic heterogeneity even in the same family. Reduction in 11-hydroxylation activity decreases corticosterone and cortisol secretion. Increased ACTH stimulates androgen synthesis. Typical laboratory findings are elevation in serum 11-deoxycortisol (DOC) and decrease in serum PRA. Hypertension and hypokalemia are explained by the increased production of metabolites such as 11-deoxycortisol and 11-deoxycorticosterone [32]. However, hypertension does not always correlate with serum DOC levels [32, 33]. In neonatal period, hypertension may usually not be detected due to the suppression of renin–angiotensin system and renal mineralocorticoid resistance. Hypertension occurs in the first few years of life in two-thirds of the cases, but there are cases reported with severe hypertension in the very first days of life and hypokalemia is also common. In addition, 17-OHP, androstenedione, and testosterone levels may be high. While heterozygotes generally give normal responses to ACTH stimulation, serum 11-deoxycortisol, and 11-deoxycorticosterone levels may be elevated [34].

Female patients who suffer from hirsutism and menstrual irregularities may have non-classical 11-beta hydroxylase deficiency. However, it is quite rare. Only 40% of women with elevated DOC levels and hyperandrogenemia have been reported to express partial activity of this enzyme [35]. In the late-onset form of this enzyme deficiency, hypertension does not usually occur.

Diagnosis

High levels of basal or corticotropin-induced 11-deoxycortisol and deoxycorticosterone levels are diagnostic. In affected individuals, these metabolites are at least three times above 95th percentile for age [36]. Serum 11-deoxycortisol level is generally over 7 ng/ml. Increased metabolites lead to water and salt retention and consequently suppression of plasma renin. Progressive virilization, acceleration in somatic development, and bone maturation occur in both sexes. In cases, when there is no mutation in 21-hydroxylase gene, 11-B-hydroxylase deficiency should be sought first. More than 50 mutations have been identified in the *CYP11B1* gene to date [8]. Genotype and phenotype relationships have not yet been demonstrated. Though difficult, prenatal diagnosis is possible.

Treatment

Treatment of CYP11B1 deficiency is similar to that of simple virilizing form of CYP21 deficiency. Virilization is prevented with glucocorticoid replacement by suppressing corticotropin release and androgen production. During this suppression, since DOC release is also suppressed, hypertension improves. However,

patients with persistent hypertension may additionally require antihypertensive therapy. Treatment follow-up is performed with serum DOC, 17-OHP, and PRA levels, as well as adjustments made according to clinical findings. Surgical treatment approach is the same in cases with atypical genitalia in both enzyme deficiencies.

Gender Determination and Surgical Treatment

Gender determination has to be decided for each case by discussion of benefits and drawbacks of each option in short and long terms. Gender identity must also be supervised by specialists if the child's age is appropriate. Irreversible corrective surgical approaches should be delayed whenever possible, considering the risk of future rejection of assigned gender in ambivalent cases. The decision should be explained to the family, and an informed consent form should be signed.

Affected virilized females may exhibit some masculine patterns of behavior, but difficulties such as inability to accept their female roles or gender identity problems are rare. ESPE 2002 consensus proposes to assign a female gender, even if the virilization degree of the affected neonate is Prader V [37].

The Aims of Surgical Intervention in Virilized Females

In general, the purpose of genital surgical treatment is to achieve functional genitalia, enable future fertility expectations, and avoid urinary tract infections and incontinence. Its aims in virilized females are to reconstruct the best female genital appearance, to enable satisfactory future sexual life, to increase the fertility chance, to prevent hematocolpos, and to reduce the risk of recurrent urinary tract infections. Clitoroplasty is recommended in the first year of life and vaginoplasty is at puberty [38]. However, the operation should not be rushed in mild and moderate clitoromegaly in order not to decrease clitoris sensitivity. Adequate medical treatment prevents the progression of virilization and even some regression of clitoromegaly may occur. In clitoroplasty, glans and dorsal neural band should be preserved. It is important to prevent loss of sensitivity in this region. It is recommended that corrective surgery in the female direction should not be performed until the adolescent period, recurrent genital examinations, and vaginal dilatation should be minimized until puberty, and operations should be performed in experienced surgical centers (>3–4 cases per year) [38]. Surgery can be performed at an early age in the presence of vagina in low confluence, but the time of operation is controversial in the presence of vagina in high confluence. Although the risk of stenosis is high in delayed operations, dilatation is disturbing during infancy and childhood. However, there is no evidence-based guideline about ideal surgery time, need for additional surgery, optimal surgical technique, and surgical side effects.

In cases that have been raised as males because of late diagnosis, psychological sex may have been shaped in male direction, and this is a major problem. However, today, the preference of the child and approach of family and the social environment are recommended being taken into consideration. Long-term follow-up results of these cases are devoid of data.

The Effects of Contemporary CAH Treatment in Childhood on Future Reproductive Life

Studies with adult patients show that fertility is reduced, especially in females with classical simple virilizing 21-OHd CAH [39]. However, there are also recent studies that report higher infertility rates in women with salt-wasting forms [28]. Excess adrenal steroids due to poor hormonal control have been proposed to be the most prominent reason. Interfering factors for low rates of fertility may be listed as less sexual activity, impaired anatomy due to unsuccessful surgery and drug incompliance. Since it is not clear during which time period the high androgens are responsible for infertility, treatment, and follow-up of these patients from infancy to the end of puberty should be supervised closely. Glucocorticoids have been used in treatment since 1950s in classical CAH in pediatric groups, and hydrocortisone has been used more widely in 1990s instead of potent glucocorticoids. The circadian rhythm has been started to be imitated since 2000s. Although there are different dosage applications, in large series of randomized controlled studies, superiority of any one scheme has not been shown. To date, in pediatric group, treatment has always focused on growth. The goal has been to suppress androgens, as otherwise the final height will be affected due to rapidly advanced bone age. The later the cases are diagnosed, the older their bone ages are.

Treatment occasionally leads to excess glucocorticoid exposure, which is an undesirable effect. Cushingoid appearance may develop as a result of overtreatment. It can negatively affect growth and fertility and can also result in hypertension, and metabolic syndrome. It is quite difficult to achieve the balance between over-treatment and under-treatment.

Fertility begins to gain importance with increasing expectations of a high quality of life. Since the vast majority of CAH cases have 21-OHd, their outcomes are better understood because of their long-term follow-up experiences. Salt-wasting patients are often diagnosed at an earlier age and spend longer periods of their childhood with hydrocortisone replacement therapy, thus their androgens are generally well-suppressed. However, there are many simple virilizing type CAH patients with late diagnoses, especially belonging to the old times before neonatal screening programs. It is not known how gonads in both sexes have been affected by high androgens during the untreated period. Once long-term follow-up results of these cases have been shared, the observations have shown that high androgens may be responsible for infertility, especially in girls. Thus, the aim of reducing high androgens has become central to patient management. Similarly, high androgen levels may lead to

testicular adrenal rest tumors (TARTs) formation in male cases and may be responsible for infertility. If early suppression of androgen levels to target range is important for reproductive life and fertility, neonatal screening programs will allow simple virilized cases to be diagnosed very early and to have appropriate therapy.

The recommended hydrocortisone doses (10–17 mg/m^2/day) in current guidelines are the dose recommendations based on growth outcomes [15, 40]. Pediatric endocrinologists do concentrate on the impact of glucocorticoid dose on growth, but not so much on which level of androgens are harmful, especially with respect to ovarian effects. The androgen-fertility relationship mostly becomes an important issue in adolescence. Treatment goals should be age-specific, especially in pubertal period, and both growth and gonadal functions should be considered. Excessive treatment should also be avoided, while androgens are suppressed. All these make treatment and follow-up quite difficult. Close monitoring is essential. It is also recommended to avoid over-suppressing serum 17-OHP levels which are used to evaluate treatment adequacy. At this point, instead of 17-OHP, androstenedione levels can be guiding [41]. Since adequate suppression in metabolites is sometimes possible only with high doses of glucocorticoids, it will be beneficial to make dose changes during follow-up, considering the patient's weight, metabolic health, menstrual pattern, and clinical findings of hyperandrogenism. High follicular phase progesterone has been shown to cause anovulation and primary amenorrhea by changing GnRH pulses despite adequate glucocorticoid therapy in some cases [42]. Therefore, it is recommended to keep progesterone <2 nmol/L in adolescent girls [28]. There is still no consensus on optimal treatment and follow-up methods of these cases. The growth potential losses in early childhood periods can be overlooked in currently used standard growth curves. They often detect growth delay at puberty. One of the future treatment goals should be to develop treatment models that concentrate on growth and maturation simultaneously.

Testicular adrenal rest tumors (TARTs) are the most common cause of infertility in male patients with CAH. The detection rate in the age range of 2–18 years varies between 21% and 28% and the probability increases with age [15]. They are localized in the rete testis. If they are diagnosed early, they can regress with glucocorticoid therapy. They seem to be early determinants of infertility. They cannot be palpated but can be shown by ultrasonography. They are usually smaller than two centimeters. Small rest tissues that cannot be shown may cause narrowing in seminiferous tubules, resulting in disruption of function of the seminiferous tubules and azoospermia. Inadequate glucocorticoid replacement therapy is the typical feature of the cases with TARTs. Apart from this, there are studies reporting that androgen excess in under-treated patients without TARTs may also be the cause of infertility. It has been reported that high androgens cause hypogonadotropic hypogonadism by suppressing the hypothalamic–pituitary–gonadal axis [43]. This results in small testicles, oligospermia, and subfertility. If the morning testosterone level is low in a postpubertal male patient with CAH, this should cause suspicion. The ratio of androstenedione/testosterone >2 can be used in pre-diagnosis [15].

Many studies conducted to date report that women with CAH are less sexually active and have fewer children [44, 45]. In addition to poor hormonal control,

unsuccessful anatomical and functional genital reconstruction (impaired clitoral structure, vaginal stenosis) may also be the cause of low fertility in these patients. Failure of surgery and vaginoplasty requiring surgical revision has been frequently reported in CAH. It is important that the clitoral nerves are not damaged whenever the corrective operation is performed whether early or late. Connection of the vagina with the perineum should be performed before the onset of menstruation, but not later than the beginning of puberty. In patients with mild virilization, surgical reconstruction should be postponed; on the other hand, early surgical correction should be recommended for girls with severe virilization with single aperture urogenital sinus. Surgery should be performed in experienced centers. Randomized controlled trials for the best age and best surgical method have not been conducted to date [37].

Apart from these, psychosocial factors can cause a decrease in fertility. Studies have been reported that girls with CAH exhibit more male-specific behaviors than their peers, but generally do not show gender identity confusion [46].

Prenatal androgen levels, age at diagnosis and at introduction of treatment, presence of psychological supportive procedures are very important points that impact on outcomes and are the principal determinants of childhood approach. Although pre- and perinatal hyperandrogenism is important, there is no evidence that it is the main determinant of sexual identity development.

Gender identity of classical CAH cases in girls with some exceptions is female, but the demonstration of any male typical tomboy behavior pattern is high. They usually prefer hopping-fighting games in juvenile play patterns, and they like to play with cars and weapons. Playmates are usually boys [47]. Data are variable in selection of sexual partners during adulthood. Homosexual and bisexual orientations are higher than normal society [48].

Treatment and follow-up are life-long in patients with classical CAH. With early diagnosis and appropriate postnatal therapy, the probability of normal life is gradually increasing. Pediatric endocrinologists should consider transition of patients to adult clinics as soon as they become young adults with a protocol. Otherwise, the cases may face with many problems such as adrenal crisis, TARTs in men, high or insufficient glucocorticoid doses. Multidisciplinary approach and follow-up should definitely be maintained in the adolescent period, as well. The recommended median age of transfer to adult clinic is 19 years (between 17 and 27 years) [49].

Evidence-based protocols for follow-up and treatment are required for childhood and adolescent CAH. If optimal approaches are not defined in childhood for restoration of fertility, it will not be possible to improve fertility and quality of life in adulthood.

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Diagnostic Challenges in Nonclassical Congenital Adrenal Hyperplasia

Héctor F. Escobar-Morreale and Manuel Luque-Ramírez

Introduction

Nonclassical congenital adrenal hyperplasia (NCAH) is a common autosomal recessive disorder that is caused by mutations in both alleles of the genes encoding 21-hydroxylase (*CYP21A2*), 11-β-hydroxylase (*CYP11B1*), or 3-β-hydroxysteroid dehydrogenase (*3βHSD2*) enzymes. Vast majority of subjects diagnosed with NCAH have mutations in both alleles of *CYP21A2* that result in a 30–50% reduction in the activity of 21-hydroxylase [1, 2]. Genetically confirmed nonclassical forms of 11-β-hydroxylase or 3-β-hydroxysteroid dehydrogenase deficiencies are extremely rare [1]. Hence, this chapter will focus on the challenges associated with the diagnosis of 21-hydroxylase deficient (21-OHd) NCAH.

Reasons to Diagnose NCAH

The clinical spectrum of NCAH is particularly wide, ranging from asymptomatic subjects—so-called cryptic forms frequently identified during family studies of affected subjects—to a variety of clinical presentations that may include growth acceleration or premature pubarche in children, adolescent and adult androgen excess, or even incidentally discovered adrenal lesions such as adrenal hyperplasia or

H. F. Escobar-Morreale (✉) · M. Luque-Ramírez
Diabetes, Obesity and Human Reproduction Research Group, Hospital Universitario Ramon y Cajal and Universidad de Alcalá and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) and Centro de Investigación Biomédica en Red Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain
e-mail: hector.escobarm@uah.es

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adenomas [1, 2]. Many of these patients do not require any treatment or may benefit better from symptomatic treatment with drugs used in other androgen excess disorders such as oral contraceptives or antiandrogens [1]. Because only a minority of patients requires treatment with glucocorticoids—i.e., children with advanced bone age—it is unclear that, from a clinical perspective, screening for NCAH is truly needed.

The most important reason to diagnose subjects with NCAH derives from the pathophysiology of the disorder. As stated above, NCAH only develops in subjects carrying mutations in both alleles of *CYP21A2* that, overall, reduce but do not suppress 21-hydroxylase activity markedly or totally (Table 1). This situation may occur in homozygotes for "mild" mutations associated with the NCAH phenotype, but most commonly appears in double heterozygotes for *CYP21A2* mutations in which one of the alleles suppresses 21-hydroxylase activity markedly, namely a "severe" allele [1, 3, 4]. In such a case, the NCAH phenotype depends on the residual enzymatic activity determined by the transcription of the mild allele carrying the less severe mutation, but possibility exists that a severe allele may be transmitted to the progeny and may cause classical CAH in newborn [5].

According to Wilson and Jungner criteria [6], a screening program is justified when "the condition sought should be an important health problem"—a newborn with CAH may even die in the immediate neonatal period if unrecognized, a possibility particularly important in boys in whom virilization of external genitalia does not raise the suspicion of CAH as usually happens in girls; "there should be an accepted treatment for patients with recognized disease"—gluco- and mineralocorticoid replacement therapy usually save these newborns; "facilities for diagnosis and treatment should be available"—diagnosis of NCAH can be done at any clinical practice with blood sampling facilities; "there should be a recognizable latent or early symptomatic stage"—genotyping of couples may be performed well-before conception of the progeny; "there should be a suitable test or examination"—serum 17-alpha-hydroxyprogesterone (17-OHP) measurements are available in most clinical settings nowadays; "the test should be acceptable to the population"—basal and ACTH-stimulated serum 17-OHP measurements and genotyping lack relevant side effects; "the natural history of the condition, including development from latent to declared disease, should be adequately understood"—the mechanisms and mode of the inheritance of 21-hydroxylase deficiency are clearly understood nowadays as to provide appropriate genetic counseling to parents; "there should be an agreed policy on whom to treat as patients"—evidence-based clinical guidelines for the management of NCAH [1] and classic CAH [7] are available; "the cost of case finding (including diagnosis) should be economically balanced in relation to possible expenditure on medical care as a whole"—even though 17-OHP assays and genotyping are not exactly cheap, the cost–benefit balance is favorable enough as to justify neonatal screening in many countries; and "case finding should be a continuing process and not a 'once and for all' project"—current clinical guidelines recommend screening for NCAH in very prevalent disorders such as the polycystic ovary syndrome (PCOS). Hence, in our opinion, detection of carriers of severe *CYP21A2* alleles, with the aim of providing adequate genetic counseling, fully justifies screening for NCAH even if no immediate consequences for the treatment of the individual patient may arise from such a diagnosis.

Table 1 *CYP21A2* mutations associated with non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency when present in homozygosis or double heterozygosity

Nucleotide changes[a]	Protein	Exon	Clinical phenotype	Activity in vitro[b] (%)
g.89C>T	p.P30L	1	NCAH/SV	60
g.140A>G	p.Y47C	1	NCAH	–
g.185A>T	p.H62L	1	NCAH	45
g.734A>G	p.H119R	3	NCAH	32
g.739A>C	p.K121Q	3	NCAH/SV	14
g.749G>A	p.R124H	3	NCAH	–
g.772C>T	p.R132C	3	NCAH	35
g.817T>C	p.C147R	3	NCAH/SV	–
g.929C>T	p.R149C	4	NCAH	36
g.929G>C	p.R149P	4	NCAH	23
g.981T>C	p.L166P	4	NCAH	–
g.987C>A	p.T168N	4	NCAH	–
g.996T>A	p.I171N	4	NCAH	–
g.1153T>A	p.I194N	5	NCAH	33
g.1343C>T	p.R224W	6	NCAH	52
g.1362T>C	p.I230T	6	NCAH	63
g.1380A>G	p.R233G	6	NCAH	8
g.1588T>C	p.V249A	7	NCAH	–
g.1683G>T	p.V281L	7	NCAH	50
g.1689A>C	p.M283L	7	NCAH	–
g.1689A>G	p.M283V	7	NCAH	16
g.1744C>A	p.S301Y	7	NCAH	–
g.1752G>A	p.V304M	7	NCAH	46
g.1981C>A	p.L317M	7	NCAH	–
g.1981C>G	p.L317V	7	NCAH	–
g.2012A>G	p.D322G	8	NCAH	18
g.2058G>A	p.R339H	8	NCAH	50
g.2063C>T	p.R341W	8	NCAH	5
g.2064G>C	p.R341P	8	NCAH/SV	0.7
g.2138C>T	p.R366C	8	NCAH	37
g.2147C>T	p.R369W	8	NCAH/SV	46
g.2286C>G	p.N387K	9	NCAH	–
g.2296G>A	p.A391T	9	NCAH	38
g.2344G>A	p.D407N	9	NCAH	73
g.2512G>A	p.E431K	10	NCAH	–
g.2524C>T	p.R435C	10	NCAH	–
g.2578C>T	p.P453S		NCAH	50–68
g.2597C>A	p.P459H	10	NCAH	–
g.2640G>T	p.M473I	10	NCAH	85

(continued)

Table 1 (continued)

Nucleotide changes[a]	Protein	Exon	Clinical phenotype	Activity in vitro[b] (%)
g.2657G>T	p.R479L	10	NCAH	–
g.2665C>T	p.P482S	10	NCAH	70
g.2669G>C	p.R483P	10	NCAH	–
g.2669G>A	p.R483Q	10	NCAH	1.1

[a]Numbering starts from A in the initiation codon
[b]Using 17-OHP as substrate
NCAH non-classic congenital adrenal hyperplasia, *SV* simple virilizing classic congenital adrenal hyperplasia
Updated from http://www.cypalleles.ki.se/cyp21.htm (last accessed Nov 1, 2016) and [3]. Reproduced from Carmina et al. [1]. Copyright Oxford University Press 2017, with permission

Indications for the Screening of NCAH

As stated above, NCAH may remain asymptomatic to the extent that as many as 90% of affected subjects may remain undiagnosed according to recent estimations [2]. Most patients with "cryptic" NCAH are diagnosed within family studies of newborns with CAH identified by neonatal screening programs, further supporting that CAH is frequent in the progeny of parents with NCAH [5]. Moreover, retrospective analysis of mothers of CAH children diagnosed with "cryptic" undiagnosed NCAH in family studies have detected symptoms attributable to androgen excess in many of them—including menstrual irregularity, hirsutism, acne, and alopecia [8]—highlighting the importance of diagnosing NCAH before pregnancy and delivery even in women with mild hyperandrogenic symptoms. Less frequently, asymptomatic NCAH is diagnosed after genotyping of relatives of patients with NCAH [1].

The symptoms and signs that should drive the screening for NCAH vary as a function of sex and age [1]. In prepubertal girls and boys, premature pubarche, accelerated growth, and early onset of adult apocrine odor should prompt for NCAH testing, particularly because hydrocortisone administration may be beneficial for the control of these symptoms in some of these children [1, 2]. Male patients remain mostly asymptomatic throughout adolescence and adulthood—testicular adrenal rest tumors are quite uncommon in NCAH patients—except for the occasional diagnoses being made during the study of adrenal incidentalomas [9]. The latter may also apply to female patients [9], even though symptoms and signs of androgen excess, including asymptomatic hyperandrogenemia, remain the main indications for NCAH screening in adolescent and adult women, even after menopause [1]. A recent meta-analysis established a 4.2% (95% confidence interval 3.2–5.4%) prevalence of NCAH in series of adolescent and adult women with signs and symptoms of androgen excess [1].

Current Challenges with Diagnostic Methods and Assays

Serum 17-OHP Concentrations

17-OHP being the main substrate for 21-hydroxylase enzyme, its increased circulating concentrations are the most widely used biochemical marker of 21-hydroxylase deficiency. The increase in 17-OHP concentrations in patients with NCAH is, however, much smaller compared with that observed in patients with classic CAH, explaining why most NCAH cases are missed by neonatal screening programs [1]. As a consequence, the diagnosis of NCAH is usually made later in life.

The assay used to measure circulating 17-OHP is of paramount importance for the diagnosis of NCAH. While high-throughput liquid chromatography–mass spectrometry (LC/MS) assays have become a reality in clinical practice during the last decade in some countries, 17-OHP concentrations have been measured—and still are in most clinical practices worldwide—by immunoassays.

In humans, 17-OHP circulates in nanomolar ranges and these very low concentrations, together with the relatively poor immunogenicity of steroid hormones, and the marked structural similarities that characterize adrenal and gonadal steroid hormones and precursors, explain the certain limitations in terms of sensitivity and cross-reactivity of immunoassays when measuring steroid hormones and precursors such as 17-OHP [10]. Nowadays, LC/MS methods, with their superior sensitivity and specificity, are considered the gold standard for steroid hormones in clinical and research settings, yet the majority of these measurements are still carried out using immunoassays for practical issues such as technical ease and cost and availability of commercial reagents [10].

Aside from the major challenge of making LC/MS widely available for the measurement of 17-OHP, one of the most important unsolved issues nowadays is the urgent need for a reappraisal of circulating 17-OHP cut-off concentrations used for the diagnosis of NCAH. Most consensus documents and guidelines [1, 7] establish 2 ng/ml (6 nmol/l) as the basal cut-off 17-OHP level. When higher levels are obtained early in the morning, and during the follicular phase of the menstrual cycle, they should prompt suspicion for NCAH. Such a suspicion needs confirmation by performing a standard 250 μg iv cosyntropin test that diagnoses NCAH when 17-OHP concentrations are above 10 ng/ml (30 nmol/l) after stimulation [1, 7, 11] (Fig. 1).

Even though other cut-off values have been proposed over time—in our experience using a commercial non-extracted radioimmunoassay, basal 17-OHP value should be decreased to 1.7 ng/ml (5.1 nmol/l) for 100% sensitivity [4]—the values mentioned above have been mostly derived from the data obtained by using high-quality radioimmunoassays that applied pre-analytical solvent extraction of samples to avoid cross-reactivity with other steroid molecules and have never been confirmed either for nonextracted radio-, enzyme linked-, and fluorescent-based immunoassays or, particularly, for LC/MS assays.

