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REVIEW PAPER

A recent overview of non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency: pathophysiology, recognition, and management

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ABSTRACT

Congenital adrenal hyperplasia (CAH) is defined as a group of congenital, autosomal, and recessive disorders associated with functional limitations or deficiency of one of the key enzymes in the biosynthesis of steroid hormones leading to hypocortisolaemia and most frequently excessive androgen production. The most common abnormality is associated with 21-hydroxylase deficiency. Activity at the level of 20–70% of normal enzyme function leads to the development of a non-classic form of congenital adrenal hyperplasia (NCAH). In contrast to classic CAH, patients with NCAH manifest normal or partially mild cortisol deficiency and moderate hyperandrogenism without a deficit of mineralocorticoids. Due to nonspecific symptoms, NCAH is often never recognized or is misdiagnosed with polycystic ovary syndrome (PCOS). Recognition can be based on the measurement of 17-hydroxyprogesterone (17OHP) for screening purposes, but the cosyntropin stimulation test with a measurement of 17OHP should be the gold standard. Pharmacological treatment is initiated only in those who manifest significant clinical symptoms.

KEY WORDS:

non-classic congenital adrenal hyperplasia, NCAH, androgen excess, NCAH diagnosis.

INTRODUCTION

Steroid hormone synthesis (Figure 1) is a multistep process that takes place in the gonads, the cortex of the adrenal glands, and some peripheral tissues (brain, adipose tissue). The adrenal gland cortex consists of 3 layers. The outer zona glomerulosa is responsible for the synthesis of mineralocorticoids with the most important representative, aldosterone. In the largest, middle layer (zona fasciculata), glucocorticoids are synthesized with the most important representative, cortisol. The inner zona reticularis is responsible for the adrenal androgens production, mainly dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEA-S) [1, 2]. It should be also noted that the biosynthesis of steroid hormones is regulated by many factors, such as the transcription and post-translational modification of steroidogenic enzymes, the mechanisms of negative feedback loop of the hypothalamic-pituitary-adrenal axis, the ratio between free and bound circulating steroid compounds, and peripheral metabolism of steroids [3, 4].

In the process of steroidogenesis, 2 types of enzymes are involved: cytochrome P450 (CYP450) and steroid dehydrogenases (HSDs) [5]. Dysfunction of the enzyme involved in one of the intermediate stages of steroid biosynthesis in congenital adrenal hyperplasia (CAH) caus-

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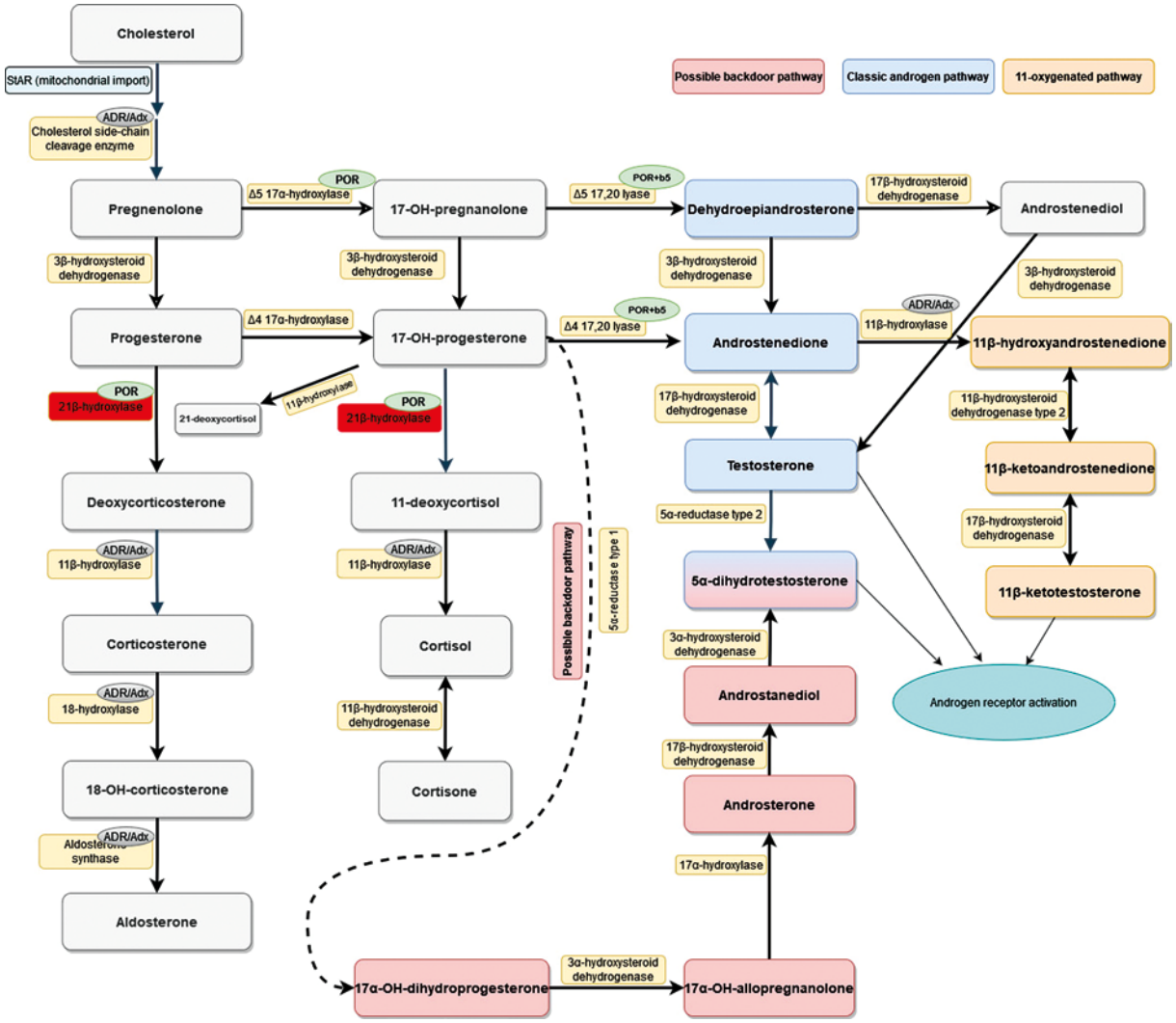