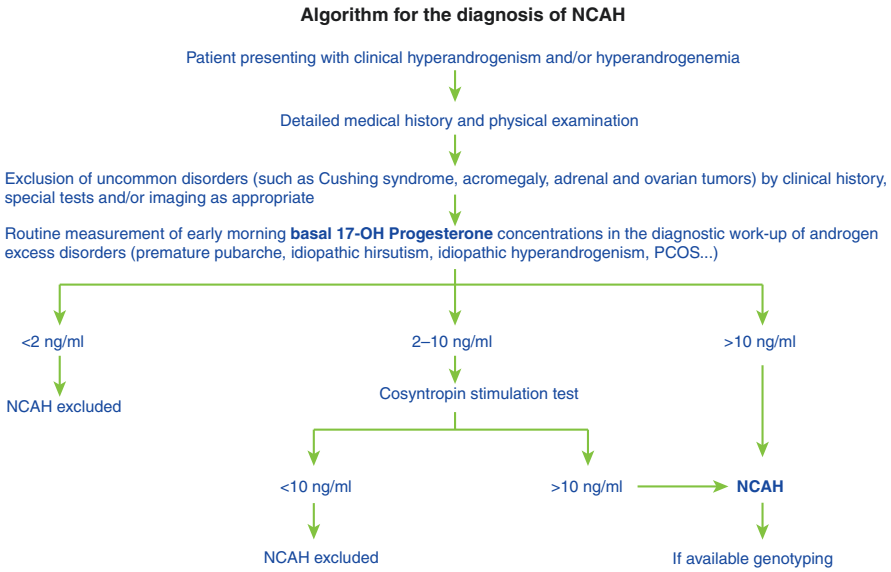

Fig. 1 Algorithm for the diagnosis of NCAH. Reproduced from Carmina et al. [1]. Copyright Oxford University Press 2017, with permission

Few available data strongly suggest that LC/MS derived 17-OHP concentrations are much lower than those obtained using immunoassays [12] and that, for the diagnosis of NCAH, the cut-off value of cosyntropin-stimulated 17-OHP concentrations using LC/MS should be decreased to 3 ng/ml (9 nmol/l) [12], a striking reduction if compared with 10 ng/ml (30 nmol/l) cut-off value recommended nowadays [1, 7, 11].

Molecular Genetic Analysis of CYP21A2

Genotyping of CYP21A2 is recommended to confirm NCAH diagnosis and, more importantly, to provide adequate genetic counseling to carriers of severe alleles [1]. The *CYP21A2* locus is quite complicated, and because one allele can carry multiple mutations, merely identifying two *CYP21A2* mutations in the absence of biochemical evidence does not permit establishing a diagnosis of NCAH. Also, restricting *CYP21A2* genotyping to the 10 or 12 most frequent *CYP21A2* mutations is no longer a valid approach, considering the very large number of different mutations associated with both CAH and NCAH nowadays [1]. Furthermore, the possibility of complex *CYP21A2* alleles carrying more than one mutation highlights the need of confirming that mutations are on opposite alleles in some cases, an issue that may require discriminating the specific maternal and paternal mutations by genotyping the parents [1]. Multiple genetic testing strategies such as PCR-based mutation detection methods, DNA sequencing, and multiplex ligation-dependent probe

amplification may be needed to accurately ascertain the *CYP21A2* genotype [1], but this approach is not always available in clinical practice. Hopefully, external quality assessment schemes such as that recently implemented by the European Molecular Genetics Quality Network in Europe [13] will improve the accuracy, interpretation, and standardization of the reporting of *CYP21A2* genotyping results.

Adrenal Steroid Profiles

Progressive implementation of LC/MS assays for steroid hormones and precursors currently facilitates the study of steroid profiles as accurate markers of adrenal disorders, including 21-hydroxylase deficiency [14, 15]. Panels of circulating steroids that, aside from 17-OHP, include among other steroids metabolites such as 21-deoxycortisol, 16α-hydroxyprogesterone, 11β-hydroxyprogesterone, and 21-deoxycorticosterone that are markedly altered in patients with 21-hydroxylase deficiency, have been proposed to provide better discrimination in the diagnosis than classic approaches [15–17]. To this regard, a very recent study indicates that a panel comprising 17-alpha-hydroxyprogesterone and 21-deoxycortisol concentrations—which are increased—and corticosterone concentrations—which are decreased—using basal unstimulated blood samples, has showed perfect discrimination between NCAH patients and controls [18]. If confirmed in larger studies, clinical implementation of LC/MS-based adrenal steroid profiling may represent an outstanding advance in the diagnosis of NCAH.

Conclusions

Nonclassical CAH due to 21-OHd is a relatively common disorder in children and adults. Clinical manifestations are quite variable, ranging from asymptomatic individuals to hyperandrogenic symptoms in women indistinguishable from those of PCOS. Even though the diagnosis of NCAH does not always result into specific changes in treatment or management, identification of these subjects is important because they frequently carry *CYP21A2* alleles that may cause classic CAH in the progeny. Nowadays, the diagnosis of CAH relies on the demonstration of a 17-OHP concentration above 10 ng/ml (30 nmol/l) either at basal conditions or after cosyntropin stimulation, with genotyping of *CYP21A2* serving as confirmation and being essential for genetic counseling and fertility planning. Hopefully, recent advances in LC/MS-based adrenal steroid profiling will improve and simplify the diagnosis of NCAH in the near future.

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Mechanisms of Reproductive Dysfunction in Classical and Nonclassical Congenital Adrenal Hyperplasia: From an Endocrinologist's Perspective

Inan Anaforoglu

Abbreviations

11B-OH	11-Beta-hydroxylase
11B-OHd	11-Beta-hydroxylase deficiency
17-OH	17-Alpha hydroxylase
17-OHd	17-Alpha-hydroxylase deficiency
17-OHP	17-Hydroxyprogesterone
17-Preg	17-Alpha-hydroxypregnenolone
21-OH	21-Hydroxylase
21-OHd	21-Hydroxylase deficiency
3-BHSD2	3-Beta-hydroxysteroid dehydrogenase type 2
3BHSD2d	3-Beta-hydroxysteroid dehydrogenase type 2 deficiency
ACTH	Adrenocorticotrophic hormone
AII	Angiotensin II
CAH	Congenital adrenal hyperplasia
CLCAH	Classical lipoid congenital adrenal hyperplasia
DHT	Dihydrotestosterone
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
HCG	Human chorionic gonadotropin
HPG	Hypothalamic–pituitary–gonadal
HPO	Hypothalamic–pituitary–ovarian
IVF	In vitro fertilization
LCAH	Lipoid congenital adrenal hyperplasia
LH	Luteinizing hormone

I. Anaforoglu (✉)
Department of Endocrinology and Metabolism, Mehmet Ali Aydınlar University Faculty of Medicine, İstanbul, Turkey

M. E. Ertorer (ed.), *Fertility and Reproductive Outcomes in Different Forms of Congenital Adrenal Hyperplasia*, https://doi.org/10.1007/978-3-030-82591-1_5

NADPH	Nicotinamide adenine dinucleotide phosphate
NCAH	Late onset or nonclassical CAH
NLCAH	Nonclassical lipoid congenital adrenal hyperplasia
PCOS	Polycystic ovary syndrome
POR	P450 oxidoreductase
StAR	Steroidogenic acute regulatory protein
SV	Simple virilizing

Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders arising from defective steroidogenesis caused by mutations of genes which encode enzymes of cortisol biosynthesis: 21-hydroxylase (21-OH), 11-beta-hydroxylase (11B-OH), 17-alpha-hydroxylase (17-OH), 3-beta-hydroxysteroid dehydrogenase type 2 (3-BHSD2), steroidogenic acute regulatory protein (StAR), P450 oxidoreductase (POR), P450 cholesterol side chain cleavage enzyme. Decreased cortisol production leads to increased levels of adrenocorticotrophic hormone (ACTH) which results in bilateral adrenal hyperplasia. There is an imbalance between glucocorticoids, mineralocorticoids and sex hormones because of blockage in pathways caused by enzyme deficiencies.

21-Hydroxylase Deficiency

The 21-OH deficiency (21-OHd) is the most common form accounting more than 90% of all CAH cases. There is a broad spectrum of clinical pictures ranging from salt-wasting (SW) to simple virilizing (SV) form, to milder forms according to the severity of mutations on 21-OH gene. The SW and SV forms are classified as classical CAH, while milder forms are named as late onset or nonclassical CAH (NCAH).

Defective 21-hydroxylation impairs glucocorticoid and mineralocorticoid synthesis resulting in increased androgen synthesis and diverts the glucocorticoid–mineralocorticoid pathways to excess androgen synthesis with accumulating precursors most notably 17-hydroxyprogesterone (17-OHP). Progesterone and 17-OHP levels proximal to 21-OH enzyme are elevated. Decreased level of cortisol leads to continuous stimulation of ACTH causing bilateral adrenal hyperplasia. There is increased androgen production by chronical ACTH stimulation, as there is no block in androgen synthesis pathways [1]. The 17-OHP is converted to 5-alpha-pregnane-3-alpha-17alpha-diol-20-one (pdiol) which is then converted to dihydrotestosterone (DHT) by 5-alpha-reductase and 3-alpha-reductase activities of AKR1C2/4 in the alternative pathway. Increased levels of DHT have been thought to contribute to prenatal virilization of female fetuses with classical 21-OHd CAH [2]. Under normal circumstances, direct conversion of 17-OHP to androstenedione is at very low

levels in humans, and this alternative pathway becomes active to metabolize accumulated 17-OHP in 21-OHd. Other steroid hormones, 21-deoxycortisol and 16-alpha-hydroxyprogesterone, 11-ketotestosterone, 11-ketoandrostenedione, also accumulate because of 21-OHd [3, 4].

Affected females with 21-OHd NCAH do not have ambiguous genitalia and usually present later in life with signs of androgen excess or may remain asymptomatic lifelong. Presentation depends on the sex of the affected infant, as well. Nonclassical CAH with 21-OHd has a wide spectrum at the onset of clinical presentation. It is before the age of 10 years in 11% of the cases and between 10 and 40 years for the 80%. Girls with classical CAH, either SW or SV, are born generally with ambiguous genitalia of varying degrees. Accumulation of androgenic metabolites causes in utero virilization. The androgen receptors in genital skin which are activated by prenatal exposure of adrenal androgens promote clitoral enlargement, labial fusion, urogenital sinus septation. The clinical presentation may vary from minimal clitoromegaly, being the most common finding, to male appearance with penile urethra and bilateral undescended testes. Fused labia majora and single perineal orifice are also common findings among affected girls, accompanied with normal internal genitalia [5]. Although uterus can be visualized by ultrasound, the ovaries may not be detected because of their small size [6]. Development of external genitalia of affected boys is normal, except some subtle findings like hyperpigmentation of the scrotum and enlarged fallus. Although female infants are detected with genital ambiguity in early hours of life, males are usually detected with symptoms of adrenal insufficiency, vomiting, hypotension, hyperkalemia, and hyponatremia in the following days [1, 5].

Premature pubarche (the presence of pubic hair, axillary hair, or apocrine odor developing before 8 years in girls and 9 years in boys) may be the presenting sign both for girls and boys with NCAH or some SV types. Other presenting signs of NCAH include cystic acnes, accelerated growth, and advanced bone age. Hirsutism, amenorrhea, oligomenorrhea, chronic anovulation, and infertility are the most frequent symptoms for adult female patients with NCAH. There is scarce information in current medical literature about males with NCAH; they are usually detected through family studies. Adult males do not present with impaired gonadal function, they usually have normal sperm counts. Still they may have short stature, oligospermia, and decreased fertility due to adrenal androgen excess [1].

It may be difficult to distinguish women with 21-OHd NCAH and polycystic ovary syndrome (PCOS) as they share similar clinical features. Insulin resistance, hyperinsulinism, hirsutism, and polycystic ovarian morphology which are characteristics of PCOS are very common in patients with NCAH. Patients may also develop “a secondary PCOS” state [7, 8]. They share similar pathophysiologic mechanisms at the hypothalamic–pituitary–ovarian (HPO) axis. Basal 17-OHP and ACTH stimulation test, in case, are useful to discriminate between these two disorders. Genetic screening test may be needed in some cases [9].

During childhood, concerns focus on preventing adrenal crises or virilization. In adulthood, treatment focuses on metabolic abnormalities, preventing adverse effects of treatment and improving fertility.

Female Patients with 21-Hydroxylase Deficiency: Fertility and Pregnancy

There are some contradictory results about fertility rates of patients with classical CAH. In early studies, in 1987, Mulaikal and colleagues had reported substantially decreased fertility rates in classical CAH in their cohort of 80 female 21-OHd patients (38% with SV form and 2.5% with SW). Only half of the patients had been heterosexually active. Poor hormonal control with hirsutism had been common besides insufficient vaginal introitus in 35% of these patients [10]. Although Jääskeläinen and colleagues have reported decreased fertility rates for their cohort of 29 patients with classical CAH compared to general population (child rate; 0.34 vs 0.91; $p<0.001$), they have emphasized on better rates of fertility compared with previous studies [11]. They have reported 13 pregnancies in 9 women and 10 healthy live births, all females have harbored SV form of the disease. Six women have become pregnant after human chorionic gonadotropin (HCG) stimulation, seven have conceived spontaneously. Females with well-controlled adrenal androgen secretion and normal serum progesterone levels have had regular menses (5/16; 31%), whereas under-replaced women with high progesterone levels have experienced irregular menses (11/16; 69%). Neither of them has demonstrated PCOS [11]. Common outcomes of the studies about reproduction rates in women with classical CAH are their decreased sexual activity and reduced desire to conceive.

Fertility in patients with 21-OHd NCAH is also mildly reduced. According to a recent study among 190 NCAH women, 187 pregnancies have occurred in 85 women. Pregnancies have resulted in 141 births in 82 cases. The estimated infertility incidence has been 11% in patients with NCAH. Ninety-nine pregnancies (52.9%) have occurred before the diagnosis of NCAH; 96 pregnancies spontaneously and 3 with ovulation inducers, whereas 88 have occurred after diagnosis (11 spontaneously and 77 with hydrocortisone treatment). The rate of miscarriages has been 6.5% for pregnancies after glucocorticoid treatment versus 26.3% before glucocorticoid treatment [12]. Pregnancy rates have been detected to be between 65% and 95% among NCAH patients who seek for medical attention. It is difficult to estimate the exact fertility rates because most NCAH women are asymptomatic and can spontaneously conceive before NCAH diagnosis, so they do not need treatment [12–15].

Several factors are proposed to explain the reproductive dysfunction in women with 21-OHd. Excess adrenal steroid production in poorly controlled patients, anovulation, negative effects of genital surgery, and reduced heterosexual partnership are the most obvious ones.

Androgen and progesterone—which is also an androgen precursor—overproduction and prenatal exposure to sex steroids are suspected to interfere with the reproductive axis of the patients. The most important factor is the adrenal steroid excess especially in poorly controlled patients. Menstrual irregularities and anovulation affect a majority of women with classical CAH [16].

Follicular development is directly suppressed by excess adrenal androgens leading to compromised ovulation. They prevent embryo implantation by causing endometrial molecular alterations [17].

Bioactive androgens and progestins seem to modulate HPO axis function through different molecular mechanisms that have not been clearly defined. Gonadotropin releasing hormone (GnRH) is secreted in a pulsatile manner by hypothalamic GnRH neurons to regulate pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release. There are multiple neuroendocrine and environmental signals regulating the function of GnRH neurons. Progesterone has a critical role in regulating GnRH pulse frequency. Bachelot and colleagues have reported reduced LH pulse frequency and amplitude in poorly controlled anovulatory 21-OHd patients, whereas LH pulses have been found to be normal in well-controlled ones. Among poorly controlled patients, 17-OHP, testosterone, progesterone, androstenedione levels have been higher and FSH levels lower. The authors have suggested that hormonal control is a key factor and there is no neonatal programming of disrupted gonadotropic axis and women with classical CAH may have normal LH pulsatility with optimal glucocorticoid replacement, undertreatment may be responsible for hypogonadotropic hypogonadism [18]. However, even with optimal glucocorticoid replacement therapy, high progesterone levels have also been detected in follicular phase of 21-OHd CAH women with oligomenorrhea and infertility [19]. It may be necessary to increase the glucocorticoid doses to higher levels to achieve pregnancy.

Excess androgens may disrupt HPO axis via many other ways. High levels of androgen may impair the GnRH/LH pulse generator and women with CAH can experience dysregulated HPO axis function resembling PCOS [19, 20]. Increased GnRH pulse frequency increases the frequency and amplitude of LH over FSH production. Increased LH/FSH leads to high levels of androgen secretion by ovarian theca cells and polycystic ovarian morphology as in PCOS in some of the patients. Exposure to in utero androgen excess has been proposed to alter the imprinting of neuroendocrine mechanisms that control kisspeptin and GnRH neurons [21]. Similarities between PCOS and CAH, oligomenorrhea associated with chronic anovulation, clinical or biochemical evidence of hyperandrogenism, polycystic ovarian morphology, make one think about the possibility of a shared mechanism in dysregulation of HPO axis. Demonstration of polycystic changes in ovaries of female to male transgender individuals with testosterone treatment is another example [22]. Thus, either as a result of treatment or endogenously as happens in CAH, elevated androgens may cause polycystic ovarian morphology [23].

Increased adrenal progesterone production affects fertility via impeding ovulation and implantation by altering GnRH pulsatility and interfering with endometrial development [24, 25]. Besides changing the GnRH pulse, increased concentrations of progesterone also disrupt endometrial thickening and ovulation, resulting in unfavorable cervical mucus, embryo implantation, and sperm migration, thereby acting as a form of contraception. Continuous high levels of progesterone may adversely affect both the quality of oocytes and implantation [26].

Excess androgens are aromatized to estrogens, this may also suppress HPO axis, leading to anovulation and irregular menstrual cycles [27]. Androgens also act

through the androgen receptors which are expressed in theca cells, granulosa cells, and oocytes [28]. They affect follicular growth during different stages of development. Normally, androgens promote initial growth of small antral follicles, whereas hyperandrogenism may cause follicular arrest and failure in selection of a dominant follicle. Androgens also induce stromal hyperplasia and rigidity by stimulating the extracellular matrix [26, 28].

Adrenal rest tissue is relatively common in men with CAH; however, ovarian adrenal rests have been uncommonly reported among women [29].

Other underlying reasons for reduced fertility in females with CAH may be higher rates of homosexuality and unsatisfactory intercourse due to inadequate vaginal introitus resulting from unsuccessful genital reconstructive surgery. Girls with CAH have more interest in terms of sports, toys, and play behavior in a masculine manner. Most women have clear female sex identification and gender dysphoria is rare despite masculinized behavior [30, 31]. Although the exact frequency is not clear, up to 25% of women with CAH report homosexual interests or orientation. There is an increased rate of bisexual and homosexual orientation compared with the general population. There is a low frequency of desire to get married and perform a traditional child-care role among these women [32]. In utero exposure to high levels of androgens is proposed to affect gender-related behavior. The severity of mutation has been found to relate with gender-atypical behavior [33] (Table 1).

Women with classical CAH may have additional problems; in utero exposure to excess androgen levels affect the development of external genitalia, resulting in urogenital sinus, labial fusion, varying degrees of clitoral hypertrophy making sexual intercourse uncomfortable. Additional issues related to sexual activity are length of vaginal introitus, lack of lubrication, pain with penetration, lack of clitoral sensitivity and anxieties about sexual performance, and genital appearance [1, 34]. Postsurgical complications or difficulties may also contribute to reduced fertility in

Table 1 Pathophysiological mechanisms of reproductive dysfunction in females with 21OHd CAH

- In utero virilisation due to accumulation of androgenic metabolites:
  - Clitoral enlargement, labial fusion, urogenital sinus septation
- Tonic oversecretion of androgens aromatized to estrogens resulting in:
  - Loss of gonadotropin cyclicity—Augmentation of pituitary sensitivity to LHRH and increase LH release-mimicking PCOS—Anovulation or dysovulation
- Ovarian hyperandrogenism with secondary PCOS
- Increased adrenal androgen production; suppression of follicular development (a negative effect on aromatase activity in granulosa cells?)
- Inhibitory effect of increased adrenal progesterone production:
  - Alteration of the rhythm and amplitude of GnRH pulses
  - Interference with ovulation, endometrial development, implantation, poor nidation capacity
  - Diminished sperm-tubal motility and thickening of cervical mucus
- Increased homosexual interests or orientation
- Reduced desire to conceive
- Decreased sexual activity and unsatisfactory intercourse due to inadequate vaginal introitus resulting from unsuccessful genital reconstructive surgery

women with classical CAH. Surgical procedures include clitoroplasty, vaginoplasty, and labiaplasty for opening the vaginal introitus, bringing the urethral meatus closer to the perineum, reducing the size of enlarged clitoris, allowing for menstrual flow, enabling tampon use and vaginal intercourse, and preventing urinary tract infections. In the past, clitoroplasty often with vaginoplasty had been performed regularly in early childhood. The second surgical operation had been performed to correct vaginal stenosis in adolescents then. Studies have reported urinary incontinence, vaginal stenosis, inadequate vaginal opening, inadequate introitus, and painful intercourse in up to 50% of these patients, probably contributing to lower sexual activity. Loss of sensitivity after clitoroplasty contributing to sexual dissatisfaction have been common. For these reasons, surgical procedures for patients with classical CAH have evolved in time bringing new techniques and surgical approaches such as preserving innervation and clitoral sensation. Timing of the surgery is still a matter of debate. The performance of current surgical approaches cannot be evaluated in a short time as it will take time to emerge. There are few studies in literature with different surgical procedures with low number of patients included, thus conflicting results make comparison impossible [35, 36].

Male Patients with 21-Hydroxylase Deficiency

Fertility issues are not limited to women, men are also affected. Recent studies have shown reduced fecundity and fertility rates in men with classical CAH [16, 37]. In a study (CaHase Study) consisting of 65 adult men with classical CAH, 37% of patients have attempted for fertility and 67% of them have been successful, being lower than the general population [16]. The largest series to date from French has included 219 men, the rate of having a child has been lower than normal French population (51% vs. 79%, respectively) [37]. Unfortunately there are very limited data about the fertility of nonclassical patients.

Men face a dual problem in means of fertility. Elevated adrenal steroid production especially androgen and progesterone can lead to hypogonadotropic hypogonadism by interfering with FSH and LH secretion. Testicular adrenal rest tumors (TARTs) being the main culprit in reduced fertility are common in men with CAH. Hypogonadism and TARTs can lead to oligospermia together.

During intrauterine development, adrenal glands are located very close to gonadal structures and adrenocortical tissue may even adhere to gonads. Testicular and ovarian descensus along the course of their supplying arteries occur before the separation of these two tissues and appearance of adrenal niche. Thus, adrenal tissue may descend with the gonads and may form an ectopic adrenal tissue within the gonads. This aberrant adrenal gland then may give rise to TARTs in men with CAH. It seems unlikely for a male to develop TARTs, if he has no adrenal rest cell within his gonads. These tumors are ACTH-dependent. They respond to persistently elevated plasma ACTH levels and may regress in response to glucocorticoid therapy [38]. There must be factors other than ACTH that contribute to TARTs formation, as these tumors may

be detected even in CAH males with normal or suppressed plasma ACTH levels. m-RNA expression of adrenal specific enzymes CYP11B1 and CYP11B2, and besides ACTH receptors, angiotensin II (AII) receptors have also been shown in testicular tumors of CAH patients with quantitative PCR [39]. This indicates that TARTs growth in CAH cases may be stimulated not only by ACTH but also by high AII levels in SW patients with poor hormonal control. Benvenga and colleagues have shown LH receptors on TARTs tissue which may explain the increasing TARTs frequency in pubertal period. Besides high ACTH concentrations due to poor hormonal control, high pubertal LH levels may also aggravate TARTs [40].