FIGURE 1. Schematic of steroid hormone synthesis and possible mechanisms that contribute to androgen excess in NCAH. CYP21A2, which is partially deficient in NCAH, is shown in red. With reduced 21-hydroxylase activity, 3 pathways lead to androgens. First, the classic pathway from cholesterol to dehydroepiandrosterone remains intact. Second, 11-oxygenated androgen pathway, increment in 17OHP leads to increased androstenedione and testosterone synthesis, which is minimal with normal 21-hydroxylase activity. Third, the backdoor pathway, which is an alternative biosynthetic route that leads to the production of DHT with omission testosterone and is active in the testes during male development in the foetus but seems to have low activity in healthy children and adults. These result in activation of the androgen receptor and the appearance of hyperandrogenism symptoms [15, 16]

StAR – steroidogenic acute regulatory protein, ADR – adrenodoxin reductase, Adx – adrenodoxin, POR – P450 oxidoreductase, b5 – cytochrome b5, OH – hydroxy.

es insufficient cortisol secretion. Lower level of blood cortisol, the most potent natural glucocorticoid, induces the hypothalamus and pituitary gland to secrete corticoliberin (CRH) and adrenocorticotropin (ACTH), respectively, hormones that indirectly (CRH) and directly (ACTH) stimulate the secretory activity of the adrenal cortex. This vicious circle results in a persistent elevated ACTH level, leading to adrenal hyperplasia, accumulation of steroid hormone precursors prior the action of dysfunctional enzymes, and an undesired shift in hormone synthesis toward other steroidogenic pathways. If there is excessive androgen production, some symptoms of hyperandrogenism become visible [6–8]. However, non-classic CAH (NCAH) patients typically have normal basal levels of ACTH, cortisol, and mineralocorticoids, but mildly elevated adrenal androgens [9]. Nevertheless, the lack of sufficient cortisol rise after adrenocorticotropic hormone (ACTH) stimulation are reported in one-third of NCAH individuals [10]. Congenital adrenal hyperplasia was described for the first time by an Italian physician almost 150 years ago. He published an autopsy report on a 44-year-old man who had external male genitals, internal female reproductive organs, significantly enlarged adrenal glands, and died during repeated episodes of vomiting and diarrhoea due to an apparent Addisonian crisis [11]. Since then, many types of CAH associated with impaired function of enzymes involved in the synthesis of steroids have been described. Data from new-

borns screened around the world indicate the prevalence of the classic form of CAH from 1 : 10,000 to 1 : 15,000 births in the Caucasian race and even 1 : 282 in a small and closed ethnic group of Yupik-speaking Eskimos [6, 12]. The most frequent cause of CAH is 21-hydroxylase deficiency, approximately 8% of CAH cases are associated with 11-hydroxylase deficiency (affecting one in 100,000 patients), and further with 17-hydroxylase deficiency, P450 oxidoreductase deficiency, or lipoid congenital adrenal hyperplasia due to StAR protein deficiency [1, 13]. The first case of NCAH was reported in 1957 in a 26-year-old married woman who had regular menstruation periods, properly developed genitalia, and manifested signs of hirsutism and acne [14].

CLASSIFICATION OF CONGENITAL ADRENAL HYPERPLASIA

Within CAH, on the basis of clinical symptoms and diagnostic tests, we distinguish 3 forms of the disease: classic salt-wasting (SW CAH), classic without salt loss – simple virilizing form (SV CAH), and NCAH [7, 15]. As shown in Figure 1, 21-hydroxylase deficiency alters the conversion of 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol, and progesterone to 11-deoxycorticosterone, key precursors in the cortisol and aldosterone synthesis pathway. The accumulated excess of 17-hydroxyprogesterone is metabolized into 21-deoxycortisol or incorporated into a properly functioning androgen production pathway in which 21-hydroxylase plays no role [16].

SW CAH is the most severe form of the disease, which accounts for 75% of all classic CAH cases. It manifests itself when mutations in the *CYP21A2* gene are so extensive that 21-hydroxylase almost completely loses its enzymatic activity (< 2%) [17]. This leads to a life-threatening impairment not only of cortisol but also of aldosterone synthesis, which is responsible for regulating sodium homeostasis. If left untreated, low levels of mineralocorticoids induce hypovolaemia, hyponatraemia, hyperkalaemia, hyperreninaemia, developmental disorders, weight loss, seizures, and eventually neonatal death 1–4 weeks after birth. Early diagnosis is crucial to increase the child's chances of survival, and so newborn screening programs for 21-hydroxylase deficiency have been introduced in many countries (for the first time in the USA in 1977 and in Poland in 2016) [18, 19]. This allows the early initiation of glucocorticoid and mineralocorticoid replacement therapy (combined with periodic monitoring of renin and aldosterone plasma levels).

A slight increase in the enzymatic activity of 21-hydroxylase compared to SW CAH leads to the development of SV CAH, which is 25% of the classic form of CAH. As in the case of SW CAH, the accumulation of steroid precursors causes an overproduction of adrenal androgen precursors, resulting in the development of ambiguous genitalia in girls of varying severity. Aldosterone synthesis is sufficient to prevent the onset of salt loss and adrenal crisis; therefore, mineralocorticoid replacement therapy is not necessary in normal conditions (without additional stress factors) [17]. It should also be noted that male patients, when screening tests are not performed, are often diagnosed with a delay of several years, after the manifestation of long-term hyperandrogenism symptoms.

NCAH is one of the most common autosomal recessive genetic disorders, which very often, especially in men, is never diagnosed [20]. The frequency in the population is estimated at 1:200 to 1:2000 cases, which is up to 50 times higher compared to the classic form of CAH [21, 22]. Among women with signs and symptoms of androgen excess, the prevalence of NCAH is 4.2% [9]. NCAH is often called latent congenital adrenal hyperplasia. The term was created to define patients who have been diagnosed by family genetic testing or 24-h urine steroid profiling, but who do not present symptoms of the disease [23, 24]. NCAH occurs when the enzymatic function of 21-hydroxylase is affected to a lesser extent (20–70% of normal enzyme function) [25]. Aldosterone and cortisol levels are sufficient to support vital functions. Androgens produced by the adrenal cortex, such as DHEA and androstenedione, are relatively weak agonists of androgen receptors, but they may be transformed peripherally into more potent androgens – testosterone and dihydrotestosterone [26]. Hyperandrogenic NCAH with similar clinical presentation can also be caused by a defect in other enzymes, such as impairment of 11β-hydroxylase activity or 3β-hydroxysteroid dehydrogenase deficiency [15, 27–29]. However, the vast majority of NCAH cases are associated with mutations in the *CYP21A2* gene, which encodes 21-steroid hydroxylase [30]. The remaining cases are very rare; therefore, in practice, it can be assumed that NCAH is caused by 21-hydroxylase deficiency [9, 31]. The characteristics of both CAH forms, classic and non-classic, are shown in Table 1.

Improperly treated or untreated NCAH causes postnatal hyperandrogenism leading to progressive virilization in boys and girls, including premature pubic and axillary hair development, acne, advanced skeletal age, rapid somatic development, and premature puberty [32–34].

It should be emphasized that the classification of CAH described above is intended to systematize the possible variants of the disease related to genetic background, and in clinical practice, distinguishing a single pure form of CAH based on clinical symptoms is often difficult, due to the intermingling equivocal symptoms of the disease. This is particularly difficult when distinguishing between SV CAH and NCAH, especially in males with one severe mutation and one non-classic allele. Furthermore, NCAH symptoms may be indistinguishable from those associated with polycystic ovary syndrome (PCOS) or premature adrenarche [20].

TABLE 1. Differentiating features of congenital adrenal hyperplasia (CAH) and non-classic form of congenital adrenal hyperplasia (NCAH)

Feature	Salt-wasting CAH	NCAH
Prevalence	1 : 10,000 to 1 : 15,000	1 : 200 to 1 : 2000
Main cause	CYP21A2 deficiency (< 2% of normal activity)	CYP21A2 deficiency (20–70% of normal activity)
Genitals after birth	Different degree of masculinization in girls	Normal
Sexual development disorders	Yes	No or mild form
Salt-wasting	Yes	No
Prenatal masculinization of female foetuses	Yes	No
Postnatal masculinisation	Yes	No or mild
Hormones profile in serum	Cortisol ↓, ↑ 17OHP ↑ 21-deoxycortisol, ↑ androstenedione ↑ renin ↑ ACTH	Slightly ↓ cortisol Slightly ↑ 17OHP
Steroid hormones profile in urine	↑↑ 17-OH-pregnanolone, ↑↑ pregnanetriol ↑↑ pregnanetriolone ↓ cortisol metabolites ↑↑ androgen metabolites ↑ Ratio: pregnanetriolone to 6α-OH-tetrahydrocortisone	Slightly ↑17-OH-pregnanolone, ↑ pregnanetriol ↑ pregnanetriolone ↔ cortisol metabolites ↑ androgen metabolites