They resemble Leydig cell tumors with features consistent with steroid secreting cells on electron microscopy. Although, in many patients, the diagnosis of CAH is made before the detection of TARTs, they can be the first manifestation of the disorder. Young age at presentation, bilateral tumors, and shrinkage with glucocorticoid therapy should make the physician consider about CAH instead of Leydig cell tumors. These tumors are always benign and usually bilateral. They are located within rete testis, thus can cause mechanical obstruction of the seminiferous tubules. Only tumors larger than 2 cm are detectable by palpation because of their location. Preservation of gonadal functions is associated with tumor size.

These lesions are classified into five different developmental stages (Fig. 1):

Stage 1 Adrenal rest cells are within the rete testis, but not detectable by scrotal ultrasonography (US).

Stage 2 Adrenal rest cells may be visible by scrotal US as small hypoechogenic lesions.

Cumulative exposure to ACTH (and AII) and the number of their receptors on adrenal rest cells are the main determinants of timing of onset of cell growth.

Stage 3 Compression of rete testis occurs due to the growth of adrenal rest tissue. Oligo- or azoospermia may be found in pubertal or postpubertal CAH patients due to the obstruction of seminiferous tubules. Fibrous strands can be visible as hyperechogenic lesions at US. Low inhibin B and elevated FSH and LH levels may also be observed as signs of gonadal dysfunction. This stage of TARTs is still responsive to high doses of glucocorticoids.

Stage 4 Progressive obstruction of rete testis takes place due to hypertrophy and hyperplasia of adrenal rest cells. Induction of fibrosis and focal lymphocytic infiltration occur. Small tumors located within the rete testis form lobulated structures separated from the residual testicular tissue by fibrous bands. High doses of glucocorticoids are usually no longer effective in decreasing tumor size at this stage. The ACTH and AII dependency of these tumors are lost in time with dedifferentiation of the adrenal rest cells. Peritubular fibrosis indicating early testicular damage can be found in the surrounding testicular tissue.

Stage 5 Irreversible damage of testicular parenchyma due to chronic obstruction occurs.

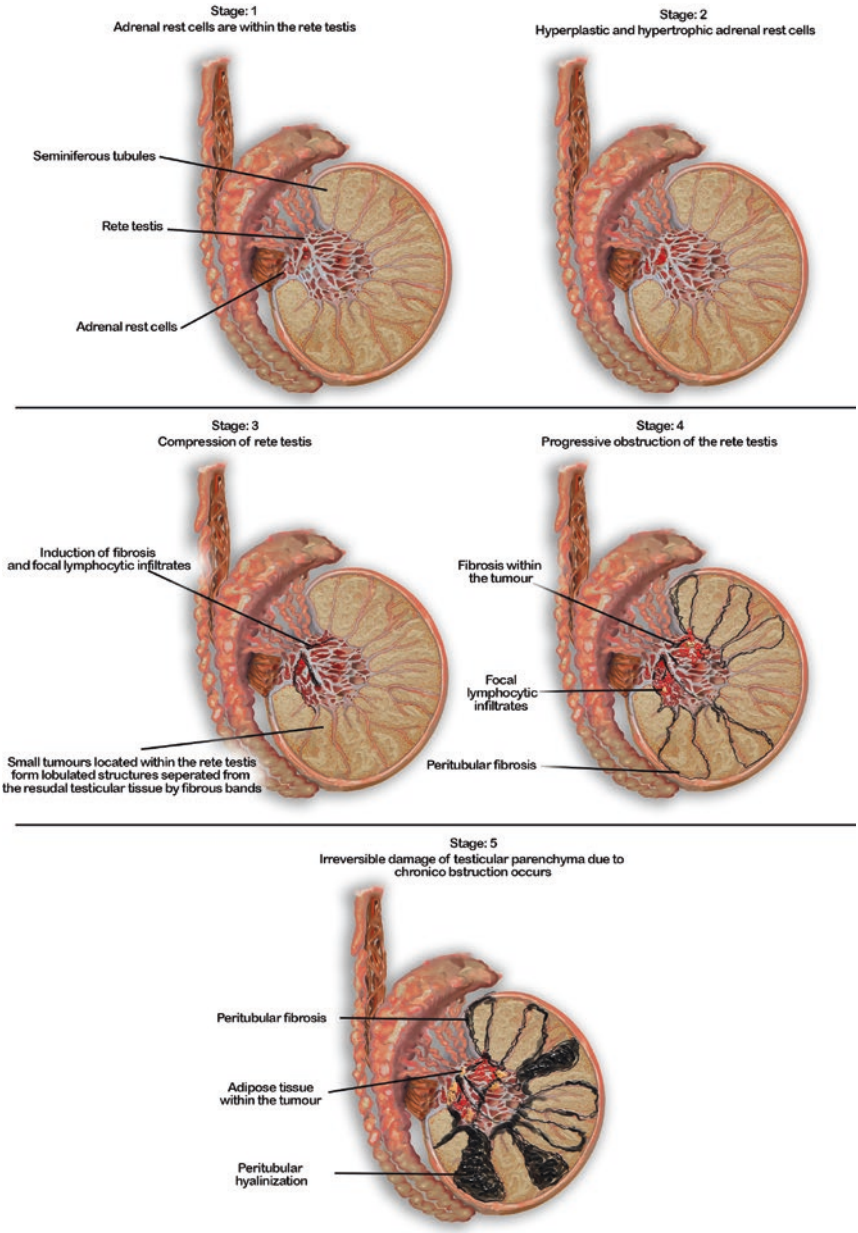

Fig. 1 Histological demonstration of different developmental stages of testicular adrenal rest tumors (TARTs) (The illustration has been drawn by Eren Arik, graphic designer)

Glucocorticoids are the treatment of choice in TARTs for preserving gonadal functions. Intensifying glucocorticoid therapy may result in ACTH suppression leading to reduction in TARTs size and improved testicular function in Stages 2 and 3 [39, 41].

Case reports of infertile male CAH patients with TARTs treated with high doses of glucocorticoids have been published. Although successful pregnancies have been reported, failure of this treatment and serious side effects after long-standing therapy has been observed, as well. In addition to glucocorticoid treatment, mineralocorticoid supplementation has to be performed in order to suppress AII-induced tumor growth [39, 42, 43].

The TARTs are not only anatomical lesions, they can cause functional impairment of the testes. Low levels of plasma testosterone and poor semen quality can be found in affected males. They may interfere with the function of normal testicular tissue directly via mechanical compression or by impairing local steroid production. Besides, high levels of adrenal androgens that are aromatized to estrogens peripherally or in the central nervous system, may suppress gonadotropin secretion, resulting in hypogonadotropic hypogonadism. However, high FSH levels may also be found, indicating a severe primary testicular problem. Semen analysis should be performed, as normal serum FSH levels do not indicate normal semen production. Azoospermia accompanied by a large testicular tumor located in the mediastinum of testis at US most probably points to a mechanical problem. Cases with heterozygous or homozygous for deletion or conversion of the *CYP21* gene are at the highest risk for developing large TARTs, as these mutations are associated with complete loss of enzymatic activity. Early detection and treatment of TARTs should be aimed, especially in these patients.

Extratesticular obstruction is the common cause of obstructive azoospermia as a complication of infections or surgery and is mostly located at the epididymis or vas deferens. Sperm cells are localized in the compensatory dilated and enlarged epididymis, and phagocytized spermatozoa are resorbed then. However, TARTs are localized near to the mediastinum of testis and cause compression of seminiferous tubules, leading to obstructive azoospermia and irreversible damage of the surrounding testicular tissue. Thus, efferent flow in the seminiferous tubules is chronically obstructed by large TARTs residing in the mediastinum of testis proximal to the epididymis without epididymal dilatation. In a study examining the testicular biopsies of seven 21-OHd CAH patients with long-lasting bilateral TARTs, decreased tubular diameter, varying degrees of peritubular fibrosis and tubular hyalinization accompanied with severe decrease in the number of germ cells have been detected. It has been proposed that large TARTs residing proximal to the epididymis may cause chronical obstruction in efferent flow in seminiferous tubules without the usual compensatory dilatation of the epididymis. Long-lasting obstruction of seminiferous tubules may result in hypospermatogenesis and peritubular fibrosis. Additionally, steroid hormones produced by TARTs may be toxic to Leydig cells and germ cells. Tubular hyalinization caused by massive deposition of collagen fibers inside the seminiferous tubules and complete loss of germ and Sertoli cells are the characteristic features of TARTs in end stage. The intertitium of the testes

are observed to contain normal or slightly reduced number of Leydig cells which is contrary to ischemic hyalinization. Therefore, TARTs may represent a very specific cause of obstructive azoospermia [38, 41, 44, 45]. In a surgical study evaluating the gonadal functions of CAH males with longstanding TARTs at stage 5, testis-sparing surgery has failed to improve gonadal functions. Persistence of gonadal dysfunction in these cases has indicated irreversible damage of the surrounding testicular tissue. However, the damage caused by the surgery itself cannot be excluded. The authors of that study have concluded that the relief of pain and discomfort caused by TARTs should be the only indication for surgery. Testicular biopsies are advised to be performed before surgery, in order to evaluate the surrounding testicular parenchyma [46]. Normal testicular parenchyma has been detected in testicular biopsy of a male with CAH and bilateral TARTs, taken at some distance from the tumor during successful treatment of infertility with intracytoplasmic sperm injection. It can be proposed that tumor obstruction-induced testicular damage may start around TARTs with progressive involvement of surrounding parenchyma. This observation underlines the importance of size and duration of TARTs matter, as well as the location of these tumors [45].

Poor hormonal control with increased adrenal androgens and progesterone leads to increased levels of estrogen produced by aromatization, resulting in hypogonadotropic hypogonadism. Overtreatment with glucocorticoids may also cause an increase in body mass index that potentiates the suppression of hypothalamic–pituitary–gonadal (HPG) axis. Obesity is an independent risk factor for reduced fertility via abnormalities of semen parameters in otherwise healthy men. Aromatization of androgens to estrogens in adipose tissue leads to dysregulation of HPG axis and decreased semen quality [34, 47]. Metabolic syndrome among patients with CAH has been found to be related to high glucocorticoid doses, as well as with lower fertility and fecundity rates [16].

11-Beta-Hydroxylase Deficiency

11-Beta-hydroxylase deficiency (11B-OHd) is the second most prevalent CAH form characterized by mutations in *CYP11B1* gene. Due to the enzymatic defect at the level of *CYP11B1*, decreased conversion of 11-deoxycortisol to cortisol and 11-deoxycorticosterone (DOC) to corticosterone takes place in zona fasciculata. This pathway is under the control of ACTH. Accumulation of hormone products above the blockage breaks the negative feedback control system, and the resultant high ACTH levels divert the precursors into intact pathways. This determines the hormonal profile of 11B-OHd which is characterized by elevated androgens as in 21-OHd, in contrast accompanied with high mineralocorticoid precursors. Decreased plasma cortisol with elevated plasma levels of DOC, 11-deoxycortisol, and androgens are present [48]. Augmented ACTH-driven corticosterone excess which takes over the cortisol's place as a glucocorticoid prevents adrenal insufficiency in contrast to 21-OHd.

Hyperandrogenemia may result in precocious puberty in both sexes. Premature adrenarche and hirsutism are common presentations of nonclassical forms of 11B-OHd (11B-OHd NCAH) in children. Low renin hypertension is usually absent in 11B-OHd NCAH at young ages, but hypertension develops with aging, and does not correlate with the severity of mutation. Females with classical 11B-OHd CAH forms present with ambiguous genitalia, virilization, and low renin hypertension. Those with nonclassical forms are born with normal genitalia, and in childhood, they may present with signs and symptoms of hyperandrogenism, while during adulthood, women may exhibit with acne, hirsutism, menstrual disturbances, and infertility [49–51].

Precocious puberty and TARTs can be observed in males [49–52]. Gynecomastia has been reported up to 28.6% [52]. The pathophysiology of gynecomastia in 11B-OHd is not clear. It has been attributed to the anti-androgenic effects of DOC and mineralocorticoids which bind to androgen receptors and aromatization of adrenal androstenedione to estrogen [53, 54]. The TARTs are frequently (up to 94%) present in adolescent and adult males with 11B-OHd, as in 21-OHd CAH males, and are usually accompanied with impaired spermatogenesis and Leydig cell failure [55].

It is difficult to define a clear distinction between classical and nonclassical 11B-OHd forms, because the disorder hormonally presents a continuum as in 21-OHd. Nonclassical 11B-OHd is also characterized by reduced cortisol synthesis accompanied with elevated adrenal androgens. Premature pubarche, acne, hirsutism or menstrual irregularity, and polycystic ovaries may be the features of affected girls in the absence of genital ambiguity. The phenotype of 11B-OHd NCAH is clinically indistinguishable from 21-OHd NCAH; therefore, its real prevalence may be underestimated. Recently, two new mutations of 11B-OHd NCAH have been added to eight known mutations, in which arterial hypertension does not occur often [56, 57].

There may be no close correlation between phenotype and genotype in classical and nonclassical 11B-OHd forms. Significant variations in the severity of hypertension, degree of virilization, and plasma levels of 11-deoxycorticosterone and 11-deoxycortisol can be observed even among same mutation carriers. Baş F and colleagues have also recently shown no definite correlation between specific mutations and clinical or laboratory findings at diagnosis in their study on 28 cases from 25 unrelated families with classical 11B-OHd (14 46,XY males, 14 46,XX females) [52]. This may cause difficulty at distinguishing 21-OHd from 11B-OHd, when hypertension is not present.

Despite varying degrees of virilization of external genitalia, internal genital structures are normal for a female in classical 11B-OHd. It is not an exceptional occasion to misassign such a female as a male at birth. 46,XX infants with high Prader scores can mistakenly be diagnosed as male. There are some case reports in which these patients continued to be boys even after the late diagnosis of 11B-OHd, and internal genital organs have had to be removed [58–60]. Females with 11B-OHd are more virilized than those with 21-OHd. However, the extent of masculinization correlates poorly with the accompanying hyperandrogenemia [49].

Lessons learned about reproductive dysfunction in 11B-OHd in both sexes are mostly driven from the publications about 21-OHd. Mechanisms of reproductive

dysfunction in females with 11B-OHd share many common features with 21-OHd. Adrenal overproduction of androgens and progestins resulting in hypogonadotropic hypogonadism, ovarian hyperandrogenism causing a PCOS-like picture, ovarian adrenal rest tumors (rarely), genital reconstructive surgery, and psychological factors (disturbed psychosexual development, reduced interest in sexual activity and loss of strong maternal feelings) are the contributing factors.

Pregnancies in genetically confirmed 11B-OHd women are rarely reported [61].

17-Alpha-Hydroxylase and 17,20-Lyase Deficiency

17-Alpha hydroxylase (17-OH) deficiency (17-OHd) represents <1% of all cases of CAH and is characterized by low renin aldosterone hypertension, hypokalemia, and impaired production of sex hormones. The CYP17A1 enzyme catalyzes both 17-OH and 17,20-lyase activities in adrenals and gonads. Hypergonadotropic hypogonadism and absence of pubertal development are detected in both genetic sexes. Male pseudohermaphroditism and primary or rarely secondary amenorrhea in 46,XX patients are the characteristic features of complete 17-OHd [62].

Complete deficiency of CYP17A1 is characterized by androgen and estrogen deficiency with mineralocorticoid excess. Neither androgens nor estrogens are produced from the gonads in complete 17-OHd. Lack of formation of 17-hydroxysteroid substrates for 17,20-lyase reaction and accompanying poor or absent 17,20-lyase activity result in impaired androgen production. Estrogens are derived from aromatization of androgens via the aromatase enzyme (CYP19A1). Accordingly, all cases with complete17-OHd exhibit sexual infantilism at birth. They cannot develop secondary sexual characteristics later on. 46,XX cases with 17-OHd have internal Mullerian structures with streak gonads. Due to defective androgen production resulting in undervirilization, 46,XY karyotypes exhibit a blind vaginal pouch. The patients are recognized due to hypokalemia, hypertension, or delayed puberty, and the disorder usually remains undiagnosed until adolescence or early adulthood [62].

Mild 17-OHd may present with irregular menses and subfertility in females and low-normal testosterone levels with slightly elevated gonadotropins and possibly oligospermia in males. Partial 17-OHd cases can produce small amounts of sex hormones. In females, hypertension and hypokalemia are generally accompanied with amenorrhea and some secondary sexual characteristics. The 17-OHd CAH females are usually thought to be anovulatory; however, there are affected females who have had spontaneous menarche with cyclic menses [63]. Males exhibit incomplete masculinization signs, such as hypospadias with bifid scrotum or micropenis [62]. Isolated 17,20-lyase (desmolase) deficiency has first been described in boys with disorder of sexual development (DSD) and in some of their sisters. It is characterized by sex steroid deficiency resulting in absent or disturbed pubertal development in both genders and 46,XY (DSD), male undermasculinization. Normal cortisol levels have been found to accompany with the low basal and cosyntropin-stimulated DHEA levels in these boys. Their androstenedione, testosterone, and

DHT have remained low after HCG stimulation, as well [64]. Simsek and colleagues have reported a 13.5-year-old girl with isolated 17,20-lyase (desmolase) deficiency presented with lack of pubertal development, primary amenorrhea, and growth retardation. She has exhibited ovarian cysts on ultrasonography. High gonadotropins cause ovarian cysts in these cases, pointing to the fact that estrogens are required not only for inducing puberty but also for suppressing ovarian cystic changes [65]. Purest form of isolated 17,20-lyase deficiency is characterized by the mutations in cytochrome b5 (*CYB5A*) gene which encodes the allosteric activator b5. The latter enhances the 17,20-lyase activity ten-fold selectively by facilitating the interaction of CYP17A1 with its electron donor POR. However, 17,20-lyase activity of these cases reaches only about 10% of normal, and this is not sufficient to prevent 46,XY DSD. There is a selective decrease in DHEA and androstenedione biosynthesis, and in all of 17-OH activities of affected cases, cosyntropin-stimulated cortisol values are normal [64]. Hypergonadotropic hypogonadism and infertility are the characteristic features of CYP17A1 mutations, resulting in decreased enzymatic activity. Low levels of gonadal steroids are accompanied with impaired spermatogenesis and folliculogenesis. Ovarian pathology demonstrates arrested folliculogenesis with primary and secondary oocytes. Pathologic evaluation of testicular tissue of affected males exhibit arrested spermatogenesis and testicular atrophy with interstitial cell hyperplasia [63, 66]. Reproductive capacity of women affected by this form of CAH is limited, because of high serum progesterone levels, resulting in suppressed cellular proliferation of endometrium. Chronically elevated progesterone levels are blamed for irreversible immaturity of the endometrium. Moreover, gonadal function of homozygote mutation carriers is well preserved as young adults, but decreases with advancing age. Fertility of the affected women is proposed to be determined by the residual activity of 17-OH/17,20-lyase enzyme, age of the patients when glucocorticoid replacement is introduced for progesterone lowering, and types and doses of estrogen replacement for inducing cycles. Accordingly, the 17-OH/17,20-lyase enzyme-deficient case of Matsuzaki and colleagues, who has been introduced to glucocorticoids at the age of 26 years, has exhibited poor endometrial response on both histologic and US examinations following nine courses of continuous estrogen replacement. In spite of low serum levels of E2, success has been achieved in ovarian follicular development, although ovulation induction has repeatedly failed [67]. However, Ben-Nun and colleagues have succeeded in having a viable pregnancy in a woman with 17-OHd who has been first given glucocorticoids at the age of 16 years. Successful in vitro fertilization (IVF) has been reported, despite low intrafollicular E2 concentrations [68]. There are 17-OHd females in literature reported to have regular menstrual cycles, although mostly have ceased early. Miura and colleagues have reported four cases who have had regular menses, even one of them has had a prolonged menstrual bleeding episode requiring total hysterectomy. Her ovarian biopsy has revealed absence of corpora lutea and follicles [69]. Araki and colleagues have reported about the menstrual states of 15 cases with 17-OHd, one of whom has experienced regular menses [70]. Levran and colleagues, in their study on four infertile females with combined partial 17,20-lyase and 17-OHd, diagnosed by clinical and hormonal

profiles, have reported live birth of triplets after transfer of cryopreserved embryos. All cases have been phenotypically normal with sufficient secondary sexual features, normotensive, and normokalemic, but have had hypomenorrhea. They have presented with primary infertility, anovulation, and persistent cervical dysmucorrhea. They have had the story of multiple unsuccessful IVF cycles till the diagnosis of partial 17-OHd. Addition of dexamethasone for controlling progesterone production to standard IVF protocole has resulted in high fertilization and cleavage rates (50% and 65%, respectively). However, serum estrogen levels have remained low and progesterone levels have been high throughout the cycles despite aggressive therapy. Complete suppression of endogenous sex steroid production using GnRH analogs and dexamethasone prior to exogenous estrogen and progesterone administration, followed by the standard IVF procedure under gonadal and adrenal suppression mentioned above, has succeeded in a triplet pregnancy that has ended with three live births. This is the only case report of infertile 17-OHd cases resulted in successful live birth; however, the genetic mutation of the case has not been described in detail. The authors have recommended considering combined partial 17,20-lyase and 17-OHd in differential diagnosis of infertile women with cervical dysmucorrhea accompanied with normal uterine cervix and regular menstruation [71]. Hypoplastic uteri are common in 17-OHd females and contribute to uterine dysfunction resulting in impaired fertility [70, 72].

Male Patients with 17-Alpha-Hydroxylase and 17,20-Lyase Deficiency

Poor gonadal androgen production contributes to arrested spermatogenesis and infertility in males. There is no report of fertility in males with 17-OHd, but Araki and colleagues have reported normal testosterone production in three of 22 males with 17-OHd [70].

3-Beta-Hydroxysteroid Dehydrogenase Type 2 Deficiency

There are two types of 3-beta-hydroxysteroid dehydrogenase (3-BHSD) enzymes encoded by two similar genes. The 3-BHSD1 is expressed in placenta and multiple peripheral tissues, including skin and mammary glands, and 3-BHSD2 is expressed in adrenals and gonads. In all reported cases of 3-BHSD deficiency (3-BHSDd), the 3-BHSD2 enzyme is affected, as 3-BHSD deficiency type 1 would result in spontaneous loss of pregnancies in first-trimester because of the disruption of placental progesterone biosynthesis. The 3-BHSD2 enzyme is necessary for the synthesis of mineralocorticoids, glucocorticoids and androgen precursors in adrenals, and for the synthesis of testosterone in gonads. In children, 3-BHSD2 expression is low both in ovary and testis. Increasing gonadotropins during puberty cause increased

expression of both 3-BHSD2 and 3-BHSD1. Majority of the androgens in both genders, about 40% in men, and most of the estrogens in children and in anovulatory or postmenopausal women arise from extragonadal steroidogenesis [73, 74].

There is sexual dimorphism in the expression of 3-BHSD2 enzyme in fetal gonads; therefore, sexual development and reproductive functions are affected differently in males and females. Deficiency of 3-BHD2 constitutes less than 0.5% of all CAH patients. Severe deficiency impairs steroidogenesis both in adrenals and gonads, resulting in SW in both sexes and incomplete masculinization of the external genitalia in males and mild virilization in females [73, 74]. Labia majora and/or clitoris enlargement are mild virilization signs, whereas displacement of the ureteral orifice, as happens in other CAH types, is a severe sign.