GENETIC BACKGROUND OF CONGENITAL ADRENAL HYPERPLASIA

The genetic cause of 21-hydroxylase deficiency is quite well understood. The 21-hydroxylase gene (*CYP21A2*) is a complex gene located on the short arm of chromosome 6, within the HLA locus and adjacent to the genes of the fourth component of the complement, a region where genetic recombination occurs frequently [35–39]. About 95% of the mutations identified in CAH patients are accounted for by 17 mutations, mainly deletions and insertions, which result from the crossing or conversion of the pseudogene *CYP21A2P* with the *CYP21A2* gene. For this reason, the diversity of genetic mutations in patients with CAH is relatively small [40]. The genotype-phenotype correlation in CAH is generally good, especially for the genotypes for SW CAH (100%) and SV CAH (95%) but only 70% for NCAH [41]. The genetic cause of NCAH has a biallelic nature, and most patients are heterozygotes with different mutations in the 2 alleles. NCAH patients might harbour one severe mutation (classic) and one non-classic allele or 2 non-classic alleles, and the phenotype is considered to be determined by the milder mutation of the 2 affected alleles [42, 43]. The patients in the first group had higher levels of both basal and stimulated 17OHP, as well as a higher basal concentrations of testosterone and androstenedione, compared to those with mild mutations on both alleles. Nevertheless, apart from the earlier presentation of androgen excess, patients with a more severe mutation on the other allele do not show differences in clinical symptoms [44, 45]. The most common genetic cause of NCAH is V281L, which comprises 73–87% of cases [46]. Other NCAH-associated missense mutations include P453S, P30L, R339H, or R369W, and combinations of mutations (heterozygous vs. homozygous) can yield different phenotypes [36, 47, 48]. Parents with NCAH have a 1.5% to 2.5% risk of having an offspring with classic 21-hydroxylase deficiency (21OHD). Characterizing the predominant phenotype for a given genotype can be helpful in genetic counselling for parents at risk of having a child with CAH [39, 49].

CLINICAL SYMPTOMS OF NON-CLASSIC FORM OF CONGENITAL ADRENAL HYPERPLASIA

The clinical symptoms of NCAH are related to increased concentrations of androgens (mainly excessive production of androstenedione) and progestogens (17OHP and progesterone) [50]. These hormones produced by the adrenal cortex can exert direct and indirect effects or can be precursors for more potent androgens, especially testosterone and 5α-dihydrotestosterone. Recent studies have described adrenal cortex as being able to secrete small amounts of testosterone directly through the action of 17β-hydroxysteroid dehydrogenase type 5 on androstenedione, but the clinical importance of these small quantities of adrenal testosterone is largely unknown [51]. As shown in Figure 1, in healthy individuals, adrenal androgens are synthesized primarily via the classic Δ5 pathway, in which 17α-hydroxypregnenolone is converted to DHEA and then subsequently to androstenedione, testosterone, and 5α-dihydrotestosterone. This route through DHEA is often downregulated in CAH [52]. In patients with 21OHD, ACTH- dependent androgen excess comes mainly from the Δ4 pathway, which has

been found to be the predominant source of elevated androgen level, and excess 17OHP is converted directly to androstenedione. 17OHP can also be converted through a multiple-step cycle called the alternative or backdoor pathway to 5α-dihydrotestosterone, bypassing DHEA and testosterone. Adrenal androgens can also derive from androstenedione or 21-deoxycortisol via the 11-oxygenated androgen pathway, for which the initial steps depend on 11β-hydroxylase activity. These 2 metabolites can be converted to 11β-hydroxyandrostenedione, and then further to 11-ketoandrostenedione, 11-ketotestosterone, and finally to 11-ketodihydrotestosterone. The last 2 hormones have comparable (approx. 40%) affinity to the androgen receptor as testosterone and 5α-dihydrotestosterone, respectively. Most metabolic steps that lead to the synthesis of 11β-hydroxyandrostenedione occur in the zona reticularis of the adrenal cortex; while 11-ketotestosterone and 11-ketodihydrotestosterone are derived mainly from peripheral conversion [32, 52, 53].