Androgens, testosterone, and DHT production is required for normal development of external genitalia of a 46,XY fetus. The critical period of male sexual differentiation is between the eight and twelfth weeks of gestation. Androgens are required for penile development including the urethra, and fusion of labial-scrotal folds during that time. Leydig cells of fetal testes start to express 3-BHSD2 beginning from the eighth week of gestation till the end of the pregnancy. Testosterone production from Leydig cells is impaired in 3-BHSD2d and DHT production is reduced by classical and backdoor pathways. The lesser production of androgens explains the undervirilization of external genitalia in affected 46,XY individuals. Depending on severity of the mutation, the spectrum of incomplete masculinization may vary from severe hypospadias, micropenis, bifid scrotum, undescended testis to complete feminization of external genitalia. In females, the critical period of sexual differentiation is also between eight and twelfth weeks of gestation. Due to the DHEA accumulation and conversion to androgens by the normal 3-BHSD1, females with 3-BHSD2d exhibit normal or mildly virilized external genitalia. Even severe androgen exposure can only lead to mild clitoral enlargement in females after that period. Estrogen is derived almost exclusively from the placenta during fetal life. Ovaries do not contribute to estrogen biosynthesis until puberty and remain quiescent throughout fetal life and childhood. 3-BHSD2 expression begins after 28 weeks of gestation in fetal ovary. Thus, 3-BHSD2d does not cause severe DSD in 46,XX individuals [75].

Similar to 21-OHd, the classical presentation of 3-BHSD2d is with salt-wasting and adrenal crisis, high-renin hypotension, and hypoglycemia. Impaired steroid synthesis in gonads and adrenal glands, and increased DHEA concentrations, elevated ratios of Δ5-steroids over Δ4-steroids are the main characteristics of the disorder. The typical steroid profile of 3-BHSD2d may be misdiagnosed as 21-OHd at birth, due to the high levels of 17-OHP. This is due to the conversion of accumulating Δ5 steroids from adrenals by the intact peripheral or placental 3-BHSD1. Mineralocorticoid and glucocorticoid replacement suppresses the hypothalamo–pituitary–adrenal axis (HPA) during infancy and childhood. However, androgen precursors (DHEA and androstenediol) are secreted from the testis at puberty under the stimulation of gonadotropins. These products serve as substrates for testosterone and estrogen production [76]. In adult males with 3-BHSD2d, high amounts of androgen precursors (DHEA and androstenediol) are converted to androstenedione

and testosterone in peripheral tissues by 3-BHSD1. Then HSD17B1, HSD17B5, and CYP19A1 enzymes catalyze the conversion of those products to estrogens, resulting in gynecomastia. Testosterone replacement therapy causes regression of gynecomastia via activation of negative feedback suppression of gonadotropins [76, 77].

There is a broad spectrum of clinical presentation of 3-BHSD2d depending on the severity of the genetic lesion, from severe SW form in neonates to mild menstrual disorders in elder females. It is unlikely that a genital atypia of mild virilization may result in sexual misassignment of a female infant with 3-BHDS2d. Premature pubarche, hirsutism, and menstrual irregularities, including oligomenorrhea and primary amenorrhea may be the symptoms of non-SW phenotype diagnosed at pre- or post-pubertal ages among girls [75]. Most females with 3-BHSD2d CAH show progressive feminization at appropriate age with menstruation. In contrast, Zachmann and colleagues have reported a girl with severe 3-BHSD2 mutation who has had no spontaneous breast development at the age of 14.7 years. She has required gonadotropin injections and estrogen treatment to develop full feminization after unsuccessful treatment with glucocorticoid replacement. However, her menstrual cycles have ceased following the withdrawal of estrogen and progesterone replacement treatment, then she has developed ovarian cysts [78]. Spontaneous pubertal development has been reported in many males with 3-BHSD2d. This is attributed to peripheral conversion of DHEAS to testosterone by intact 3-BHSD1 and HSD17B5 activities [79].

Gonadal dysfunction, azoospermia, arrested spermatogenesis, gynecomastia, and infertility have also been reported previously in adults with 3-BHSD2d. There are only a few papers about long-term follow-up of 3-BHSD2d, most of them with good therapeutic responses, for instance, restoration of menstruation in females [75–78]. In their testicular biopsy materials performed on a 15-year-old male with 3-BHSD2d, Burckhardt and colleagues have reported few germ cells in both testes (about 5–10% tubules in a cross-section) and no spermatogenesis. Any immature fetal gonocytes or premalignant germ cells have been observed pointing to low malignancy risk [76]. There are affected male cases having normal testicular histology and spermatogenesis in literature, as well. There is a complex relationship between genotype and gonadal phenotype in severe 3-BHSD2d. Thus, it is difficult to perform predictions about fertility. Alos and colleagues have reported two severe 3-BHSD2d cases, one of whom has been a 46,XY boy born with ambiguous genitalia. However, he has been reported to undergo normal masculinization at puberty, but has still found to be azoospermic in his adult ages. The other case has been a 46,XX patient who has admitted with acute adrenal failure as an infant. She has presented premature adrenarche starting at the age of 4 years, breast development, and menarche with regular menses that have occurred at 10.2 years [80]. Guran and colleagues, in their study, on a large group of genetically proven 3-BHSD2d with classical form, described the genotype–phenotype interaction of the disorder. There have been 31 patients with homozygous 3-BHSD2 mutations from 24 families (12 female and 19 male patients). All of them have been diagnosed before 2 years of age, mostly in the newborn period, with adrenal insufficiency with or without

ambiguous genitalia. All of the males have exhibited undermasculinization findings regardless of homozygous pathogenic changes of 3-BHSD2, even one of them has been born with female genitalia. Only one female has exhibited mild cliteromegaly as a sign of virilization. The authors have concluded that 3-BHSD2d rarely causes ambiguous genitalia in females, even in the presence of severely impaired 3-BHSD2 activity. In the same study, it has been reported that 11 patients (five girls and six boys) have had a history of premature pubarche regardless of severity of the mutation. Two of those five girls then have had central precocious puberty, and one of them has exhibited irregular menses and PCOS later on. The TARTs have been observed in two brothers. This is probably due to stimulation of adrenal rests by elevated ACTH, but the prevalence of TARTs in 3-BHSD2 deficiency is unknown as there is limited number of patients reported [77].

Recommendations for the follow-up of cases with 21-OHd are valid for the ones with 3-BHSD2d. Many children with premature pubarche, and females with hirsutism and menstrual irregularities who have exhibited exaggerated Δ5-steroid production after ACTH stimulation test and elevated 17-OHP to cortisol ratios have been proposed to have nonclassical 3-BHSD2d, which is proposed to be mild and late-onset. However, genetic studies have failed to detect any mutation in *3-BHSD2* gene in these patients [81–83]. This has been attributed to the presence of either a PCOS-related disorder or an unidentified intraadrenal 3-BHSD over-activity.

P450 Oxidoreductase Deficiency

P450 oxidoreductase deficiency (PORd) is a recently defined, the most complex and rare form of CAH. The enzyme POR is an important electron donor from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to all microsomal P450 cytochrome (CYP) enzymes including CYP51A1 (lanosterol-alpha-14-demethylase) and squalene monooxigenase that are involved in cholesterologenesis, 17-OH (CYP17A1), 21-hydroxylase (CYP21A2), and P450 aromatase (CYP19A1) which are involved in steroidogenesis [84]. P450 aromatase is responsible for the conversion of androgens to estrogens. As POR supports the activities of a wide variety of enzymes, PORd has a broad range of clinical phenotypes. It also affects nonendocrine systems, such as skeletal development and drug metabolism. Varying degrees of DSD in both sexes that are accompanied with skeletal malformations, glucocorticoid deficiency, and maternal virilization during pregnancy can be observed [85]. There has been no PORd patient carrying null mutations on both alleles reported, indicating that such a genotype is not compatible with life.

The majority of reported patients with PORd have Antley–Bixler syndrome phenotype which is characterized by craniosynostosis, radioulnar or radiohumeral synostosis, bowed femur and some other variable skeletal disorders. Milder POR mutations are not accompanied with Antley–Bixler syndrome phenotype, but present with hypogonadism and/or infertility [86, 87]. The common Japanese mutation

R457H, either as homozygotes or heterozygotes, typically causes the most severe skeletal findings and virilization of the mother during pregnancy, suggesting fetoplacental aromatase deficiency. It has been shown to associate with virilization of 46,XX patients, and most likely normal development of male genitalia [88–91]. However, skeletons of cases carrying the common European mutation A287P are less affected, and their mothers are less severely virilized during pregnancy, pointing to spared fetoplacental aromatase activity. It may be concluded that the R457H mutation severely affects aromatase activity, whereas A287P does not. The residual enzymatic activities are found to be higher in cases affected by the European mutation. Krone and colleagues, in their study performed on 30 PORd patients, have nicely showed that severe malformations are associated with major loss-of-function mutations on one of the affected alleles, but mild to moderate malformation phenotypes are caused by homozygosity or compound heterozygosity for missense mutations [92]. There is a wide clinical spectrum of PORd which may be explained by differential inhibition of various POR-dependent enzymes by various mutations. Inhibition of CYP17A1 results in decreased androgen production and explains undermasculinization of the males with such PORd. However, 46,XX babies are born virilized without postnatal progression of virilization. This is due to DHT production via the activation of alternative "backdoor pathway" secondary to CYP21A2 deficiency which is active only during prenatal period. The majority of reported 46,XX cases have shown different Prader stages below IV and have had female sex assignment. The activity of the alternative pathway mentioned above declines, leading to sex steroid deficiency in both sexes [93].

The POR deficiency can cause ambiguous genitalia in both sexes. Undermasculinization of 46,XY males is typical due to low 17,20-lyase activity resulting in reduced androgen synthesis. 46,XX females are frequently virilized at birth; however, this virilization is not progressive postnatally, as it happens in untreated girls with 21-hydroxylase deficiency [94, 95].

Loss of aromatase activity due to inactivating CYP19A1 mutations in fetus presents with maternal virilization due to placental aromatase deficiency and virilization of the female fetus. Maternal virilization is reversible and resolves at the postpartum period [96]. There is limited number of reports about pubertal development of PORd cases. Idkowiak and colleagues have reported seven cases with PORd (five females and two males) who have had absent or incomplete pubertal development. All of them have had either normal or low adrenal androgen levels. Five of them have exhibited gross skeletal deformities at birth. Significant pubertal impairment and ovarian cysts have been the characteristic presentation of the females. Four of five girls have had primary amenorrhea with elevated gonadotropins. Ovarian cysts have resolved in two females with the introduction of glucocorticoids and GnRH agonists followed by estrogen/progestin therapy. One female patient has had ovarian cyst rupture despite the appropriate therapy mentioned above. Male cases have had slightly delayed pubertal development with testicular volumes appropriate for age. Their testosterone levels have been within normal limits in the presence of mildly elevated gonadotrophins, pointing to

compensated hypogonadism. This finding may indicate that sex steroid production in the testicles is less dependent on fully functional POR than ovaries or adrenals during puberty [97]. Fukami and colleagues have reported similar findings [89].

Primary hypogonadism resulting in excessive LH-mediated ovarian stimulation is obviously the main underlying pathology of PORd in females. High gonadotropins resulting from estrogen deficiency due to the mutant POR may impair the steroid synthesis and metabolism [91]. Additionally, disrupted meiosis-activating sterols in follicular fluid may be a contributing factor for ovarian cyst development. These may explain the resistance of these cysts to medical therapy, requiring long-acting glucocorticoids in addition to sex steroid replacement for controlling excess LH secretion [91]. During puberty, females can present with delayed development of sexual characteristics, significant hypergonadotropic hypogonadism and large ovarian cysts with a tendency to torsion [97]. In addition to glucocorticoid replacement therapy which is determined in accordance with cortisol response to stress, females may require estrogen replacement during puberty. Estrogen patches are recommended in order to avoid hepatic first-pass metabolism. Since CYP3A4 metabolizes estrogens and glucocorticoids, it is likely that individuals with POR mutations may have a reduced hepatic clearance of these hormones, thus may have higher circulating hormone levels than expected [98]. The POR-dependent enzyme CYP51A1 is highly expressed in human gonads. It has been shown to be upregulated by gonadotropins, pointing to its critical impact on oocyte maturation at puberty [99]. Accordingly, its inhibition results in oocyte arrest in vitro [100]. Low androgen levels are detected in infants and children with PORd. However, circulating androgen levels have been found to be normal in pubertal PORd males. This finding can be attributed to the upregulation of cytochrome B5 (CYB5) which is normally expressed in human adrenal and testicular tissues pre- and postnatally. This enzyme acts as an allosteric facilitator of POR and CYP17A1, thus facilitating CYP17A1 17,20-lyase activity. Its expression starts in adrenal zona reticularis with adrenarche. It can start 17,20-lyase activity significantly in testicular Leydig cells and increase androgen production during adrenarche and puberty despite partially impaired POR function [91].

Phenotypically mildly affected patients with infertility have also been reported in literature. Sahakitrungruang and colleagues have reported an 18-year-old female diagnosed to have PORd during the evaluation of primary amenorrhea. She has exhibited normal basal gonadotropins, but has had abnormal ACTH-stimulated cortisol, 17-OHP, progesterone, and androstenedione levels. This case has indicated that there may be mild forms of PORd that may remain undiagnosed. Atypical genitalia may require surgical intervention. However, information about the clinical course of the disease in adulthood and the long-term consequences for fertility remain unknown. In PORd females, hypergonadotropic hypogonadism causing polycystic ovaries in infancy or childhood may be the presenting complaint in adulthood. The ovarian cysts are associated with hypoandrogenemia which is in contrast to classical PCOS phenotype [91].

Lipoid Congenital Adrenal Hyperplasia

Steroidogenic acute regulatory protein (StAR) facilitates cholesterol transfer from outer mitochondrial membrane to the inner membrane of adrenal and gonadal steroidogenic cells, where it becomes substrate for cholesterol side chain cleavage enzyme P450scc. This is the first step of steroidogenesis. It mediates the rapid actions of ACTH and AII on adrenals and of LH on the gonads, thus resulting in rapid rises in circulating concentrations of steroids in response to acute physiological stimuli [101]. Lipoid congenital adrenal hyperplasia (LCAH) is an autosomal recessive disorder caused by biallelic loss-of-function mutations in StAR gene, resulting in disrupted conversion of cholesterol to pregnenolone [102].

There are classical and nonclassical types of LCAH. Null StAR mutations cause severe impairment in adrenal and gonadal steroidogenesis resulting in classical LCAH (CLCAH). The clinical picture is characterized by primary adrenal insufficiency of early onset within the very first few days of life and female appearing external genitalia regardless of genotype [103]. Gonadal failure appears much earlier in males than females. This gender dimorphism is attributed to low StAR activity of ovaries during fetal and prepubertal period compared to the high activity of fetal testes. Ovarian quiescence delays the accumulation of cholesterol in the cytoplasm and subsequent ovarian cellular damage. This is a reasonable explanation for the inability of 46,XY males with LCAH to develop normal male appearing external genitalia and spontaneous puberty. Human chorionic gonadotropin stimulation during early pregnancy also facilitates the cholesterol accumulation and cellular damage to the testes [104, 105].

46,XX female cases with LCAH exhibit spontaneous pubertal development and menarche. Impaired residual steroidogenic capacity causes follicular atresia due to the accumulation of ovarian cholesterol esters following menarche. This inevitably results in irregular anovulatory menstruation and premature menopause. Histopathological examination of the ovaries of postpubertal LCAH girls demonstrates infiltration of macrophages in stroma and lipoid deposits both in theca cells and macrophages [106].

Peripubertal 46,XX cases with LCAH have been shown to have high gonadotropin levels, predominantly LH, with the formation of large ovarian cysts, causing ovarian torsion, even rupture in classical cases. Shima and colleagues have described three Japanese 46,XX patients with StAR mutations who subsequently have developed anovulatory cycles and bilateral ovarian cysts. Increased sensitivity of anterior pituitary to secrete more LH in response to low circulating estrogen levels in LCAH cases has been proposed. This results in high LH/FSH ratios. The relatively normal FSH levels of the cases are explained by inhibin-induced suppression of FSH produced by the existing follicles in ovaries that are not yet affected by StAR deficiency. This is valid until there is significant ovarian cellular damage [106]. There is growing evidence that ovarian cystic formation in females with LCAH is because of chronic anovulation. Women with polycystic ovaries demonstrate polycystic ovaries, high LH:FSH ratio. But they differ from LCAH females with their elevated androgen levels [106, 107].

Nonclassical LCAH (NCLAH) cases usually present with primary adrenal insufficiency, and later in infancy or childhood, mineralocorticoid deficiency may or may not accompany. This is due to their partially spared residual StAR activity which enables some steroidogenic capacity. Males may have normal or underdeveloped external genitalia due to some testosterone-producing capacity. Accordingly, Baker and colleagues have reported three NLCAH cases; all have had primary adrenal insufficiency on admission. The two 46,XY males have exhibited normal appearing male external genitalia with descended testes [105]. There are 46,XX patients with LCAH who feminize spontaneously in adolescence. This finding may be attributed to the pubertal arousal of ovaries that are silent since fetal life by trophic stimulation [108, 109]. Classical LCAH 46,XX cases inevitably have difficulties in achieving and maintaining pregnancy. They cannot ovulate as they cannot produce enough estrogen to induce LH surge. They cannot maintain pregnancy even though they can succeed in conceiving, because of their inability to produce sufficient progesterone. Corpus luteum produces mainly progesterone until the seventh or eighth weeks of gestation, then placenta takes the progesterone-producing task. Progesterone-producing capacity of the corpus luteum in early stages of pregnancy is not sufficient for the maintenance of pregnancy in classical LCAH females. There is limited number of pregnancies in CLCAH in current literature that conception has been achieved with clomiphene therapy or in vitro fertilization and maintained with progesterone replacement therapy during the early stage of the pregnancies [110–113]. There is scarce information about the ovarian functions and pregnancy outcomes of NLCAH cases. However, considering about the residual hormonal activity, the clinical picture is supposed to be lighter. Accordingly, Hatabu and colleagues have reported four Japanese women with NLCAH that have presented with overt primary adrenal insufficiency in early childhood. All of them have had spontaneous menarche and have had enough estrogen-producing capacity to provoke LH surge and probably to induce ovulation. Two of them have had successful pregnancies (one spontaneously and the other with clomiphene induction) and delivery without early progesterone replacement therapy. Despite the accompanying primary adrenal insufficiency that has started in early childhood, all four of them have exhibited normal estrogen and progesterone production at young adulthood. It has not been still clarified why primary adrenal insufficiency starts long before ovarian failure in NLCAH. It may be attributed to the varying degrees of contribution of StAR-dependent steroidogenic pathways to total steroidogenic capacity. Time-dependent accumulation of cholesterol esters in ovaries resulting in ovarian failure may be another reasonable explanation [114].

Conclusion

Congenital adrenal hyperplasia is still among one of the most challenging endocrine disorders due to its rarity and heterogenous clinical spectrum. Restoration of normal linear growth and puberty are the treatment goals in children, whereas management of regular menses, prevention of hyperandrogenic signs, and preservation of fertility are aimed for adolescents and adult cases.

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Reproductive Dysfunction in Classical and Nonclassical Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

Enrico Carmina

Introduction

Patients with congenital adrenal hyperplasia (CAH) both with severe (classical CAH) and mild (nonclassical NCAH) forms have alterations in reproductive function including reduced fertility and low number of live births. However, there are important differences between these two groups of patients.

In this short review, only the alterations in reproductive function of patients with 21-hydroxylase deficiency (21-OHd) will be discussed because they represent almost all of the patients in reproductive clinical settings.

Reproductive Function in Classical 21-OHd CAH Patients

In girls with classical 21-OHd CAH, the age of puberty onset is often earlier than in general population, and in some patients, precocious puberty may be present [1]. Its prevalence is not well determined but mainly depends on incomplete suppression of adrenal androgen production. However, in affected males, the ages at pubertal onset and pubarche are usually similar to that observed in general population [1].

Menstrual irregularities (oligomenorrhea, primary or secondary amenorrhea) are common in women with classical 21-OHd CAH and generally result from overproduction of adrenal androgens including 11-oxygenated C-19 androgens and progestins and rarely from ovarian adrenal rest tumors [2, 3]. Often, secondary PCOS is observed with increased ovarian androgen production and anovulatory cycles.

E. Carmina (✉)
Department of Health Sciences, Child and Mother Care, and General and Specialist Medicine, University of Palermo, Palermo, Italy
e-mail: enrico.carmina@ae-society.org

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Infertility in classical 21-OHd CAH women is common and depends not only on anovulation but also on increased circulating progesterone concentrations during the follicular phase which impact on endometrial receptivity, tubal motility, and cervical thickness [1–4].

In males, primary gonadal failure due to testicular adrenal rest tumors (TARTs) may be observed [5], and regular evaluation for TARTs using ultrasonography is recommended from the start of puberty or even earlier when poor hormonal control is present [1–3]. When TARTs are present, counseling about cryopreservation of semen should be offered [6]. Secondary gonadal failure due to poor hormonal control is relatively uncommon in males with classical 21-OHd CAH [1–3].

In all patients with classical 21-OHd CAH, optimal hormonal control is a key factor for adequate gonadal function. Therefore, regular measurements of adrenal and gonadal steroids and/or their metabolites should be performed, and normalization of these parameters is essential for avoiding or improving gonadal dysfunction and impaired fertility. Administration of sufficient substitution dose of glucocorticoids may lead to spontaneous pregnancy, but in some patients, because of the association with polycystic ovary syndrome (PCOS), induction of ovulation and sometimes in vitro fertilization program may be needed [6].

Reproductive Function in Women with 21-OHd NCAH

Puberty time is regular in girls with 21-OHd NCAH, but menstrual irregularities occurring immediately after menarche often represent the presenting symptom. In a study of 220 female adolescents, menstrual irregularities have been the presenting sign of 21-OHd NCAH in 56% of the patients, while primary amenorrhea occurs in 9% of them [7]. It means that only one third of girls with 21-OHd NCAH will have normal menses during adolescence.

About 50–70% of adult women with 21-OHd NCAH present with irregular menses and chronic anovulation [7–9], while 80% present with polycystic ovaries [8]. In past, we had reported that adult women with 21-OHd NCAH presented with inappropriate gonadotropin secretion like that found in PCOS with increased amplitude of LH pulses [10] and that gonadotropin suppression by a GnRH agonist determined improvement of hirsutism and of the androgen secretion larger than that observed by using corticosteroids [11]. Because all these patients are hyperandrogenemic, most of them will be diagnosed as PCOS, if 17-OHP assay is not performed [12].

We have recently reviewed our experience in 66 (mean age of 23±7 years) adult women with 21-OHd NCAH (Table 1) (unpublished data). Most patients have presented with moderate or severe hirsutism, while mild or moderate acne (assessed by Global Evaluation Acne-GEA scale) has been found in 40% of the patients and mild or moderate hair loss (female pattern hair loss, assessed by Sinclair scale) has been detected in 16% of the patients. Menstrual cycles have been regular in only 35% of the patients, while oligomenorrhea has been reported in 60% and amenorrhea in

Table 1 Menstrual irregularities, chronic anovulation, and polycystic ovaries in 66 adult women with 21-OHd NCAH and 275 PCOS patients (Carmina E, unpublished data)

	Menstrual irregularities (%)	Anovulation (%)	Polycystic ovaries (%)
21-OHd NCAH	65	50	75
PCOS	62	68	90

5%. Ovulatory cycles (assessed by measuring progesterone values on day 22 of the menstrual cycle) have been found in 50% of the patients with NCAH while in the remaining 50% chronic anovulation has been observed. About 75% of the cases have had polycystic ovaries and only 25% have exhibited normal ovaries.

These data confirm that secondary PCOS is present in most adult women with 21-OHd NCAH and is particularly important in determining the reproductive dysfunction of these patients. Because of PCOS, glucocorticoid treatment is often unable to improve the clinical and endocrine expression of the syndrome [12].

However, there are some interesting differences between 21-OHd NCAH patients with a secondary PCOS and PCOS women. In fact, as shown in Table 1, while the prevalence of menstrual irregularities is similar in adult women with 21-OHd NCAH and PCOS, chronic anovulation is more common in PCOS. It suggests that in women with NCAH, despite menstrual irregularities and secondary PCOS, ovulatory cycles may occur and determine normal fertility.

Fertility and Reproductive Outcome in Women with 21-OHd NCAH

In fact, in most studies, the majority of women with 21-OHd NCAH have conceived spontaneously [12–14] with only 30% of the patients complaining of infertility. Chronic anovulation is the main cause of infertility in 21-OHd NCAH women, but most patients will conceive using ovulation inducing drugs [4, 15]. Differently on that is found in adult women with classical 21-OHd CAH, only in a few adult women with 21-OHd NCAH, persistent elevated progestogen concentrations (due to excess circulating levels of progesterone and 17-OHP of adrenal origin) participate to the pathogenesis of infertility [4].

The rate of a singleton live birth is higher in 21-OHd NCAH women treated by glucocorticoids prior to conceiving than in those patients who have conceived spontaneously (86% vs. 69%, respectively); there is no difference in the rate of ectopic pregnancy, preterm birth, stillbirths, twins, or multiple pregnancies [14]. Because spontaneous miscarriage in 21-OHd NCAH women is common and has been reported in up to 25% of pregnancies, a figure that decreases to 10% in women in whom glucocorticoid treatment is started before pregnancy [14, 16], it has been suggested that glucocorticoid therapy may reduce the rate of pregnancy loss in 21-OH-d NCAH. Glucocorticoid treatment (other than dexamethasone) should be suggested to all women with 21-OHd NCAH who wish to conceive and have the story of miscarriages [12].

Reproductive Dysfunction in Males with 21-OHd NCAH

Data regarding adolescent and adult male patients with NCAH due to 21-hydroxylase deficiency is extremely limited. In fact, the great majority of male patients are asymptomatic, and most are identified during genetic screening carried out for purposes of genetic counseling. Only a few seek for medical advice because of prepubertal or pubertal manifestations [17].

In male adolescents, gynecomastia may sometimes present, but it is an uncommon finding. Wasniewska et al. have reported two boys presenting with either prepubertal or pubertal gynecomastia [18]. In both boys, gynecomastia has completely regressed 5–8 months after the institution of glucocorticoid replacement therapy.

Testicular adrenal rest tumors (TARTs) are the most important cause of infertility in male patients with classical 21-OHd CAH, but are uncommon in NCAH patients [19]. Therefore, routine measurement of 17-OHP in the evaluation of male infertility is not recommended.

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Fertility Treatment in Men with Classical and Nonclassical Congenital Adrenal Hyperplasia

Meletios P. Nigdelis and Dimitrios G. Goulis

Abbreviations

17-OHP	17-Hydroxyprogesterone
A/T	Androstenedione-to-testosterone
ACTH	Adrenocorticotropic hormone
ART	Assisted reproductive technologies
CAH	Congenital adrenal hyperplasia
CI	Confidence interval
CYP11B1	Cytochrome P450 family 11 subfamily B member-1
CYP11B2	Cytochrome P450 family 11 subfamily B member-2
DSD	Disorder of sex development
FSH	Follicle-stimulating hormone
hCG	Human chorionic gonadotropin
HPG	Hypothalamic–pituitary–gonadal
HSD17B3	Hydroxysteroid 17-beta dehydrogenase-3
ICSI	Intracytoplasmic sperm injection
INSL3	Insulin-like-3
IVF	In vitro fertilization
LH	Luteinizing hormone
MC2R	Melanocortin-2 receptor
micro-TESE	Microsurgical testicular sperm extraction
MRI	Magnetic resonance imaging
NCAH	Nonclassical congenital adrenal hyperplasia
OR	Odds ratio
TARTs	Testicular adrenal rest tumors

M. P. Nigdelis · D. G. Goulis (✉)
Unit of Reproductive Endocrinology, 1st Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, “Papageorgiou” General Hospital, Thessaloniki, Greece
e-mail: dgg@auth.gr

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Introduction

Although female infertility and its treatment is an extensively studied topic, male fertility issues in patients with congenital adrenal hyperplasia (CAH) are less described [1]. Indeed, both the disorder per se (e.g., multiple genotypes and phenotypes) and the male infertility make the individualized approach a challenging task.

Small studies in 1970s have failed to demonstrate an association between CAH and male infertility [2]. On the contrary, most recent studies have reported a significant impact of CAH on fecundity and fertility outcomes [1, 3–5]. More specifically, in a French national survey, 51% of patients with CAH have been reported to have at least one child, a prevalence significantly lower compared with the French general population (79%) [3]. A British study has reported that almost 40% of males with CAH have used fertility treatment services (24/65) with a success rate of 67% [4]. From another perspective, an analysis of the Swedish CAH registry (n = 221) has demonstrated that the odds ratio (OR) of being a biological father of at least one child has been 0.5 [95% confidence interval (CI) 0.4–0.7] for CAH males compared with controls matched for year, place of birth, and migration status [5]. An interesting point raised by the same study is that the introduction of neonatal CAH screening in Sweden has not significantly changed the likelihood of having a child, even though the OR is doubled (OR_{before} = 0.5 versus OR_{after} = 1.0) [5]. The previous study has also provided data specific for patients with non-classical adrenal hyperplasia (NCAH), a group for which evidence is even more scarce in literature [5]. More specifically, the OR of being a biological father has been 3.7 (95% CI 0.9–15) for NCAH males compared with matched controls from general population [5]. The difference between the two forms of the disease has been attributed to a variety of factors. Compared with the classical form, NCAH may not be diagnosed as often, thus hampering a properly powered estimation of infertility in these individuals [5]. Another reason can be that testicular adrenal rest tumors (TARTs), one of the most common reasons for male infertility, constitute a rather rare entity in patients with NCAH [6]. In line with this finding, the routine ultrasound screening for TARTs in patients with NCAH is not included in the Endocrine Society's most recent clinical practice guidelines [7].

An essential prerequisite of infertility treatment in men with CAH is the identification of its possible causes (Fig. 1). Some of them are attributed to the disease per se. The causes include TARTs, primary (hypergonadotropic) hypogonadism resulting from testicular dysfunction and secondary (hypogonadotropic) hypogonadism. Furthermore, sexuality problems may be related to the disease per se, given its effect on male sexuality [8]. Glucocorticoid administration may lead to increased body weight or even hypogonadism by suppressing gonadotropin secretion [8]. Finally, other infertility aspects (e.g., sexually transmitted infections, obesity, medications) may also accompany. Hence, accurate diagnosis of all comorbidities is of utmost importance in infertile men with CAH.

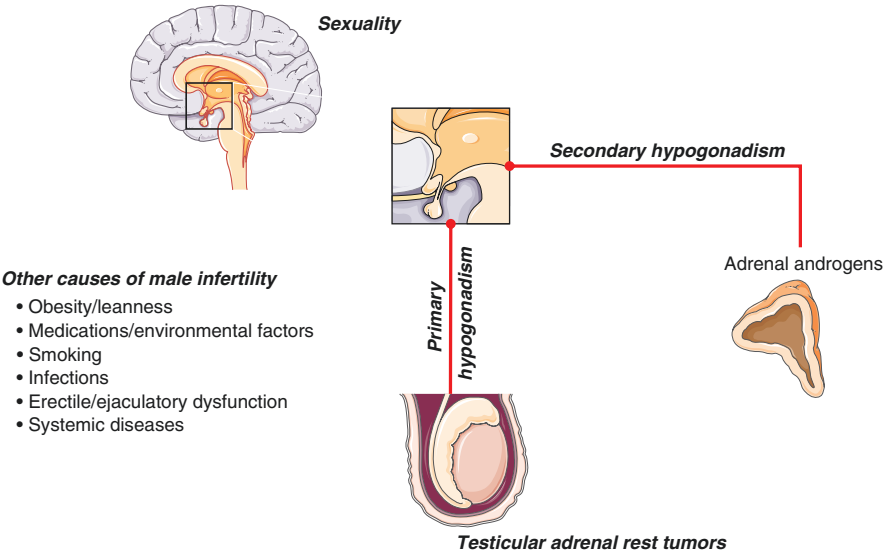

Fig. 1 The multifactorial etiology of infertility in congenital adrenal hyperplasia (CAH). Four mechanisms specific for CAH patients are identified (bold letters). Other causes of male infertility need to be taken into consideration. Parts of the figure were based on images by Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License

Testicular Adrenal Rest Tumors

Testicular adrenal rest tumors (TARTs) constitute the most common cause of male infertility among CAH patients. These tumors are benign, mostly bilateral, and require early detection to prevent permanent testicular damage [9]. There are case reports in current literature stressing on the importance of these lesions in CAH, as TARTs can be the only manifestation [10].

Embryology

The pathophysiology of TARTs development has been studied extensively in the past years. The interplay between a specific primordial cell type nesting in rete testis during fetal development and exposure to growth factors pre- and postnatally constitute the main mechanism [9].

As far as cellular types are concerned, some authors have proposed primordial adrenal cells, while others have suggested that fetal Leydig cells may divide into TARTs, as the latter also express receptors and enzymes found in TARTs [9, 11]. A seminal paper by Val et al. has introduced a new concept in the embryology of TARTs [12]. More specifically, fetal and adult mouse testes have been demonstrated

to possess adrenal-like cells, expressing adrenal-specific markers; cytochrome P450 family 11 subfamily B member-1 (Cyp11B1) and Cytochrome-21 (Cyp21), and responding to adrenocorticotropic hormone (ACTH) and human chorionic gonadotropin (hCG). The authors have suggested that a common steroidogenic progenitor, originating in the area between the mesonephros and the gonad, can be responsible for TARTs formation in CAH [12]. Based on these findings, Engels et al. have hypothesized the presence of a pluripotent steroidogenic cell in humans, as well [11].

In line with the theory of pluripotent steroidogenic progenitor in humans, a study by Smeets et al. has provided molecular evidence that characteristics of both adrenal and gonadal cells are present in TARTs [13]. In gene expression analysis of TARTs, the authors have identified 1000- to 10,000-fold higher adrenal enzymes expression compared with testis. Those have been CYP11B1, cytochrome P450 family 11 subfamily B member-2 (CYP11B2), and melanocortin-2 receptor (MC2R). On the other hand, insulin-like-3 (INSL3) and hydroxysteroid 17-beta dehydrogenase-3 (HSD17B3) produced in Leydig cells have been expressed in TARTs at significantly higher levels compared with adrenal tissues. This complex steroidogenic profile can only be attributed to a less differentiated embryonic progenitor [13].

Considering growth factor exposure, ACTH's proliferative effect on primordial adrenal cells has been proposed as a possible contributor to TARTs development [9]. Compatible with the theory mentioned above, patients with NCAH rarely present with TARTs, probably because ACTH is only moderately increased. Engels and colleagues have stressed that ACTH exposure's timing is crucial and have reported that not all CAH patients with increased ACTH present with TARTs [11]. Nevertheless, TARTs have also been described even in patients with low ACTH concentrations [9]. Apart from ACTH, recent molecular analyses of TARTs tissues have suggested that angiotensin II and luteinizing hormone (LH) exposure, the latter appearing during puberty, can also play a role in the development and progression of these tumors [13].

Prevalence

A recent pooled analysis of 1315 patients by Engels and colleagues has estimated an overall prevalence of almost 40% (489 cases) [11]. Main factors associated with the presence of TARTs are patient's age, method of detection, and severity of the disease. In general, TARTs are more prevalent as the patient's age increases from puberty to adulthood [11]. Even though TARTs are considered rarer in children, a review of autopsy studies has reported seven CAH patients younger than 8 weeks, three of whom have demonstrated bilateral TARTs. Of note, TARTs have been identified in all patients older than 14 months of life [14]. More recent evidence is in line with these findings, suggesting that boys present with TARTs more frequently than expected, gonadal function [determined by follicle-stimulating hormone (FSH), LH, testosterone, and inhibin B concentrations] has not been impaired in any of the patients with TARTs compared with those without. As a result, the authors have concluded that gonadal function impairment may occur as the age progresses [15].

Furthermore, different diagnostic methods modify the estimated prevalence rates of TARTs [11]. More specifically, clinical examination fails to identify small tumors (see Diagnosis). On the other hand, ultrasonography and magnetic resonance imaging (MRI) can identify small size tumors; thus, imaging modalities have an important place in TARTs diagnosis. Finally, TARTs prevalence increases in parallel with disease severity. A genotype-specific association has been described, with salt-wasting forms demonstrating higher prevalence compared with the simple virilizing forms [11].

As far as NCAH is concerned, anecdotal reports occur [6]. Still, the review by Engels and colleagues has provided no specific estimate of TARTs in patients with NCAH [11]. Therefore, one can conclude that they constitute a rare entity.

The aforementioned data has been related to 21-hydroxylase deficiency (21-OHd). Even fewer data are available for other forms of CAH. In a study of boys and adolescent males, three out of five patients with 11-β hydroxylase deficiency have presented with TARTs. All subjects have demonstrated a suboptimal control of the disease [16]. In these patients, TARTs are associated with oligo- and azoospermia as in patients with 21-OHd [17]. Further assumptions are hard to be drawn, as evidence on TARTs and 11-β hydroxylase deficiency remains scarce.

Association with Hormonal Parameters

Given that TARTs have been described in patients with ACTH overproduction, such as Cushing's or Nelson's syndromes [18, 19], and TARTs have demonstrated ACTH-responsiveness [20], a series of studies have evaluated hormonal parameters as determinants of TARTs. More specifically, some authors suggest that the control of the disease (demonstrated by hormonal concentrations) play a role in the development of TARTs. According to Engels' pooled estimates, 58% of cases with TARTs have demonstrated inadequate hormonal control [11].

In line with the "poor control" concept, evidence is driven mostly from cross-sectional studies. Increased ACTH concentrations have been associated with TARTs presence [21]. Furthermore, in a recent multicentric European study of 121 patients, 17-hydroxyprogesterone (17-OHP) concentrations two times higher than the upper limit of the reference range have resulted in an OR for having TARTs of 28.0 (95% CI 3.1–252.5), while simply exceeding the reference range led to an OR of 18.7 (95% CI 2.2–158.1) compared with concentrations within the reference range [22]. The authors have also demonstrated that androstenedione concentrations above the reference range has been associated with 3.6 times greater odds of having TARTs (95% CI 1.0–12.7, significance not reached) [22].

Despite the evidence from cross-sectional studies on disease control parameters, a longitudinal analysis of 50 patients with classical 21-hydroxylase deficiency has failed to demonstrate any significant correlation. Pregnanetriol and serum 17-OHP concentrations have not been related to TARTs presence [23]. Similar observations have been made for long-term clinical control parameters, namely testicular volume, Tanner stage for pubic hair, and bone age [23].

As a conclusion, caution is required in the interpretation of clinical data connecting disease control with TARTs development. Most studies providing data are cross-sectional, and hence, the association should not necessarily imply causation. Furthermore, the number of patients enrolled in these studies is rather small, decreasing the power of estimates produced by them. Importantly, one needs to bear in mind that TARTs can be present even in patients with optimal hormonal control. Hence, more robust evidence is required for producing definitive conclusions, even though multifactorial etiology cannot be excluded [11].

Diagnosis

Clinical examination allows diagnosing TARTs larger than 2 cm. Ultrasonography is considered to be the diagnostic method of choice. Data on MRI stem from case reports and are rather scarce. Most TARTs are located in the rete testis and can affect one or both testicles [11]. A variety of ultrasonographic features characterizes TARTs: they can be round, lobular, or even irregular in shape, clearly defined from the testicular parenchyma [24]; the majority of them are hypoechogenic (without excluding hyperechogenic lesions) and usually demonstrate increased vascularity in color Doppler scans [24, 25].

It is important to mention that TARTs need to be differentiated from Leydig cell tumors of the testis. Their bilateralism, typical adrenal steroid secretion profile, response to glucocorticoids, and solely benign potential are characteristic features. However, Leydig cell tumors can be malignant in 10% of cases, and they exhibit Reinke crystals in pathologic specimens [11].

Staging

Even though TARTs are benign, histopathologic studies have proven that they can progress in size and cause irreversible obstructive pathology demonstrated as oligo- or azoospermia. Residual parenchyma biopsies following testis-sparing surgery (see Management options) have demonstrated impaired spermatogenesis [26]. This obstructive damage has been attributed to decreased tubular diameters, thickening of the lamina propria, peritubular fibrosis, and hyalinization [9, 26]. Furthermore, toxic damage to Sertoli and germ cells via paracrine hormone secretion by the tumor has also been implicated [9]. Apart from direct effects on testes, endocrine mechanisms have also been postulated [27]. More specifically, steroid production directly by TARTs can interfere with hypothalamic–pituitary–gonadal (HPG) axis. Blumberg-Tick and colleagues have demonstrated the secretion of 11-β-hydroxylated steroids in gonadal veins of patient with TARTs [27]. Adding to this, presence of TARTs has been correlated with increased concentrations of 11-oxygenated androgens (11-β-hydroxyandrostenedione, 11-β-hydroxytestosterone, 11-ketoandrostenedione, and 11-ketotestosterone) [28].

Table 1 Histopathologic classification of TARTs

Stage	Description	Clinical information
Stage 1	Microscopic detection of TARTs cells in the rete testes	• Regression during infant life • Clinically undetectable lesions
Stage 2	Proliferation and hypertrophy of TARTs cells	• Ultrasonographic detection
Stage 3	Continuation of proliferation, rete testes compression, testicular dysfunction	• Oligo- or azoospermia • Decreased inhibin B, increased FSH, LH • Glucocorticoids still effective
Stage 4	Lymphocytic infiltration focally, fibrosis (peritubular and in tumor)	• Glucocorticoids no longer effective
Stage 5	Irreversible damage to the testicular parenchyma: • Peritubular fibrosis • Tubular hyalinization Replacement of tumor with adipose tissue	

FSH follicle-stimulating hormone, *LH* luteinizing hormone, *TARTs* testicular adrenal rest tumors. Source: [9]

Claahsen-van der Grinten and colleagues have attempted to classify the histopathologic stages of TARTs to predict long-term sequelae better and guide therapy [9] (Table 1). Note that late stages provide little space for treatment, as testicular damage becomes irreversible.

Management Options

There are primarily two goals in the management of TARTs. The first is early detection via ultrasound screening in early stages of the disease, and the second is the reduction of tumor size with subsequent prevention of testicular failure.

As far as the first goal is concerned, informing patients about TARTs and subsequent risk of infertility remains an essential prerequisite of effective screening [11]. Moreover, periodically scanning males with classical CAH to assess TARTs development have been stressed in the most recent recommendations by the Endocrine Society [7]. Specific details, such as age at initial screening or screening frequency, remain uncaptured, as clinical data are scarce.

Based on the observation that children with CAH may present with TARTs, Claahsen-van der Grinten and colleagues have suggested that ultrasound screening must begin in childhood [29]. Engels and colleagues have proposed that screening should be conducted annually, starting at 8 years of age until adulthood. From adulthood on, biennial or triennial controls are sufficient [11]. In addition, as TARTs may cause progressive, irreversible gonadal failure, authors have advocated in favor of sperm cryopreservation in pubertal or young adult males with TARTs [11, 29, 30].

Intensification of glucocorticoid treatment is considered as the primary choice for treatment of TARTs [9, 11, 29]. The prevailing theory behind fertility

improvement is tumor shrinkage resulting from ACTH decrease after glucocorticoid administration [29]. It is important to stress that no prospective evidence is currently available [11].

Patients with second- or third-stage TARTs have been reported to achieve a decrease in TARTs size, reversal of azoospermia, normalization of hormonal profile, and pregnancies after recommencing, increasing the dosage, or altering glucocorticoid treatment to a longer-acting one [9, 31–33]. Regimens used include hydrocortisone combined with dexamethasone [31], dexamethasone [32], and fludrocortisone [33]. Prednisone may also be used [29]. Of note, patients presenting with salt-wasting phenotypes require additional mineralocorticoid replacement [34].

Glucocorticoid administration comes with certain restrictions. First of all, adverse effects need to be discussed with the patients before deciding on intensification. These include Cushingoid effects (e.g., body weight gain, striae) and reduced height (pubertal administration) [30, 32]. Of note, weight gain may also deteriorate the negative effects of CAH on fertility [6]. Furthermore, tumor shrinkage and therapeutic benefits may only be temporary for as long as intensified treatment is administered [9]. Finally, stage 4 TARTs are resistant to glucocorticoid treatment, while other treatment options remain of dubious value [9].

Surgical treatment constitutes another possible choice. Historically, orchiectomies have been considered the treatment of choice for patients with CAH, because the diagnostic conundrum between Leydig cell tumors and TARTs has prevailed. Given that orchiectomies have been radical, and the testis cannot be preserved, testis-sparing surgery has been quickly employed in TARTs surgery. Walker and colleagues have reported three cases of steroid-resistant TARTs treated with tumor enucleation resulting in a viable testis [35]. Still, the effect of surgery on fertility parameters has not been addressed [35]. A more recent study has examined testis-sparing surgery's effect on gonadal function in eight patients with stage 5 TARTs [36]. Tumor enucleation has resulted in no improvement of semen parameters, 6 and 22 months after the operation. As far as hormonal parameters are concerned, various changes have occurred, even though inhibin B concentrations have remained low in all patients. Two patients have demonstrated decrease in testosterone concentrations, indicating further gonadal damage attributed to surgery [36]. Based on the findings mentioned above, the role of surgery in the restoration of fertility remains uncertain. Hence, until new evidence is produced, surgical treatment should only be considered in cases of pain or discomfort caused by the tumor [11].

Apart from TARTs-specific treatments, classical assisted reproductive technologies (ART) have been applied in patients with TARTs. Yang and colleagues have reported the successful use of clomiphene citrate (25 mg daily) in a patient with TARTs treated with dexamethasone (initially 2 mg for 7 days, and then 0.25 mg daily) who has been unable to achieve pregnancy solely with steroid therapy. The patient's sperm number and volume have increased, and after five cycles of intrauterine insemination, the couple has achieved pregnancy [37]. Rohayem and colleagues have described the use of gonadotropin administration (hCG 1500 IU twice a week and FSH 150 IU three times a week) in a patient with persistent hypogonadotropic hypogonadism and TARTs despite steroid administration [38].

Murphy and colleagues have described intracytoplasmic sperm injection (ICSI) in a patient on prednisolone with azoospermia and TARTs. Spermatozoa have been obtained via testicular needle aspiration (TESE) and used for ICSI, which has resulted in live birth [39]. Microsurgical testicular sperm extraction (micro-TESE) has also been described in non-responsive cases with functional testicular parenchyma [30]. Kavoussi and colleagues have reported two patients with TARTs accompanied by orchialgia and azoospermia despite gonadotropic stimulation [40]. Resection of TARTs followed by TESE and ICSI leading to live birth has been reported in one patient, while the other has been opted for cryopreservation. The pain subsided in both patients [40].

In 2014, Bry-Gauillard and colleagues have reported mitotane use for fertility restoration in a 29-year-old male with azoospermia and bilateral TARTs [41]. Mitotane, a regimen inducing "chemical adrenalectomy," has resulted in normalization of hormonal profile (decrease in 17-OHP, increase in FSH, LH, inhibin B). The TARTs sizes have decreased, and 8 months after treatment, sperm counts have increased. In vitro fertilization (IVF) after ovarian stimulation has resulted in birth of a neonate, heterozygous to the mutation of the father. As the authors have concluded, mitotane can constitute as a "last resort" in patients with large, unresponsive tumors and severe sperm disorders (azoospermia) [41]. Of course, more data are required to produce definitive evidence on the drug's effectiveness in infertility treatment.

Disturbances of HPG Axis

Apart from TARTs, dysfunction of HPG axis may also cause male infertility. Hypogonadism remains a challenging concept, as males with CAH may present with normal or even increased testosterone concentrations, instead of decreased, due to excessive adrenal androgen production [42].