NCAH patients with an increased ACTH level have the same pathophysiological mechanisms of androgen excess as classic CAH, but generally NCAH individuals do not present elevated levels of ACTH or CRH or reduced cortisol. In such cases, androgen excess could be attributed to altered enzyme kinetics due to 21OHD. The less efficient conversion ability of 21-hydroxylase results in an increased precursor to product ratio and accumulation of 17OHP, regardless of ACTH levels [9]. Another cause of androgen excess in NCAH patients may be ovarian hypersecretion and peripheral conversion from steroid precursors primarily through the backdoor pathway (Figure 2) [54]. As is well known, polycystic ovarian morphology is a common finding in women with NCAH and ovarian dysfunction can, as an additional factor, leading to androgen excess in those women [21]. Excessive progesterone and androgens by the adrenal glands can cause alterations in hypothalamic-pituitary-ovarian function that promote rapid pulses of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) hypersecretion contributing to androgen excess in the ovaries [55]. Furthermore, overexpression of 5α-reductase in the ovary is often observed [56]. LH hypersecretion can initiate and sustain a vicious cycle in which LH stimulates androgen overproduction by theca cells of the ovary, exacerbating the consequences of excessive androgen secretion by the adrenal glands [55, 57].

At birth, children with NCAH have normally developed genitalia consistent with the karyotype of the subject and the 17OHP level is normal or only slightly elevated; therefore, the diagnosis is often made many years later, at puberty or even in adulthood, during treatment of other diseases [49, 58]. Newborn screening most often fails to detect children affected by NCAH. The first symptoms appear no sooner than 60 months after birth, but they can manifest at any age of a person with the burden. In a 2000 multicentre study involving 220 women with NCAH, only 11% were diagnosed before the age of 10 years, while the majority, 80%, of NCAH were diagnosed between the ages of 10 and 40 years [59].

In childhood, symptoms indicative of NCAH are early adrenarche and peripheral precocious puberty (87%), oily skin, premature development of pubic hair, acne, advanced bone age, accelerated growth, and ultimately reduced height compared to predicted [60–65]. Recent studies have shown that 5–10% of all cases with premature pubic hair and 4–25% of premature puberty cases are patients with NCAH [60, 66–68]. Early diagnosis and initiation of treatment before the age of 9 years allow the target height calculated from the parents' height to be reached [60, 70]. However, data on final height are inconclusive, and it has been reported that in untreated patients with NCAH, the target height is within the normal range in most studies [70–72], but contradictory results have also been described [73, 74]. These might suggest that elevated androgen levels in NCAH patients do not lead to significant growth reduction, which is probably more affected by the severity of the phenotype [72]. In adults, the most common symptoms are: hirsutism (60–78%), acne (33%), menstrual disorders (55%), and fertility disorders (12%) [59]. These clinical manifestations are mainly the result

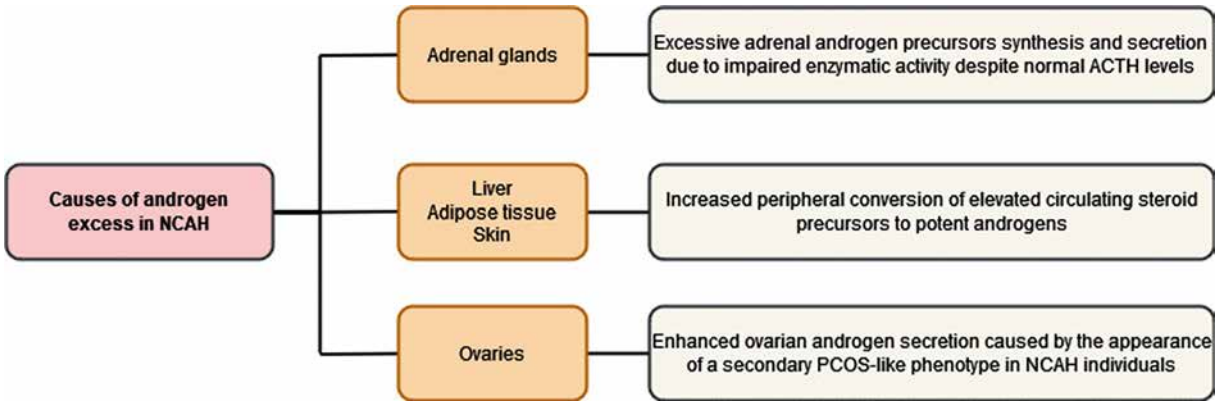

FIGURE 2. Main causes of androgen excess in patients with NCAH

NCAH – non-classic congenital adrenal hyperplasia, ACTH – adrenocorticotropic hormone.