Given that no methods to differentiate adrenal from testicular androgens have been described, Engels and colleagues have proposed using androstenedione-to-testosterone (A/T) ratio to categorize patients in three categories based on androgen production source [22]. Those with testicular androgen production present with A/T values <0.5, those with adrenal origin present with values ≥ 1, while cases in between represent significant adrenal involvement [22].

Primary Hypogonadism

The principal cause of primary (hypergonadotropic) hypogonadism in patients with CAH is TARTs causing testicular dysfunction. A study of 50 men with CAH due to 21-hydroxylase deficiency has detected increased FSH concentrations in 11 patients [30]. Nine of them (82%) have presented with TARTs, whereas only two (12%) have

had no abnormalities in testicular ultrasound [30]. Similar findings have been observed in the study by Bouvattier and colleagues [3]. Increased FSH concentrations have been associated with TARTs presence. Seminal abnormal parameters have further demonstrated testicular damage and markedly decreased inhibin B levels.

Secondary Hypogonadism

Apart from eugonadism and primary hypogonadism, secondary (hypogonadotropic) hypogonadism seems to be a common entity among patients with CAH [3, 23, 30]. Adrenal steroids, produced in excess because of CAH, can be aromatized to estrogens and interfere with gonadotropic secretion. As a result, infertility may occur [43].

The exact prevalence of hypogonadotropic hypogonadism varies among studies. Variability in definitions leads to heterogeneity in prevalence estimation. In the study by Bouvattier and colleagues, 62 of 164 (37.8%) men have presented with decreased LH concentrations [3]. King and colleagues have reported that 26 of 50 patients (52%) have had at least one low LH value [30]. A study on 22 CAH patients have demonstrated that eight of them have had decreased serum testosterone concentrations with three have also exhibited low basal LH concentrations [23]. Finally, Engels and colleagues have reported that 12 of 90 patients with CAH have presented with low LH concentrations [22]. In the same study, an A/T ratio value >1.0 have been associated with reduced gonadotropin concentrations (OR 39.3, 95% CI 2.1–732.4). Therefore, the authors have stressed the role of adrenal androgens in the pathogenesis of secondary hypogonadism. Given the retrospective nature of the data, caution is required in their interpretation [22].

As far as treatment options are concerned, even though no prospective evidence for hypogonadotropic hypogonadism exists, retrospective data suggest that commencement, intensification, or altering glucocorticoid treatment seems to restore hormonal, seminal parameters, and fertility (nine patients) [30]. This has also been demonstrated in cases with NCAH (see below). Of note, glucocorticoid administration has uncovered a hypergonadotropic hypogonadism in one patient [30]. In nonresponsive cases, gonadotropin administration and ICSI with or without micro-TESE constitute possible alternatives [30].

Nonclassical CAH (NCAH) data is less abundant, mainly there are four case reports. Augarten and colleagues have reported secondary infertility in a patient with hypogonadotropic hypogonadism and poor semen quality. Treatment with prednisolone 10 mg daily has resulted in normalization of hormonal and seminal profile and fertility restoration, resulting to his second child a few months after treatment [44]. Wischusen and colleagues have stressed the reversible nature of NCAH-related hypogonadotropic hypogonadism and infertility in a patient receiving 0.5 mg dexamethasone daily, whose spouse has achieved pregnancy [45]. Finally, Trakakis and colleagues have reported a couple with NCAH and two failed IVF attempts, both wife and the husband have been treated with hydrocortisone

30 mg daily, and pregnancy has been achieved. As the authors stated, ART should only be reserved for patients unresponsive to glucocorticoid administration [46].

Disturbances in HPG axis and semen production can be attributed to overtreatment with glucocorticoids [23]. They may be the consequence of glucocorticoids' direct effect or weight gain [34]. Increased body weight alone can lead to hypogonadotropic hypogonadism, semen disturbances, and infertility in otherwise healthy males [47]. This observation has been shown in males with CAH, as well [6]. More specifically, males with CAH and abnormal semen parameters exhibit increased total and truncal fat mass and fat/lean mass ratio compared with males with CAH and normal semen [6]. Consequently, establishing optimal glucocorticoid dosage, when intensification is not required, constitutes an important step in infertility management [34]. Weight loss and adoption of a healthy lifestyle can also support fertility [6].

Sexuality Disorders

Even though CAH constitutes a disorder of sexual development (DSD) in women, primarily due to androgen excess in fetal period, its exact effect on male sexuality remains unknown [48]. Data are limited for robust conclusions to be drawn. Apart from fertility, sexuality disorders can greatly impair well-being and quality of life [4]. Hence, identifying and treating them should be a part of CAH clinical care.

A recent multinational European cohort study of patients with CAH treated in specialized centers has demonstrated comparable age of sexual debut, frequency of intercourse and sexual satisfaction with the general population [49]. Of note, disease severity (attributed to genotype) has been shown to negatively associate with sexual activity. Furthermore, a slightly increased prevalence of sexual desire problems has been reported in the cohort. More specifically, lack of sexual desire has been reported in 17.6% of males with CAH, compared with 11.7–15.7% in general population. Excessive desire has also been more frequent (23.1% versus 5–10%, respectively). As far as premature ejaculation is concerned, it has been reported by 25% of the patients [49].

Erectile dysfunction has been also studied. The aforementioned study has reported a slightly increased prevalence (18.7%) compared with the general population (12–23%, mean 16.2%) [49]. Other studies have reported increased estimates (55% and 41%) [4, 50]. Such differences can be attributed to different methods used to measure erectile dysfunction (standardized questionnaires or self-reports). Nevertheless, patients with CAH should be screened and treated for erectile dysfunction.

In conclusion, men with CAH constitute a distinct population displaying dysfunctional aspects of sexuality. These include desire problems and erectile dysfunction. In the future, larger studies can provide more precise estimates, focus on addressing confounding factors interacting with sexuality, and assess the efficacy of different treatment methods.

Conclusion

Infertility constitutes a common problem among males with CAH. A treatment algorithm is provided in Fig. 2. Additional causes of male infertility should always be sought for and treated. The management begins in adolescence at latest with ultrasonographic controls for TARTs and sperm cryopreservation if required. The specific TARTs stage leads to appropriate therapeutic approaches. These include glucocorticoid intensification, surgery for pain relief, and classical ART strategies. Lifestyle changes may facilitate fertility. Despite TARTs, patients with CAH present with different forms of hypogonadism with variable testosterone concentrations; hence, the A/T ratio should be used for the diagnosis. Finally, sexual history should be obtained, and sexual disorders need to be addressed.

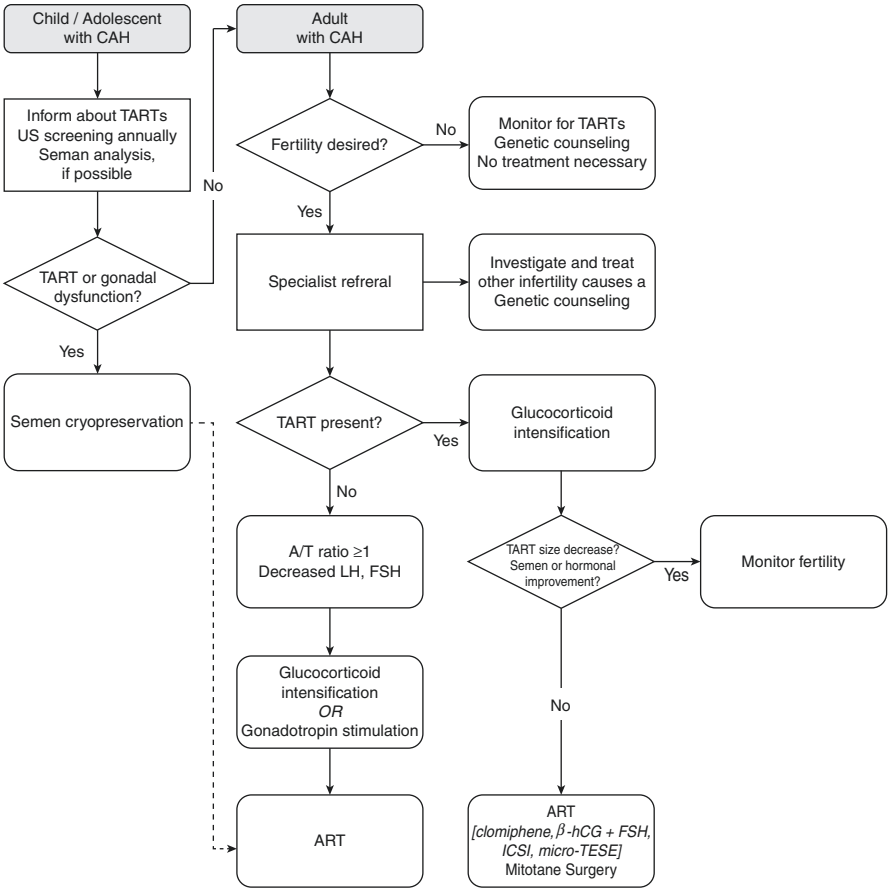

Fig. 2 Proposed CAH management algorithm for children and adults seeking fertility. [a]See Fig. 1. *ART* assisted reproductive technologies, *A/T* androstenedione-to-testosterone, *β-hCG* beta-human chorionic gonadotropin, *CAH* congenital adrenal hyperplasia, *FSH* follicle-stimulating hormone, *ICSI* intracytoplasmic sperm injection, *LH* luteinizing hormone, *TARTs* testicular adrenal rest tumors, *TESE* testicular sperm extraction

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Fertility Treatment in Women with Classical and Nonclassical Congenital Adrenal Hyperplasia

O. Pavlidi and E. M. Kolibianakis

Introduction

Although in a large proportion of infertile couples no specific cause is identified [1], in the majority, infertility can be attributed to either male or female factor. Female factor is mainly due to advanced female age, low ovarian reserve, tubal occlusion [1], endometriosis [2], uterine malformations [3], or certain endocrine disorders, such as congenital adrenal hyperplasia [4].

The term congenital adrenal hyperplasia (CAH) represents a group of autosomal recessive disorders. Genes that cause CAH are responsible for the expression of enzymes that have key roles in cortisol biosynthesis. Mutations in these enzymes are usually associated with decreased cortisol production and androgen excess [5].

The most frequently encountered form of CAH is due to 21-hydroxylase deficiency (21-OHd) (over 95% of all CAH cases). Less frequent forms are due to deficiency of 11β-hydroxylase (11β-OH), 17α-hydroxylase (17-OH), 3β-hydroxysteroid dehydrogenase type 2 (3βHSD2), steroidogenic acute regulatory protein (StAR-congenital lipoid adrenal hyperplasia), P450 cholesterol side chain cleavage (P450scc) and P450 oxidoreductase (POR).

Clinically, 21-OH deficiency (21-OHd) CAH can be categorized as classical and the nonclassical (NCAH) form, depending on the residual activity of the affected enzyme. Nonclassical form is more frequent than the classical form (incidence 1:1000 vs 1:10,000 to 1:20,000 live births, respectively) and is characterized by 20–50% of residual enzyme activity. The classical type is further subcategorized into two forms, the more frequent (75%) salt-wasting form, in which the enzyme is completely deficient, and the simple virilizing form, in which residual enzyme activity is 1–2%.

O. Pavlidi · E. M. Kolibianakis (✉)
Unit of Human Reproduction, 1st Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

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For many years, it has been considered that achievement of pregnancy in women with CAH is infrequent [6], the main responsible factors are hyperandrogenism and anovulation. In recent years, however, pregnancy rates have improved due to the availability of hormonal therapy, minimal invasive surgery and of medically assisted reproduction [7]. This chapter provides an overview of contemporary methods used for fertility treatment of women with classical and nonclassical CAH mostly due to 21-OHd as it is the most prevalent form.

Targets of Fertility Treatment in Women with Classical CAH and NCAH

Hyperandrogenaemia and Anovulation

The majority of women with CAH are characterized by hormonal imbalance which is due to alteration of the hypothalamic–pituitary–gonadal (HPG) axis, causing hyperandrogenaemia and anovulation [7, 8]. Menstrual irregularities and anovulation manifest in 30–68% of women with the salt-wasting form and in 30–75% in women with the simple virilizing form [9]. Hyperandrogenaemia, which is one of the main characteristics of polycystic ovary syndrome (PCOS) [10, 11], is often found in both classical CAH and NCAH [12]. The PCOS in these patients is secondary to the hormonal changes caused by 21-OHd [13]. In fact, 33% of women diagnosed with PCOS may suffer from NCAH [7].

Disorders of Female Anatomy in Women with Classical CAH and NCAH

Women with more severe classical forms are categorized as a specific subtype of sexual development disorders [14]. Ambiguous genitalia is frequently encountered in these women due to their exposure to high androgen levels of adrenal origin during embryonic life. Despite the fact that internal reproductive organs remain normal, clitoromegaly, labial fusion and rostral migration of urethra/vaginal perineal orifice are frequently encountered [15]. These may explain why these women, in whom homosexual orientation is common [16], tend to have fewer partners [8], decreased frequency of sexual intercourse and why they do not seek for motherhood frequently [17, 18].

Fertility Treatment in Women with Classical CAH and NCAH

Restoration of Hormonal Imbalance

Medical Treatment

A daily dose of 0.25 mg dexamethasone has been suggested for the treatment of anovulation, hirsutism and acne in women with NCAH. Treatment for 3 months has been associated with a reduction in irregular menstruation and acne, while a longer period of treatment (about 30 months) is required for the management of hirsutism [19, 20]. Women with the salt wasting classical CAH form need mineralocorticoid replacement therapy in addition to dexamethasone [5, 7]. In contrast to women with NCAH, they are usually anovulatory and are characterized by elevated progesterone levels in follicular phase which is suggested to be less than 2 nmol/L [4, 21, 22].

Glucocorticoid treatment in adults is usually adjusted on a per patient basis, attempting to find the minimum effective maintenance dose determined by a combination of clinical and biochemical markers [23]. In a series of 80 female patients with mostly 21-OHd CAH, 37% with the salt-wasting form have been treated with hydrocortisone (median 30 mg, range 15–40), 53% with prednisolone (median 7 mg, range 4–10), 4% with dexamethasone and 5% with a mix of glucocorticoids. All of these patients have been administered fludrocortisone (median 175 μg, range 50–400). In addition, 12% of patients with simple virilizing CAH have received no treatment, 42% have been treated with hydrocortisone (median 20 mg, range 15–30), 31% with prednisolone (median 5 mg, range 3–75), 3% with dexamethasone, 12% with a mix of glucocorticoids and 38% with fludrocortisone (median 100 μg, range 0–200) [23].

Surgical Treatment

Bilateral laparoscopic adrenalectomy is known to restore the hormonal imbalance, especially in patients with the classical CAH form, and this may be superior to medical therapy in some cases [24]. However, according to the Endocrine Society, it should be considered only in selected cases of infertile females with the salt wasting classical form, where medical treatment has failed. Bilateral laparoscopic adrenalectomy decreases the risk of virilization and allows treatment with lower glucocorticoid doses. However, it may induce the loss of residual adrenal function and lead to adrenal crisis [20].

Restoration of Female Anatomy

The majority of patients with classical CAH are sexually active and satisfied following urogenital reconstructive surgery, which is characterized by a low incidence of complications. However, there are patients who complain about sexual impairment and a low satisfaction experience [25]. Vaginal stenosis is frequent (25%), while additional complaints include impaired clitoral sensitivity, dyspareunia and low frequency of sexual intercourse with a low female sexual function index score [25].

In the past, feminizing genitoplasty had usually been performed in early infancy or during the patient's first year of life. The skin at this stage of development is exposed to high post-natal residual maternal and placental oestrogen that influence positively the healing process, decreasing scar formation [26]. However, recent data show that feminizing genitoplasty in early life may be associated with loss of sexual sensitivity and dyspareunia during the adult life [27, 28]. In addition, procedures that aim restoring female anatomy, especially when performed in patient's early life, are associated with legal and ethical concerns, since individual autonomy and informed consent by the patient herself are considered essential during counselling [28].

Ovulation Induction

Although restoration of ovulation is possible with surgical and/or medical treatment with glucocorticoids and mineralocorticoids, some women with classical CAH and NCAH forms will remain anovulatory. Ovulation induction can be achieved in these patients with the addition of clomiphene citrate (CC) in the treatment scheme [4, 29–33]. However, the sole addition of CC has also been reported to result in ovulation in undiagnosed patients with late onset 21-OHd [34]. Studies about ovulation induction in women with various CAH forms are listed in Table 1.

Assisted Reproduction Technologies (ART)

In Vitro Fertilization (IVF)

Women with classical CAH and NCAH forms can be treated with IVF in order to achieve pregnancy. Ovarian stimulation in combination with a freeze-only strategy [35] can be used in these patients to manage the adverse effects of elevated progesterone on endometrial receptivity [36, 37]. No solid conclusions can be drawn regarding the probability of pregnancy after IVF, since currently the existing information is restricted to either case reports or small series of patients [38–53]. Details of studies about IVF in women with classical CAH and NCAH may be seen in Table 2.

Table 1 Studies about ovulation induction in women with various congenital adrenal hyperplasia forms

Study (reference)	Patients	Age (years)	CAH type	Ovulation induction	Outcome
Birnbaum and Rose [29]	48	–	Classical CAH[a] 11β and 21-OHd	Prednisolone/ clomiphene citrate and prednisolone only	64% pregnancy rate
Jain [30]	1	24	21-OHd[b]	Prednisolone/ clomiphene citrate	Live birth
Khoury et al. [31]	1	26/28[c]	Classical CAH StAR gene mutation CLAH[d]	Glucocorticoids/ mineralocorticoids/ clomiphene citrate	Live birth
Purwana et al. [32]	1	30	NCAH[e] 21-OHd	Hydrocortisone/ clomiphene citrate	Live birth
Simm and Zacharin [33]	1	26	Classical CAH 11β-hydroxylase deficiency	Glucocorticoids/ mineralocorticoids/ clomiphene citrate	Live birth
Laohaprasitiporn et al. [34]	1	31	NCAH 21-OHd	Clomiphene citrate	Live birth

[a]Classical congenital adrenal hyperplasia
[b]21-Hydroxylase deficiency
[c]The patient underwent two trials at the age of 26 and 28 years
[d]Congenital lipoid adrenal hyperplasia
[e]Nonclassical congenital adrenal hyperplasia

Preimplantation Genetic Diagnosis

An important option in women with various classical CAH and NCAH forms treated by IVF is the ability to screen embryos with preimplantation genetic testing for monogenic disorders (PGT-M). This is valuable when both parents are carriers of a severe CAH mutation or if one parent is affected by CAH, and the other one is a carrier. In this case PGT-M allows the transfer of only normal embryos [17, 20, 54].

Oocyte Donation

Only scarce data are available regarding achievement of pregnancy after oocyte donation in patients with CAH, which are restricted only to case reports. A viable twin pregnancy after oocyte donation has been reported to occur in a woman with 17-OH deficiency after multiple embryo transfers. This pregnancy has been complicated by pre-eclampsia at 25 weeks associated with adrenal insufficiency and has resulted in an emergency caesarean section [55].

Table 2 Details of studies about IVF in women with classical CAH and NCAH

Study (reference)	Patients	Age (years)	Enzyme deficiency	Outcome (live births)
Classical CAH				
Albarel et al. [38]	1	25	StAR gene mutation CLAH	1
Bianchi et al. [39]	1	29	17-OHd	1
Falhammar et al. [40]	1	43	17-OHd	1
Kitajima et al. [44]	1	24	17-OHd	2
Levran et al. [45]	5	41 38 38 33 –	17-OHD/17,20 desmolase deficiency	1/5
Matsuzaki et al. [46]	1	26	17-OHd	0
Neuwinger et al. [47]	1	28	17-OHd	0
Pariente et al. [48]	1	30	17-OHd	0
Pellicer et al. [49]	1	31	17-OHd	0
Rabinovici et al. [50]	1	30	17-OHd	0
Sertedaki et al. [51]	1	25	StAR gene mutation CLAH	1
Song et al. [52]	1	28	PORD P450 oxidoreductase deficiency	1
Nonclassical CAH				
Falhammar et al. [41]	1	31	21-OHd	0
Jiang et al. [42]	25	32.2 ± 4.4 (mean ± SD)	21-OHd	19/39[a]
Kawarai et al. [43]	1	38	21-OHd	1
Trakakis et al. [53]	1	32	21-OHd	0

[a]Positive human chorionic gonadotropin/embryo transfer

Treatment During Pregnancy in Women with Classical CAH and NCAH

Glucocorticoid treatment enables conceiving, prevents early pregnancy loss and improves pregnancy outcomes in many 21-OHd CAH cases. In a study inquiring 187 pregnancies of 85 women with 21-OHd NCAH (2/3 have had mild/severe mutations), glucocorticoid treatment has clearly found to lower miscarriage rates; 26.3% vs 6.5% [56]. Another study about 187 pregnancies of 75 women with 21-OHd NCAH (84% homozygous for V281L mutation; a mild mutation), glucocorticoid treatment has been found to shorten the time to conceive; 10.2 ± 11.4 months vs 3.3 ± 3.4 months (p = 0.02) [57]. Females with 21-OHd who wish to conceive can achieve pregnancy with adequate suppression of follicular-phase serum progesterone less than 2 nmol/L (0.6 ng/mL) and early morning 17-OHP below 8.0 ng/ml

and androstenedione within normal limits [22, 56, 58]. Hydrocortisone 20–25 mg/day or prednisone 2.5–5 mg/day is recommended for maintenance once pregnancy occurs.

Pregnancy in women with classical CAH and NCAH should be closely monitored, in order to avoid over- and undertreatment, if glucocorticoid and/or mineralocorticoid therapies are given [4].

Changes in Hormonal Values

During pregnancy, women with 21-OHd show a significant increase of steroids hormones, which should be taken into account when interpreting results using trimester-specific reference intervals for healthy women. In addition, changes in hormonal levels vary with time in this population. For instance, 17-hydroxyprogesterone levels rise progressively, while androstenedione levels rise up to 80% by the end of the first trimester and subsequently remain steady until the end of the gestation. Moreover, progesterone levels are four times lower in the first trimester as compared to the third trimester of gestation [59]. As maternal 17-OHP is also elevated in normal pregnancy, it is no use in monitoring glucocorticoid treatment in 21-OHd CAH.

Treatment During Pregnancy

During pregnancy, hydrocortisone and prednisolone represent the most appropriate treatment options in women with CAH, since both are metabolized by placenta and exert no adverse effect on foetus [18, 60–62]. Treatment dose varies according to gestational age. Progesterone, due to its anti-mineralocorticoid effect, necessitates the use of higher doses of fludrocortisone during the third trimester [63]. In addition, daily hydrocortisone dose can be increased by 20–40% (5–7.5 mg) in the third trimester, if the pregnant CAH case exhibit signs and symptoms of adrenal insufficiency [64].

Complications During Pregnancy

Overtreatment with glucocorticoids in pregnancy can lead to gestational diabetes, hypertension, excessive weight gain, bruising, oedema and pre-eclampsia. On the other hand, in case of undertreatment, hyperemesis and adrenal crisis may occur [65]. If adrenal crisis occurs during pregnancy, management do not differ compared to non-pregnant patients [4].

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Prenatal Diagnosis and Treatment in Congenital Adrenal Hyperplasia

Zehra Yavas Abali and Tulay Guran

Introduction

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive disorders caused by enzymatic defects in adrenal steroidogenesis. Cortisol deficiency causes elevation in adrenocorticotropic hormone (ACTH) concentrations and overproduction of adrenal androgens in virilizing forms of CAH [1]. 21-Hydroxylase deficiency (21-OHd) is the most common type and constitutes for more than 90% of CAH.

The microsomal enzyme 21-hydroxylase (P450c21) is encoded by *CYP21A2* gene located on chromosome 6p21.3 region. Recombinations between the active gene *CYP21A2* and pseudogene *CYP21A1P* are responsible from most of the mutations. *CYP21A2* and *CYP21A1P* have a distance of 3.1 kB, and they are highly homologous with a sequence similarity of 96–98%. Unequal crossing over during meiosis can result in duplications or deletions in the active gene. Gene conversions can transfer deleterious mutations from *CYP21A1P* to *CYP21A2* and may induce enzymatic defects. Due to these chromosomal arrangements of the active gene and pseudogene, deletions and gene conversion events are fairly common in 21-OHd [1–3]. Consequently, complexity of this locus makes genotyping difficult in 21-OHd. Accuracy of molecular diagnosis in CAH, particularly for 21-OHd, is crucial for appropriate genetic counseling of families for their future offsprings. It is recommended that genetic testing for *CYP21A2* must be provided by certified molecular laboratories with the experience to analyze this gene [4].

Z. Yavas Abali
Department of Pediatric Endocrinology, Pendik Research and Training Hospital, Marmara University, Istanbul, Turkey

T. Guran (✉)
Department of Pediatric Endocrinology and Diabetes, Marmara University, School of Medicine, Istanbul, Turkey

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In females with virilizing CAH, like 21-OHd, 11β-hydroxylase deficiency (11β-OHd) also causes genital ambiguity via the increase in adrenal androgens [5]. Adrenocortical function arises about the 7th gestational week (gw), and a female fetus with CAH is exposed to elevated concentration of androgens at a critical time of sexual differentiation between the 8th and 12th gw [6]. This prenatal androgen exposure results in clitoromegaly, labioscrotal fusion, and common urogenital sinus. Therefore, sex assignment may be difficult, and multiple corrective genital surgeries may be required in the postnatal period [7]. Suppressing adrenal androgens in female fetuses with CAH has been of a major interest for a long time [8].

Prenatal diagnosis and treatment of CAH and long-term outcomes of prenatal treatment will be reviewed in this chapter.

Pretenatal Diagnosis

Couples who are at risk of having a child with CAH often ask for genetic counseling for their future offsprings. An accurate diagnosis of CAH requires correct molecular genetic diagnosis of the index case in the family. The risk of transmitting disease to a potential fetus, all the updated information about the disease, and its consequences should be provided to the parents during counseling.

The aim of prenatal diagnosis in CAH is to commence prenatal treatment in early gestation to prevent virilization of external genitalia in affected female fetuses. Eventually, the need for genital surgery and the risk of sex misassignment and gender confusion may be reduced. Until today, several approaches for prenatal determination of fetuses with CAH have been used. Owing to advances in molecular genetic methods, more-targeted approaches will increasingly become available.

Hormonal Measurements in Amniotic Fluid

Improvement of techniques in culturing fetal cells from amniotic fluid and implementation of amniocentesis (AS) has enabled present-day prenatal diagnosis [1, 9]. Previously, diagnosis of CAH had been based on hormonal measurements of amniotic fluid. In early studies, excess amniotic fluid 17-ketosteroids and pregnanetriol concentrations had been detected in a 40th gw affected fetus [3, 10]. The technique had been improved by detection of elevated testosterone and 17-hydroxyprogesterone (17-OHP) in the amniotic fluid of affected fetuses in the second trimester [11]. Amniotic fluid androstenedione and 21-deoxycortisol concentrations had also been additional useful assays [12]. Because the AS is performed later than critical period of sexual differentiation, this approach does not prevent the virilization of a female fetus, so it is no longer applied. Other limitations are unreliability if the mother is treated with dexamethasone (androgen production is suppressed in fetal adrenal), and 17-OHP may not increase significantly in some types of 21-OHd [6].

HLA Haplotyping in Amniotic Fluid

CYP21A2 and *CYP21A1P* are located in close proximity to the major histocompatibility complex (HLA). Before the cloning of *CYP21A2* gene, HLA typing had been the preferred technique for the diagnosis of 21-OHd prenatally. This technique is based on the genetic linkage disequilibrium, by which proximally located genes are inherited together [6, 13]. The first prenatal diagnosis of CAH by HLA typing of fetal cells had been reported in 1979 [14, 15]. This technique cannot be applied before the second trimester due to the requirement of AS. Since genital organogenesis has already occurred at this period, the possibility of treating an affected female fetus and preventing virilization is missed [16–19]. The HLA haplotyping had also been not useful in cases with a misdiagnosed proband or without a proband [18]. Intra-HLA recombination events unlinking the HLA and the *CYP21A2* gene had been reported, as well. Some HLA alleles are expressed weakly in cultured amniocytes [6, 18]. Therefore, the HLA typing procedure is no longer applied.

Molecular Genetic Analyses

Sequencing of the *CYP21A2* gene is frequently used for the molecular diagnosis of 21-OHd in the postnatal period. However, for prenatal diagnosis, only when the mutation in a previously affected child or parent is clearly identified, *CYP21A2* sequencing procedure becomes reliable.

Congenital adrenal hyperplasia can be diagnosed by chorion villus sampling (CVS) at 10th to 12th gw and by AS at 16th to 20th gw. The former is the currently preferred procedure over AS that allows the diagnosis in the first trimester of pregnancy. The earliest possible prenatal diagnosis using these methods is later than 9th gw, meaning that male and unaffected female fetuses will also be treated unnecessarily. In addition, both AS and CVS are invasive procedures, with a risk to the mother and fetus to a certain extent. Therefore, CAH must be diagnosed before the start of genital organogenesis, so that only the affected female fetus will be treated [5].

The course of sexual differentiation in a female and application of diagnostic procedures according to gestational weeks are illustrated in Fig. 1.

Recent advances in *CYP21A2* genotyping have made molecular analysis of fetal DNA, the ideal diagnostic method for fetuses at risk for 21-OHd, possible [16]. During prenatal diagnosis, in a small percentage of cases, problems occur mostly due to undetectable mutations, allele dropout (ADO), or maternal DNA contamination [16, 20, 21]. Analysis of a CVS material may create a false-positive or false-negative result if the fetus and placenta are discordant genetically. However, this is not a problem in AS because fetal cells that have been shed into the amniotic fluid are directly evaluated [22].

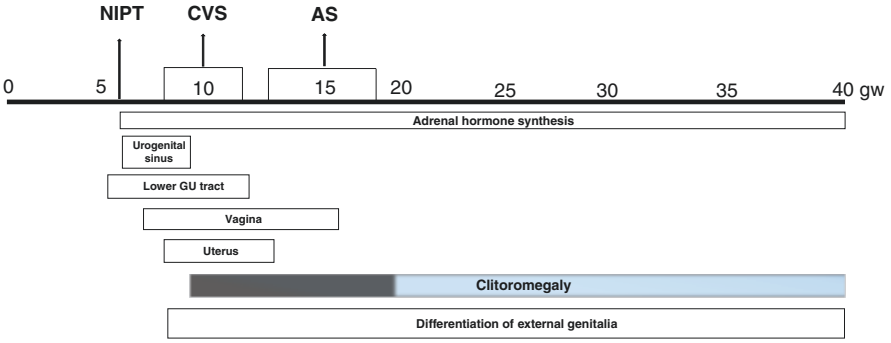

Fig. 1 Sexual differentiation in a female. Adrenocortical function arises about 7th gw. NIPT by using cell-free fetal DNA in maternal plasma allows earlier diagnosis and treatment initiation around 6–7 gw. By using invasive diagnostic procedures like CVS or AS, time of treatment initiation coincides after the external genitalial differentiation starts.*gw* gestational week, *NIPT* noninvasive prenatal testing, *CVS* chorion villus sampling, *AS* amniocentesis, *GU* genitourinary

Cell-Free Fetal DNA (cffDNA)

Prenatal treatment in CAH should be commenced before the differentiation of external genitalia before 8th gw. Noninvasive prenatal testing (NIPT) including cell-free fetal DNA (cffDNA) from maternal blood is a promising procedure that can be applied for the prenatal diagnosis of 21-OHd [2].

Lo and colleagues [23] have been the first to describe the high concentration of cffDNA in maternal plasma. In early periods of gestation, a mean 3.4% of total DNA in maternal plasma is of fetal DNA. In maternal blood, fetal DNA molecules circulate among a background of maternal DNA sequences. Because the maternally inherited fetal allele is genetically identical to maternal sequences, the allele that the fetus inherits from the father is fetal-specific sequence in maternal blood.

Fetal genotype can be assessed through detection of a unique molecular characteristic of paternally inherited fetal allele by using cffDNA. Y chromosome or the sex determining region Y (*SRY)* gene, and paternally inherited mutations or polymorphisms are examples of these unique features. If the parents carry a different mutation, prenatal assessment of CAH may be provided by excluding the paternal mutation. Furthermore, detection of a wild-type allele in the father may rule out the disease, if the parents have the same mutation [16].

The first successful sex determination in a fetus at risk for 21-OHd has been reported by Rijnders and colleagues by the detection of *SRY* gene in maternal blood [24]. Bartha and colleagues [25] have accurately identified the male fetus as early as 6th gw. An algorithm for prenatal diagnosis has recommended *SRY* testing at 5th gw. Serial testing is suggested until 11th gw or until a male fetus is detected in two different samples. If the fetus is found to be 46,XY, then CVS is not required [26]. This protocol has the advantage of reducing the need for invasive diagnostic procedures and also the time of exposure to unnecessary treatment in male fetuses [16].

In some reports, prenatal sex determination by *SRY* can be determined as early as 4 weeks and 5 days [27]. Although reports of sex determination by NIPT using cffDNA show great promise, confirmation of the results is necessary before such a protocol can routinely be applied.

Fetal sex determination is a relatively clear procedure and depends on the presence or absence of Y chromosome sequences [2, 27]. However, sex determination only avoids the unnecessary treatment in males, not in unaffected females. Molecular genetic diagnosis is necessary to prevent treatment of unaffected female fetuses. Mutation detection is technically difficult because most of the mutations in *CYP21A2* are caused by gene conversion events, so they are also present in fetal (and maternal) DNA. Next-generation sequencing (NGS) technology has been used for the detection of single-nucleotide polymorphism (SNP) haplotypes around the *CYP21A2* gene. By using this technique, millions of reads of small fragments (100 bp) are created and organized into longer sequences. It is straightforward to detect paternal alleles if they are not present in maternal plasma. To identify the maternal allele is difficult because the maternal allele of the fetus is present only in 2% excess over the other allele. High-sequencing depth is necessary for correct quantitation, but this is expensive and technically difficult by whole-genome sequencing (WGS). According to recent approaches, a few hundred kilobases around the *CYP21A2* locus have been amplificated [28] or DNA has been captured from the target region before sequencing [29]. Both of these decrease the amount of sequencing. Fetuses as early as at the 8th gw have been accurately genotyped. These techniques are still in improvement phase but evolving rapidly.

Validation of targeted massively parallel sequencing (MPS) for noninvasive diagnosis of CAH using cffDNA has been first reported in 2014 [30]. In this study, 14 families, each with an index case with classical 21-OHd and parents with at least one mutant *CYP21A2* allele, have been evaluated using NIPT. It has been concluded that the fetal sex and CAH genotype are correctly detected by using this method. In a family, CAH status has been detected from maternal plasma as early as 5 weeks and 6 days. It is also suggested that this new noninvasive technique will prevent risks caused by invasive procedures like CVS or AS. The superiority of this method is the treatment of the affected female fetus only, thus preventing unnecessary treatment in unaffected fetuses [30]. It should be emphasized that cffDNA testing does not fully replace the invasive diagnostic procedures and confirmation is still required [31].

Massive parallel sequencing is more complicated than detecting only specific *CYP21A2* mutations. This technique necessitates targeted MPS of genomic DNA from the affected proband and parents. The SNPs on both sides of the *CYP21A2* locus allow to build haplotype blocks that are required to detect paternal and maternal allele characteristics. Dosage analysis quantifies the DNA inherited from the father and the mother, taking into account the mother's own alleles as well as those she has transmitted to the fetus. If the fetus has inherited a CAH-linked haplotype, a linked SNP will show more DNA than if not [32]. It is difficult to differentiate the fetal and maternal alleles in the maternal blood. These technical problems limit extensive use of cffDNA for the diagnosis of fetal monogenic

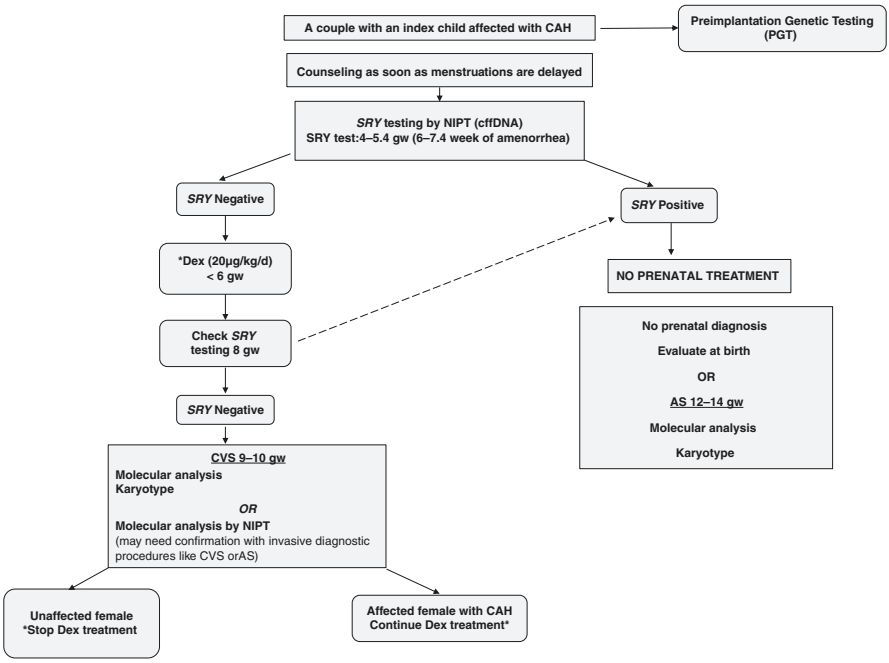

Fig. 2 Illustration of an algorithm for prenatal diagnosis and treatment of CAH combining NIPT with invasive diagnostic procedures. *Prenatal dexamethasone treatment is still a controversial and experimental treatment. Therefore, a specific treatment protocol is not routinely recommended by clinical guidelines. *CAH* congenital adrenal hyperplasia, *NIPT* noninvasive prenatal testing, *PGT* preimplantation genetic testing, *gw* gestational week, *Dex* dexamethasone

disorders [33]. However, there have been recent successful reports about sequencing the fetal genome using cffDNA as early as at the first trimester [32, 34]. As genomic sequencing technologies evolve, challenges such as accurate identification of the fetal alleles will be defeated. Diagnosis of single-gene disorders like CAH from cffDNA in the maternal plasma is an exciting improvement in fetal genetic diagnosis, despite ethical and technical challenges in interpreting fetal genome data [32, 33].

The protocol for prenatal diagnosis and treatment of CAH combining NIPT with standard invasive diagnostic procedures has been described in Fig. 2.

Preimplantation Genetic Testing (PGT)

It has been about 30 years after the first report of preimplantation genetic testing (PGT) in human embryos [22, 35]. Preimplantation genetic testing for monogenic diseases (PGT-M, previously preimplantation genetic diagnosis, PGD) offers a

precious reproductive option for families at risk of having a child with a single-gene disorder like CAH [36]. Genetic abnormalities have been determined in preimplantation embryos before the embryo transfer in PGT, so that only unaffected embryos are transferred [16]. There are three PGT approaches, (1) polar body biopsy, using female gametes (oocytes); (2) blastomere biopsy, using the 3-day, 6- to 8-cell cleavage stage embryo; and (3) trophectoderm biopsy, using the 5-to 6-day blastocyst (contains ~120 cells) [37].

Polar Body Biopsy

Genotype of the oocyte can be determined by the analysis of the first and second polar bodies [38, 39]. In PGT for CAH, the first polar body from a heterozygous carrier mother having a mutant allele should be complemented by a primary oocyte having the normal allele. Oocytes with normal genetics can be fertilized in vitro and transferred. First polar body biopsy can occur before fertilization, thus offers the possibility of preconceptional diagnosis. The second polar body is not a preconceptional biopsy since it is not extruded until the oocyte is fertilized.

Recombination events must be considered when diagnosing monogenic disorders. If recombination between sister chromatids does not occur, the two chromatids of a single chromosome in the first polar body will be identical and complementary to the oocyte containing the homologous chromosome. The second polar body (chromatid only) will thus be identical in genotype to the oocyte. However, if crossover involves the chromosomal region containing the chromosome 6 and the 21-hydroxylase locus, the single chromosome in the first polar body will show a different allele on each of its two chromatids (heterozygosity). Biopsy of the second polar body will be necessary in this condition, because the genotype of the segregated oocyte cannot be predicted. For this reason, in almost all centers, both the first and second polar bodies are biopsied [39].

Paternal genotype cannot be assessed by polar body biopsy, so there will be loss of efficiency if the paternal genotype transmitted to the fetus cannot be evaluated. However, carrying a wild-type maternal allele will mean that the fetus is at least heterozygous (unaffected). Description of the normal maternal locus will rule out an affected embryo.

Cleavage Stage Embryo Biopsy

The zona pellucida (glycoprotein layer surrounding the cleavage stage embryo) is breached by mechanical, laser, or chemical means to extract a cell containing DNA (blastomere). Only a single cell is typically removed because even one fewer cell at this stage is believed to reduce survival of the embryo as much as 10% [39, 40].

Blastocyst Biopsy

The cleaving embryo (morula) has enlarged by 5–6 days (cell number ~120). The embryo exists in the form of a *blastocyst*. The inner cell mass (embryo per se) is readily differentiated from the trophectoderm (placenta per se). The blastocyst biopsy is the preferred procedure in PGT. At this stage, biopsy is technically easier compared to cleavage stage or polar body and more DNA can be obtained [41].

Importantly, specific markers around the gene of interest having the pathogenic variant are used to screen for the presence of the pathogenic variant itself rather than sequencing. Embryos that carry either no copies of the affected allele or only one copy (indicative of carrier status) can then be implanted [42].

When PGT is applied for single-gene disorder like 21-OHd, SNPs or short tandem repeats (STRs) should be used for linkage analysis. This is required to exclude allele dropout (ADO), that may include the mutant allele. Explanations for ADO are dsDNA breaks or failure of host DNA to anneal with its intended primer. This problem will cause the conclusion that the single allele has been the only allele present in embryo. If ADO is involved in a mutant recessive allele and has not been recognized, the embryo will be assumed as (homozygous) normal. The ADO results from preferential allele specific amplification and can lead to misdiagnosis of the genetic status of the oocyte/embryo [43]. Therefore, PGT procedures should include several linked polymorphic microsatellite markers flanking the disease gene in order to reduce these problems [44].

Invasive diagnostic testing to confirm the results of PGT is strongly encouraged because the methods used have technical limitations that include the possibility for false-negative results [22].

Although prenatal diagnosis of 21-OHd CAH is the most studied and reported one, the same molecular approaches and PGT procedures may be applied for other types of CAH, including 11β-OHd, 3β-hydroxysteroid dehydogenase deficiency (HSD3B2), and for other rare forms.

Prenatal Treatment

Considerable interest has focused on prenatal treatment of CAH, particularly on 21-OHd, for many years. Nevertheless, prenatal treatment is still regarded as controversial and experimental for several reasons.

Treatment of a mother during pregnancy to reduce virilization of a female fetus with CAH had been first reported by David and Forest, in 1984 [45]. Dexamethasone is the preferred glucocorticoid for prenatal treatment because of its long half-life, its ability to cross the placenta (not metabolized by placental 11β-HSD2) and suppress fetal ACTH secretion, consequently decrease androgen concentrations.

The original experimental approach for prenatal treatment of 21-OHd has been to use dexamethasone in early pregnancy (before 6th gw). This approach requires

approval of parents who are heterozygous for a *CYP21A2* mutation. After fetal sex determination by AS or CVS, treatment can be terminated in 46,XY fetuses. As 21-OHd is a autosomal recessive disease, one in four children may be affected and only one in eight are affected females. Therefore, seven of eight fetuses will be unnecessarily treated during early stage of development.

In prenatal treatment protocol, the recommended dexamethasone dose is 20 μg/kg/day for maternal prepregnancy weight (maximum 1.5 mg/day). For a 60-kg mother, this dose is 1.2 mg/day, and it is approximately six times more than the physiologic dose [46, 47]. Protocols using lower dexamethasone have not been reported for prenatal treatment, and it is still not clear why such a high dose has been suggested [27]. Cortisol concentration of the fetus is low in very early gestation, rises during 8th to 12th gw during external genitalial differentiation [48], and it is only about 10% of maternal levels during midgestation [46, 47], and then increases during the third trimester. This high prenatal treatment dose results in fetal levels 30–60 times higher than normal and may cause elevation in fetal glucocorticoid concentration exceeding physiological midgestation levels [49, 50].

In a pregnancy which the fetus is treated until term, the efficiency of the treatment is monitored with maternal serum DHEAS (from 7th gw) and estriol measurements. Low DHEAS concentrations mean adequate fetal adrenal suppression, whereas low estriol concentration is a sign of maternal adrenal suppression and compliance [51]. It has been reported in several studies that treated infants with CAH have been less virilized than their untreated affected sisters, if their treatment have been initiated on time and mothers are compliant to treatment [51, 52].

To prevent genital virilization in 21-OHd or 11-OHd females, treatment must be started before the start of genital sensitivity to androgens, latest at the 7th gw or 9th week of amenorrhea [4]. This protocol results in unnecessary steroid treatment in seven out of eight potential fetuses, since fetal genotyping is usually not possible before 10–12 gw [8, 53]. The timing of initiation of dexamethasone plays an essential role in genital morphology of affected females with CAH [27]. It is suggested that dexamethasone must be continued until birth in female fetuses with CAH in order to be effective [27, 54].

The potential benefits of dexamethasone treatment are prevention or reduction of virilization of external genitalia and brain and the need for multiple corrective surgeries in the postnatal period. This treatment should be offered only to the parents who are heterozygotes with an affected child with CAH. Prenatal diagnosis cannot be performed before 6–8 gw of pregnancy while fetal sex determination may be performed from maternal plasma by analyzing cffDNA, avoiding dexamethasone treatment or early cessation of therapy in male fetuses. In female fetuses, CVS at 12–13 gw will allow the diagnosis of CAH [4].

Animal studies have shown that in utero early exposure to dexamethasone may cause adverse neurodevelopmental effects. However, human studies are less clear. Possible relationship between prenatal dexamethasone use and neuropsychological development has been reported in several studies but still no consensus has been reached [55].

Exposure to dexamethasone from 7th to 12th gw overlaps with neurogenesis and neuronal migration period [53, 56]. Epigenetic processes may also have long-term programming effects on the development of brain. Therefore, there are still questions regarding behavioral and developmental effects of prenatal dexamethasone use. Although, a Swedish study has reported good school performance and psychological well-being, concerns about memory and gender behavior are not clearly described [55, 57]. In unaffected children who have been treated prenatally short term with dexamethasone, impaired verbal working memory and social anxiety have been described [55]. A larger follow-up study analyzing cognitive outcomes in fetuses exposed to dexamethasone has not demonstrated impaired working memory. However, this study has found slower mental process in prenatally treated females with CAH compared to controls [58].

Previous researches have mostly interested in the second and third trimester effects of steroids on neurodevelopment. Synthetic glucocorticoids used to induce fetal lung maturation in fetuses at risk of preterm birth during third trimester have been shown to associate with reduction in the thickness of the rostral anterior cingulate cortex [59]. Higher prenatal maternal cortisol concentrations have been reported to be with decrease in fetal brain growth, and altered functional and structural connectivity during childhood [60–62]. The amygdala develops at early stages of fetal life, and it is especially vulnerable to early abnormalities in cortisol concentrations. Increased amygdala volume is associated with depression risk in girls [53, 62, 63].