of elevated androgen levels and are more pronounced in women. Another well-known complication of CAH, especially in men, is an increased risk of adrenal rest tumour formation, especial testicular adrenal rest tumour (TART), which develops when adrenal rest cells are stimulated by permanently elevated ACTH levels [75]. TARTs are diagnosed in up to 94% of men with CAH and have been reported at all ages, even in 6-year-old boys [76, 77]. However, the incidence of TARTs in patients with NCAH is much lower, but it remains an important cause of male infertility. In order to reduce tumour size and infertility, glucocorticoid therapy should be introduced or intensified [75]. Furthermore, the incidence of ovarian adrenal rest tumours (OART) has been reported in woman with CAH, but the prevalence is very low [78]. It should be emphasized that due to the similarity of the symptoms of NCAH and PCOS, it is difficult to distinguish between the 2 disorders solely on the basis of clinical evaluation. According to the different statistics, PCOS affects 6–15% of women of reproductive age and is diagnosed in 3–6% of teenagers. Despite many studies and trials, controversy about diagnostic criteria and recommendations for PCOS in juveniles continues. This is because the diagnostic pathological features used in adult women may be normal during physiological puberty. Several studies have described cases where PCOS was initially diagnosed in women with NCAH [79, 80]. NCAH and PCOS show similar clinical features, such as menstrual and fertility disorders and elevated androgen levels. Both diseases are also associated with obesity, insulin resistance, and dyslipidaemia [81]. In males, NCAH is often asymptomatic or only with visible acne and/or reduced fertility [47]. Table 2 presents a summary of the main clinical manifestations of NCAH according to age and sex.

DIAGNOSIS OF CONGENITAL ADRENAL HYPERPLASIA

The Endocrine Society Clinical Practice Guideline describes all issues related to the correct diagnosis and management of patients with congenital adrenal hyperplasia [82]. In countries where newborn screening is carried out, the diagnosis is based on the determination of plasma levels of 17-hydroxyprogesterone, a substrate for the dysfunctional 21-hydroxylase enzyme, which can prevent serious infant morbidity and mortality [83, 84]. 17OHP > 30 nmol/l (> 1000 ng/dl) in a random blood sample is diagnostic of classic CAH; the cut-off value is below 6 nmol/l (200 ng/dl) at 3 days in full-term infants. The immunoassays use dried blood spots on the same filter paper card taken from the baby's heel, which is also used for other newborn screening tests. This allows an unequivocal diagnosis of SW CAH, with a lower probability of SV CAH, whereas NCAH is not usually identified (slight increase in the level of 17OHP). In Sweden only 12 NCAH cases were recognized in 2.7 million screened infants whereas in Netherlands there were only 5 per 2.2 million screened infants, well below the actual prevalence in the population [55, 56]. It should be noted that immunoenzymatic techniques can give false positive results (approximately 0.5%) due to cross-reactivity of steroid conjugates and insufficient specificity of the antibodies used [85]. These phenomena are particularly common in preterm infants, sick children, children with low birth weight, and in relation to delayed functional maturation of 11-hydroxylase [86]. On the other hand, glucocorticoid treatment during pregnancy may result in false negative results [87]. Therefore, the reference values of the 17OHP concentration should be stratified by gestational age or birth weight [88–90]. A positive result should be confirmed by another, more advanced analytical method, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) or gas chromatography-mass spectrometry (GC-MS). If an LC-MS/MS assay is not available, a cosyntropin stimulation test should be performed to confirm the diagnosis before initiating corticosteroid treatment. Despite the lower probability of obtaining false positive results, which are unnecessary concerns for parents, performing the analysis by chromatographic methods is not common because it is an expensive and time-consuming technique and requires specialized knowledge. Chromatographic techniques enable the simultaneous measurement of several analytes in a sample and the determination of the precursor/product ratio (e.g. 17OHP + 21-deoxycortisol/cortisol), which significantly reduces the possibility of a false positive result, also in preterm and stressed neonates [85, 91]. Obtaining a complete steroid profile is especially recommended in

TABLE 2. The most common clinical symptoms of non-classic form of congenital adrenal hyperplasia (NCAH) in different groups of patients

Girls	Boys	Women	Men
Acne Peripheral precocious puberty and axillarche Accelerated growth Rare mild clitoral enlargement	Acne Peripheral precocious puberty Accelerated growth Gynecomastia	Acne Fertility disorders Increased risk of short stature Clitoral enlargement Menstrual disorders Hirsutism, alopecia Adrenal incidentaloma Ovarian adrenal rest tumours	Acne Fertility disorders Adrenal incidentalomas Testicular adrenal rest tumour

patients with borderline 17OHP levels after a cosyntropin stimulation test to differentiate 21-OHD from other enzyme defects [82].