In a recent study, the effect of first trimester dexamethasone treatment on brain structure alterations in adult age has been investigated [53]. The MRI scans of treated cases at risk of CAH but not having CAH (males and unaffected females) have been compared with the controls. It has been reported that first trimester dexamethasone exposure has been related with structural alterations of brain at adult age and gene methylation changes have also been detected. Altered methylation of genes associated with brain development such as brain derived neurotrophic factor (*BDNF*), glucocorticoid receptor (*NR3C1*), glucocorticoid receptor (GR) co-chaperone *FKBP5* and mineralocorticoid receptor gene (*NR3C2*) have been demonstrated in dexamethasone-treated individuals without CAH, as well [64]. Change in the methylation of some of these genes has been associated with depression [65–67]. Owing to effects of synthetic steroids on prenatal programming, alterations in brain structure may occur. Since the cognitive function and mood regulation depend on networks with high density of GR, prenatal treatment may have deleterious effects. These findings add to the safety concern of prenatal dexamethasone treatment in CAH.

Recent clinical guidelines report that prenatal dexamethasone treatment is an experimental treatment due to the safety issues. Therefore, specific treatment protocols is not recommended by Endocrine Society guidelines [4]. Prenatal treatment must be offered after Institutional Review Boards approval, at centers capable of collecting data on a large number of CAH cases; thus, risks and benefits of this treatment can be more clearly identified.

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Psychological Evaluation of Patients with Congenital Adrenal Hyperplasia (CAH)

Loukas Athanasiadis

Introduction

Congenital adrenal hyperplasia (CAH) is a condition that may have great impact on patients, family, and social environment and poses diagnostic and therapeutic challenges to the health services. The quality of life may be low in some patients with CAH. Several aspects of the patients' lives can directly or indirectly interfere with fertility and reproduction issues and with their plans to gestate. The degree of the interference for each contributing factor may vary from person to person, and the interaction of several factors produces the final outcome.

Researches on fertility issues in patients with CAH are usually short-term, and the results are sometimes inconclusive or controversial. There is also more information on females with CAH than on males.

The psychological evaluation of patients with CAH should cover a wide spectrum of issues, including sexuality and fertility. Psychological and psychiatric morbidity should be explored and treated.

Fertility issues may not necessarily be a top priority for patients and health specialists. However, there are many patients with CAH who are concerned about this issue and do desire to mother or father a child. An exploration of the factors which potentially interfere with fertility will allow health professionals to discuss this issue with the patients and their partners and plan a proper and potentially effective management.

The main factors which may interfere with or are relevant to fertility and reproduction will be discussed under subheadings listed below.

L. Athanasiadis (✉)
1st Department of Psychiatry, Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
e-mail: loukatha@outlook.com.gr

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Medical Management and Impact on Sexual Function

Impact of the disease itself and the impact of unsatisfactory medical management may impair sexual function and potentially interfere with fertility.

Unsatisfactory vaginal reconstruction and reduced clitoral sensitivity may cause sexual difficulties and sexual dysfunction and may have an effect on partner choice, relationships, marriage, and fertility [1].

Suppression of androgens following glucocorticoid overtreatment may result in low sexual desire [2]. Problematic intercourse due to gonadotrophin suppression and subsequent anovulation due to overtreatment, adrenal androgen excess with resultant anovulatory infertility due to undertreatment, elevated follicular phase progesterone levels that prevent implantation, and inadequate vaginal introitus which makes penetration difficult are the causes of low fertility rates in CAH [3–8].

Consequences of treatment may not be limited only to sexual implications, and some details are not always understood. The impact of dexamethasone treatment on brain of the fetus (for instance, on working memory), administered to prevent intrauterine virilization of female external genitalia in pregnancies at risk of CAH, is still not well understood [9].

Sexual function may also be impaired by other reasons (somatic and psychiatric comorbidity, relationship/marital problems, side effects of medications). Regular monitoring of CAH patients by a multidisciplinary team is recommended in order to offer assistance [10].

Quality of Life

Quality-of-life parameters may be associated with sexual function and fertility issues. Data on health-related quality of life (HRQoL) in patients with CAH are rather scarce and mostly refer to females [11]. Their interpretation often produces conflicting results. Most studies report worse QoL [12, 13], and some similar [14, 15] and very few better QoL [16, 17] compared to controls. Impaired QoL appears to be more common in adults than in children and may be associated with more severe forms of the disorder [18].

The QoL may be diminished when disease, the consequences of medical treatment, stress, and other factors cause sexual dysfunction [19]. Psychological support may improve QoL [20]. New treatments are also expected to improve health outcomes and QoL in patients with CAH.

Female Patients with CAH

Female patients with CAH may face serious sexual, psychosocial, and interpersonal difficulties. Fertility may be impaired. Researches indicate that the cause of infertility in these patients is often multifactorial. Many women, especially those with the

most severe mutations, may not try or are not interested in achieving pregnancy [21]. Some do not think that fertility is an important issue [22]. The reasons for this are not always clear.

The psychological effects of CAH, for instance, play behavior, spare time interests, sports, choice of profession, difficulties in sexual function, sexual interest and orientation, reduced fertility, and being happy with existing sexual life, all exhibit some degree of severity correlating with the disease severity (depending on CYP21A2 genotype, prenatal androgen exposure) [23]. Even though genotype is usually considered to be an indicator of the degree of virilization, some females with I2splice genotype may exhibit external genitalia with Prader score V and are more severely virilized than the ones who harbor the null, the more severe genotype [23]. Genotype–phenotype correlation may not always be fully observed, and this is challenging for the treating physicians.

Female patients with CAH may have impaired fertility, especially if they belong to the classical types; simple virilizing and the salt wasting CAH [24, 25]. Fertility may be impaired due to a number of reasons [20]. High prevalence of polycystic ovaries leading to ovulatory dysfunction, inadequate adrenal suppression of androgens, and elevated follicular phase progesterone levels resulting in failure of implantation may be the contributing factors [26]. The low libido of these women, fewer heterosexual relations, and diminished desire to engage in having a family can be considered as other responsible factors [27, 28]. However, most women with CAH can become pregnant with proper medical treatment [21]. Adrenalectomy may rarely be required for maintaining reduction in progesterone levels that is necessary for achieving pregnancy [29].

Non-somatic reasons may be important. In CAH women with reduced sexual interest or sexual activity, the presentation is probably not related to the early exposure to androgens which would otherwise exert greater sexual interest and sexual activity. It may be related to the fact that many of these women are not happy with their genital reconstructive surgeries that cause inadequate vaginal introitus, thus unsatisfactory, sometimes painful sexual intercourse [30].

Some girls with CAH have low interest in infants, nursing and childcare [31]. It has been hypothesized that the awareness of reduced fertility and having a chronic illness may play a role. However, there are women with CAH who do want to get pregnant and have a child [30]. Some authors conclude that the lower pregnancy and delivery rates in women with CAH are mainly due to psychosocial factors [21].

It has been indicated that psychosexual factors in women with CAH may affect behavior, sexual orientation, and core gender identity. Factors that may play a role include the amount and timing of prenatal exposure to androgens, genetic factors, age at diagnosis of the disorder, effectiveness of postnatal hormonal treatment, the age and success of feminizing surgery, psychosocial support, the attitude of the parents, and sociocultural factors [32].

Female CAH patients in developing countries may face with similar problems as women in developed countries; however, local sociocultural issues and limited availability of resources may cause additional difficulties. Lack of education, poverty, lack of proper and affordable medical care, traditional beliefs on sexuality,

limited community support, religious and cultural issues are common problems. Reports from Malaysia and Vietnam support this view [33].

A recent study on 13 Swedish women with CAH [34] investigating the identity, sexuality, and parenthood issues has focused on the topics "forming identity" (girl, tomboy, and/or a woman) and "establishing relationships" (intimate relationships, pregnancy, and parenthood). One of the main findings of this investigation is that sexual activity with a partner can be avoided, problematic, or uncomplicated among these women. Self-confidence seems to be an important determinant of this finding. Some women have reported their will for achieving pregnancy, but have also expressed their confusion about its possibility and the fear for having children. In the same study, participants have also interpreted their same-sex sexual orientation as a part of their CAH. Poor body image and not feeling gender typical seem to prevent intimate relationships. Being unable to compete with the "perfect woman" and the fear of unsatisfactory sexual intercourse via offering penetrative sex have been pointed as common problems. Education and referral to a sexology service seem to be of great help to these women [34]. This study has also demonstrated that CAH has been the main focus in the lives of some participants, whereas it has been a side issue for the others. The experiences of these women have varied, and they reflect shifting perspectives on how much the disease affects several aspects of their lives [34].

There are also interesting findings of some studies. General psychosocial adaptation, as compared to siblings, may not be different in these cases [35]. Self-reported health-related quality of life may be adversely affected, especially in women [12, 36–38]. Sexual function may also be impaired [8, 22, 39]. Women with CAH often prefer living alone [15]. Fertility may be impaired in both men and women [36, 40], although pregnancy rates are rather normal for those who receive medical care. The majority of men who seek medical care may finally father a child [40].

In conclusion, the etiology of infertility in CAH females is multifactorial, including ambiguous genitalia issues, excessive androgen secretion, hypersecretion of adrenal progesterone, PCOS, and several psychosocial factors [41]. Assisted reproduction has to overcome the androgen excess-associated anovulatory cycles and high circulating progesterone concentrations during the follicular phase. Glucocorticoid treatment alone may be very helpful for achieving pregnancy [41]. When pregnancy occurs, the pregnant CAH patient needs careful and close monitoring, preferably at a tertiary care service [10].

Male Patients with CAH

Difficulties about living with a rare chronic disease, school absences due to frequent medical procedures, consequences of glucocorticoid over or under treatment (precocious puberty, obesity, shorter final height, etc.), possible androgen effects on brain development and behavior, risks for reproductive health, and several

psychosocial and emotional issues are the factors that impact on the well-being of an individual with CAH [42–43]. Investigations on males with CAH are rather scarce. However, most of the findings gathered from females with CAH apply for males. Some studies present methodological problems and the need for further research about CAH males is evident [44].

Male sexual health may be impaired by a number of emotional and psychological factors [45]. An association has been reported between CAH and mood disorders. Behavior disturbances, anxiety, insomnia, mood disorders, and psychosis have been described resulting from treatment with glucocorticoids, as well [46]. Hyperandrogenism itself may also increase the risk of developing psychological/psychiatric pathology [47].

Self-perception of reproductive health, psychological and psychiatric health, and quality-of-life parameters have been studied on male CAH patients in a review of 11 studies [44]. Accordingly, these males have had higher negative emotionality and many problems related to internalizing behaviors (negative behaviors directed toward the self) when compared with the reference groups. Three of these studies have investigated the impact of sexual health issues and fertility on psychological health and have found that sexual well-being has ranged from impaired to normal among them. Five of six studies have reported reduced QoL ratings. However, the authors of this review have reported several methodological limitations and weaknesses in some of these studies [44].

Males with CAH and testicular adrenal rest tumors (TARTs) may suffer from intense psychological strain. The TARTs and inadequate control of the disease (under-treatment or non-compliance) may also contribute to fertility difficulties [41, 48–52]. In general, fertility in males with CAH appears to vary from normal to severely impaired when compared to control groups or to relevant national data [41, 48, 51].

A study on males with CAH has described impaired sexual well-being, the dimensions of "sexual drive," "erections," and "ejaculations." "Problem assessment" and "overall satisfaction" scores have been within the normal range [53]. In another study, fewer CAH male patients have been found to be sexually active; however, sexual satisfaction has been similar to the control group [45].

Although females with CAH are known to present the impact of androgens on several domains such as gender typical play and sex-typed toy preference, spatial abilities, aggression, occupational interest, gender identity, and sexual behavior, less is known about psychological consequences of CAH in men, assuming that they are exposed to normal androgen levels in the womb.

Sexual problems have been less frequently reported by the most severely affected CAH males [54]. They have been found to associate with psychological problems, such as anxiety and depression. Sexual orientation is generally not affected. Heterosexual orientation has been reported in 90.1% of participants, homosexuality in 3.3%, and bisexual orientation in 1.1%. More than 50% of males with CAH with a sexual partner have reported to have almost daily or at least one to two sexual activities per week [54]. Another study has found the frequency of intercourse

similar to matched controls [40]. The males have been generally happy with their sex life, having a comparable score to reference population [54]. Approximately 25% have had low/absent or excessive sexual desire. Arousal or orgasmic difficulties have been reported by 10% of the participants and 25% have had premature ejaculation. Erectile dysfunction has been reported by a quarter of the patients [54]. The participants of this study have been recruited from specialized European centers, have had mostly good hormonal control, thus have exhibited similar aspects of sexuality compared to European reference populations. They have a similar profile of sexual problems, but their sexual desire has been lower compared to controls [54]. Androstenedione/testosterone ratio and the hormones investigated have not been found to associate with sexuality. Genotype has been found to impact on the frequency of sexual activity. The more severely affected cases have had lower frequency, however mainly due to psychological problems [54]. Early attention, even during childhood, has to be given to testicular functions in order to prevent detrimental consequences in the future [9].

Congenital adrenal hyperplasia impacts seriously on the life of the affected individual and the partner. Sexual orientation, sexual function, fertility, partnership, and many other issues need to be addressed and further investigated. The diagnostic and therapeutic approach needs to be flexible, realistic, and multidisciplinary.

Psychological and Psychiatric Issues

Psychological well-being is considered to be essential for sexual well-being, and it is an intergraded component of good health status. Mood may be altered in CAH patients for several reasons. Glucocorticoids prescribed as a pharmacological treatment and not only as a substitute may cause or exacerbate psychiatric symptoms, such as behavior disturbances, anxiety, insomnia, mood disorders, or even psychotic symptoms [44].

Patients with CAH may exhibit frequent psychological/psychiatric problems such as anxiety disorders, depressive disorders, and drug/alcohol abuse. Stigmatization, fear of rejection in a sexual situation, the effect of the awareness of their anatomical problems, potential high-risk behaviors, stress, and avoidance of sexuality issues have to be taken into account by the health professionals [55].

Sexual dysfunction and mental health morbidity often present a two-way relationship. Difficulties and doubts regarding the person's psychosexual role, psychiatric morbidity (mainly anxiety and depression), and side effects of psychiatric medication are the factors that may interfere with sexual desire and function in general, as well as in patients with CAH. The interplay between other contributing factors (effect of relationship difficulties, socioeconomic problems, etc.) may also play a detrimental role. Psychological and psychiatric screening and support must be available to CAH patients in order to offer proper care for their mental health.

Sexual Orientation

Definition of sexual orientation and the way we measure it appears to be a controversial issue. This is also evident in patients with CAH. Sexual development (including sexual orientation) is programmed in brain in intrauterine life. The presence or absence of androgens seems to play a major role in the direction (traditional male or female) of development. This is a useful concept in exploring the sexuality of patients with CAH, although it has been disputed by some authors.

A systematic review of 30 studies [55] has investigated the sexual orientation of individuals with CAH who have been either assigned female (46,XX) or male at birth (46,XY and 46,XX). The results have shown that compared to females from general population, the assigned females at birth (46,XX) are more likely to not exhibit an exclusive heterosexual orientation. No assigned males have identified themselves as non-heterosexual [55].

The severity of CAH may influence on the outcomes of sexual orientation. Methodological weaknesses and limitations of the studies should be taken into consideration at interpreting the results of regarding studies.

Sexual orientation is multidimensional and includes attraction, behavior, and self-defined identity aspects ("gay/lesbian," "bisexual," "heterosexual"). There seems to be a potential major biological basis for sexual orientation, although there are still many grey areas. As noted before, the prenatal effects of sex-atypical levels of androgens or estrogens may alter sexual differentiation. The sexual differentiation of human brain most probably depends on both sex hormone and sex chromosome processes which act either in parallel or in combination.

Females with CAH often tend to prefer rough play, choose hobbies the boys have, play with typical boys' toys, choose boys as playmates, exhibit certain male-typical personality features, and have a greater likelihood to have non-heterosexual orientation. Brain organization theory focusing on the effect of intrauterine exposure to sex-atypical steroid hormones may explain "tomboyish" behavior and sexual orientation in this particular group, however not in a definite way (not everybody with CAH is homosexual).

The mechanism is complicated and multifactorial. The degree of masculinization of the genitals does not necessarily reflect the degree of masculinization of the brain. There are also individual differences at varying androgen levels that affect the masculinization of brain.

Finally, physical, medical, psychological, and relationship factors may impact on sexual orientation of CAH patients. Potential social response, negative heterosexual experiences, negative reaction of a potential partner to the appearance of ambiguous genitals, postnatal effects of increased androgens, behavioral changes as a result of living with a chronic disease, impaired clitoral sensation, and vaginal introitus abnormalities that impair intercourse may all be listed as potential contributing factors. Even repeated genital examinations may also play a role. More research is needed to further explore the sexual orientation and related issues in this particular population.

Gender Issues

Gender identity is expected to play a role in making decision for having children; however, further research is required on patients with CAH.

"Gender identity disorder" is not common among these patients. A number of factors, such as hormonal control, familial, social, and religious beliefs have impact on gender identity in CAH.

Gender assignment is a major issue. In a study, gender assignment of patients with CAH is advised to be done as soon as possible [56]. Girls with high stages of Prader scoring mostly have female identity because they are usually in good control of their disease in spite of high intrauterine exposure to androgens. Hormonal milieu after birth seems to be more important than intrauterine hormones and chromosomal aspects for gender identity.

In addition to biological factors such as pre- and postnatal hormone levels, difficulties of parents to accept their child gender can have an impact on sexual identity issues in CAH patients [57].

Gender identity seems to be flexible, and prenatal exposure to high levels of androgens in CAH patients still allows successful adoption of a female identity [22]. However, some females with CAH may report less satisfaction with the assigned female sex.

Most female CAH patients do not present major gender identity problems, such as "gender dysphoria," which in a study on 250 patients, it has been reported only in 5% [58]. In the same study, nine out of ten female CAH patients who have been raised as males have reported male gender identity [58].

Adolescents

The necessity for daily medication, close supervision of the parents, and medical interventions may also restrict the psychosocial and physical development of children with CAH, like in other patients who harbor chronic disorders.

Adolescence is a time during which major hormonal, somatic, cognitive, and psychological changes take place [59]. Adolescents with CAH face additional difficulties and may need to make important decisions regarding medical and surgical interventions. The care of these patients may be a challenging issue. Decision-making issues, transition to adult care, good health care, and quality of life are some of the issues that health professionals should take into consideration.

In adolescence, increase in adrenal androgen synthesis, increased cortisol clearance, insulin resistance, and other hormonal changes may demand reassessment of CAH treatment to prevent complications in the future, such as infertility. Potential noncompliance with treatment poses additional problems.

Surgical considerations include problems arising from former genital surgery or/and related to the planning of subsequent surgical procedures. Adolescents may be very concerned about the appearance of their genitals and their sexual performance.

Surgery during infancy may cause or may contribute to a number of genitourinary difficulties, such as urinary incontinence, vaginal voiding, impaired sensation of clitoris or clitoral pain, vaginal stenosis, sexual dysfunction, and concerns about the appearance of the genital area. Special care on a multidisciplinary basis may be needed in order to manage these problems. The specific aspects of surgical approaches and selection of optimal timing are challenging and controversial issues. A shared decision-making process is recommended and requires a proper education of patients and family.

Talking to adolescents with CAH is a sensitive issue and should include educational and problem-solving components. Transition to adult care services needs to be smooth and well planned. The goals of transition include the provision of knowledge regarding medication, pathophysiology, stress management, surgical history/options, fertility, and sexuality issues.

In a study from Sweden, the girls with CAH, but not boys, have exhibited a higher risk of not completing primary education, compared to controls. There are many potential reasons which may, at least partially, explain this finding. Possible causes (some not limited to CAH) are cognitive deficits, learning difficulties, the effect of hypoglycemia together with salt-crisis, and over-treatment with high levels of hydrocortisone which may impair cognitive performance. The negative outcome for girls may also be due to more severe psychosocial problems they have to face with [60].

Adolescents with disorders of sex development (DSD) may have difficulties in managing sexual issues especially if they have experienced repeated genital examinations and unprofessional treatment by clinicians and health services or have atypical genital appearances. They may feel insecure in their psychosexual role, may fear from being rejected, and may postpone or avoid intimate relationships [61]. Thus, women with CAH are reported to differ from controls in terms of delayed psychosexual milestones; less sexual experiences, low sexual motivation, less sexual activity and imagery, less partnership/marriage, and poor sexual self-image [62–64].

Sociocultural Factors

Sociocultural factors may also play a role in deciding about fertility issues. Significant differences regarding employment, education, marital status, and fertility have been detected in patients with CAH depending on sex and severity of the disorder [60]. They are more often on sick leave, and they more likely receive disability pension. Women in salt-wasting CAH group, but not other women or men, are more often single. Men and women with classical CAH (SW and SV forms) have fewer biological children than controls [60]. The literature describes decreased fertility and interest in infants among women with the disorder. A high homosexual orientation rate in women with CAH may also be a contributing factor. More men than women have children, however at lower rates compared to the general population. The patients even in a stable relationship are less likely to have children for reasons that have to be further investigated.

Health Care Service

Proper care and good patient–health care professional relationship may facilitate the management of several concerns including sexual, gender, and fertility problems. Care should be adapted to age, personal situation, and other factors [9].

Caring for people with DSD is a crucial, challenging, and even controversial issue. A Consensus Statement, developed by a European multidisciplinary group of specialists, proposes several recommendations for individuals with DSD, including individuals with CAH [9]:

- Psychosocial care to the families should be individualized and provide empowerment, encouragement, and time in an atmosphere of acceptance. Information for parents includes biological, medical, and gender-related aspects. It should be given in a proper manner and language. Good communication and efficient contact with support groups should be also included. Psychological counseling should include discussion of decision-making and acceptance of individual development.
- Information to the individual with the disorder should include sex, gender, and other issues and must be provided on an individual basis in relation to timing and to inclusion of regarding topics.

There may be risk of various genetic or treatment-related comorbidities, and further research is needed for providing relevant data. Roles of the members of multidisciplinary team that will manage DSD issues may be challenging. Issues that also need to be managed include social acceptance, parent–child relationship, body image, sexual function, and well-being.

Psychological care is recommended to be provided from birth [65]. Transition of care from child to adolescent/adult services should be well planned. Health care must be longitudinal and be provided on a holistic basis. Good quality of life should be the primary goal.

Recent Endocrine Society Clinical Practice Guideline strongly recommends behavioral/mental health consultation and evaluation to address any concerns related to CAH for patients and their parents [18]. An interdisciplinary team of health care professionals with experience should provide "parent/family medical education, parent/family counseling regarding psychosocial prognosis, and managing parents' distress," "assistance in gender assignment at birth in cases of marked genital virilization," "discussion about the pros and cons of gender-confirming genital surgery in infancy and early childhood," and "counseling regarding potential gender reassignment of patients with 46,XX CAH after infancy."

Stigmatization (including stigmatization by lovers), gender-atypical behavior and related problems with social fit, homosexual and bisexual attractions, and sexual dysfunction should be addressed, as well [29].

Conclusions

Congenital adrenal hyperplasia (CAH) is a chronic condition that may have influence on many domains of patients' well-being. Impact of the disorder itself and its treatment, personal experiences, attitudes of the family and health care professionals, psychological and psychiatric comorbidities (mainly anxiety and depression), sexual function and orientation issues, body image and gender issues, relationship dynamics, and the impact of medical interventions (mainly surgery of genitals) are all interfering factors. The severity of the disease also has an important role.

Sexuality, gender, partner relationship, psychiatric status, fertility, reproduction, and relevant issues must be included in a holistic diagnostic and therapeutic approach. Studies in current literature are mainly focused on females, and methodological issues and limitations are not infrequent. Well-designed studies are needed in the future.

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