The diagnosis of NCAH is not as simple as that of classic CAH. Newborn screening is highly beneficial for early detection of classic form, but most screening programs fail to distinguish those individuals affected by NCAH [85]. Many people with NCAH do not show any symptoms of the disease for a long time. The parameters of growth and maturation remain normal, reproductive function is preserved, and the diagnosis is made only as a result of related tests, most often when looking for causes of increased androgen levels [16]. The diagnosis of NCAH is possible using basal and/or dynamic hormone measurements. An effective screening test for NCAH is the measurement of 17OHP in the early morning hours in the follicular phase of the cycle. Early morning measurement is very important because the concentration of 17OHP decreases rapidly during the day. In the screening, 17-OHP concentrations of less than 2–5 nmol/l (66.6–166.6 ng/dl) in children and less than 6 nmol/l (< 200 ng/dl) in women rule out the diagnosis of NCAH [9, 92]. The result in the range of 6–30 nmol/l (200–1000 ng/dl) should be confirmed by the ACTH stimulation test (250 μg of cosyntropin injection). Then the cut-off value is 30 nmol/l (< 1000 ng/dl) [9]. In the ACTH stimulation test, the level of 17OHP (sometimes also androstenedione) is assessed before and 60 minutes after ACTH administration. The levels of these metabolites before cosyntropin administration do not differ or differ only slightly from the reference values, while they increase significantly after ACTH administration, thus establishing the diagnosis [93, 94]. When differentiating between NCAH and PCOS (Table 3), the NCAH group tends to have higher 17OHP and progesterone concentrations than women with PCOS, who show insulin resistance, obesity, polypoid ovarian morphology, and an elevated LH/FSH ratio [57, 95]. Nonetheless, 20% of patients with PCOS have elevated baseline levels of 17OHP, and in this case, an ACTH stimulation test is necessary to distinguish between these 2 syndromes [96]. A clear diagnosis can also be established using chromatographic methods such as LC–MS/MS measurement of basal follicular 21-deoxycortisol, pregnantriolone, 17OHP, and corticosterone or steroid profiling of urine from 24-h collection by the GC-MS technique [92, 95, 97].

The genetic diagnosis of CAH due to 21-OHD is recommended only if the results of the adrenocortical profile after an ACTH stimulation test are inconclusive, or the stimulation test cannot be accurately performed (e.g. patient being treated with glucocorticoids), or for genetic counselling purposes. As mentioned above, most mutations in the *CYP21A2* gene encoding 21-hydroxylase result from the exchange of genetic material during meiotic recombination or conversion between this gene and the 98% homologous inactive *CYP21A1P* pseudogene [98, 99]. *CYP21A2* genotyping allows the detection of heterozygosity for mutations of the gene, which may cause CAH in the offspring of these patients. Most NCAH patients carry one allele with a severe mutation for *CYP21A2*, so there is a 1 in 240 risk for a parent with NCAH to have an offspring with classical CAH [82]. However, the prevalence of CAH among children of women with NCAH was higher than predicted and was 2.5% for CAH and at least 15% for NCAH, presumably due to the fact that affected individuals tend to marry within their own ethnic subpopulations [100]. With normal baseline and after cosyntropin stimulation, 17OHP levels do not exclude carrier status for mild or severe *CYP21A2* mutations [67, 82]. The study by Guarnotta *et al.* confirmed previous reports that 17OHP values after the stimulation test were in the normal range in approximately 50% of heterozygous *CYP21A2* mutation carriers, indicating the unsuitability of a simple ACTH stimulation test to detect heterozygosity for 21OHD [81]. The 17OHP/cortisol ratio can be a good and simple tool to identify heterozygous CAH carriers before proceeding to genetic analysis. The study found significantly higher 17OHP/cortisol ratios compared to controls in both heterozygous carriers of the classical CAH and NCAH genes. Thus, the value of the 17OHP/cortisol ratio may be an independent biochemical predictor of gene heterozygosity. The cut-off value of this ratio is 0.03 in heterozygous patients, to differentiate from controls. This parameter shows good specificity but low sensitivity and can be used as a second-line diagnostic test to correctly distinguish healthy asymptomatic patients with a mutated form of the gene on one of the alleles [101]. 21-deoxycortisol has also been shown to be a more sensitive marker than

TABLE 3. Clinical features and biochemical parameters characterizing non-classic form of congenital adrenal hyperplasia (NCAH) and polycystic ovary syndrome (PCOS) [94, 134]

Feature	NCAH	PCOS
Hirsutism	59%	60–70%
Acne	33%	14–25%
Polycystic ovaries	25%	70–90%
Menstrual irregularities	17%	90%
Infertility	13%	25–50%
Pregnancy complications	25%	20–40%
Obesity, insulin resistance, dyslipidaemia	40%	20–85%
17OHP	↑	Normal or ↑ (20%)
17OHP after ACTH stimulation test	≥ 30 nmol/l	< 30 nmol/l
21-deoxycortisol	↑	Normal
Testosterone	↑	↑
LH/FSH > 2	9%	22–29%

17OHP, capable of detecting more than 90% of heterozygous *CYP21A2* mutation carriers. 21-deoxycortisol is produced by 11β-hydroxylation of 17OHP and is generally not excreted in large amounts (even in premature babies), and elevated levels are highly specific for 21OHD. However, this marker must be measured by advanced diagnostic methods as LC-MS/MS; therefore, its use in diagnostics is limited for the time being. Conversely, tests that evaluate the ratio of 17OHP/cortisol are readily available and simple to calculate [97, 102, 103].

It should also be emphasized that there is the possibility of a prenatal diagnosis of CAH but excluding NCAH. Chorionic villus sampling at 9–11 weeks of gestation or amniocentesis at 15–18 weeks of gestation and genetic testing allow the early diagnosis of 21OHD and the introduction of prenatal treatment [104]. Recent guidelines also recommend the genetic screening for Y-chromosomal DNA in maternal blood to exclude male foetuses from potential treatment groups [82]. However, prenatal therapy is still considered experimental and treatment should only be carried out in centres with protocols approved by institutional review boards, and the risks/benefits of the procedures should be properly discussed with the family [82]. In children with suspected disease (familial burden), treatment should be initiated no later than the ninth week of pregnancy (dexamethasone), which prevents increased androgen levels and virilization in girls. The results of the genetic test determine further treatment. Therapy is discontinued when the foetus is a boy or a healthy girl. Otherwise, treatment is continued throughout pregnancy at a dose of 20 μg/kg/day of dexamethasone, which crosses the placenta barrier and suppresses foetal ACTH secretion and adrenal androgen overproduction [105–107].

TREATMENT OF NON-CLASSIC FORM OF CONGENITAL ADRENAL HYPERPLASIA

The objective of treatment in patients with NCAH is to inhibit excessive adrenal androgen synthesis and related complications. In patients diagnosed with NCAH, pharmacological treatment is initiated only in those who manifest clinical symptoms [15, 82]. Most men with NCAH do not need pharmacological therapy, and in women it is indicated when patients have significant symptoms of hyperandrogenism and/or fertility problems. Glucocorticoid therapy in NCAH is also recommended in children and adolescents with premature onset and rapid progression of puberty or accelerated growth velocity with bone maturation significantly advanced (2 or more years), and in adolescents with overt virilization. Premature pubarche without advanced bone maturation can be observed every 6 months without pharmacological interference. Treatment is also implemented in children identified on the basis of neonatal screening tests, who develop symptoms relatively early, which is a sign of a more severe phenotype [108]. In NCAH treatment, glucocorticoids are most commonly used to inhibit excessive ACTH secretion, normalizing the cortisol level and excessive adrenal androgen production resulting not so much from cortisol deficiency but from altered enzyme kinetics [109]. During treatment, androgen levels may even be below normal values in both sexes [110, 111].

The steroids most commonly used in NCAH are hydrocortisone, prednisolone, and dexamethasone. Hydrocortisone is preferred in children due to its lower growth inhibition compared to long-acting preparations [112]. The recommended basal dose of hydrocortisone in classic CAH is 10–15 mg/m^2 of body surface area, divided into 3 or 4 daily doses [113]. Higher doses may have disadvantageous effects on growth and result in the development of iatrogenic Cushing's syndrome. Bone mineral density should also be routinely assessed. In patients with NCAH, the doses required to normalize androgen levels are often much lower [114]. Prednisolone (1–5 mg/day divided into 2 doses) is often preferred in adults due to its simpler dosing and longer action. In the case of dexamethasone, some clinicians are cautious and avoid its use altogether because of its poorer metabolic and bone profile [115–117]. Therefore, dexamethasone as well as other long-acting glucocorticoids should be shunned in childhood [118]. At the same time, as mentioned earlier, dexamethasone crosses the placenta and can be used to treat a foetus with genetically confirmed CAH. This effect may be undesirable in women with CAH/NCAH with a healthy embryo. However, some physicians choose dexamethasone as the preferred treatment option in NCAH, especially in the case of fertility problems related to TART [17, 118].

Daily glucocorticoid supplementation is recommended in patients diagnosed with NCAH, whose stimulated cortisol level is less than 500 nmol/L. This applies to approximately one-third of NCAH patients [10, 44, 45]. However, it is important to note that when glucocorticoid treatment is initiated, the hypothalamic-pituitary-adrenal axis is inhibited, increasing the risk of adrenal crisis after a severe stress stimulus. Therefore, in situations generating high stress (e.g. surgery, trauma) it may be necessary to increase the doses of glucocorticoid, and sometimes to use intravenous administration. In this case, proper education of patients and their families is important [119, 120]. More recently, modified-release hydrocortisone formulations have been introduced and experimental studies have been conducted with subcutaneous infusion systems [52, 111], which have been shown to better mimic the circadian rhythm in classic CAH. However, there is no evidence yet of their more beneficial effects on NCAH [121, 122].

In children with increased growth velocity and whose bone age is significantly higher than chronological age, GnRH analogues are helpful in treating secondary GnRH-dependent precocious puberty [57, 123]. However, such therapy should be considered an experimen-

tal treatment and is not recommended routinely except if the predicted height SD is –2.25 or below their target height [16].

Most adult men generally do not receive daily glucocorticoid therapy. The exceptions are patients with infertility, adrenal tumours, testicular adrenal rest tumours, and in the case of phenotypes intermediate between classic and non-classic phenotypes [82].

In patients with NCAH, the inclusion of mineralocorticoids (fludrocortisone) is rare and usually implemented to reduce the doses of glucocorticoids. Side effects, such as hypertension and swelling, hinder its use in the elderly, especially women over 50 years of age [17, 124]. Treatment can be terminated during early to intermediate puberty for boys and 2–3 years after menstruation in girls. In girls with persistent symptoms, prolonged glucocorticoid treatment should be avoid [16, 82, 103].

OTHER TREATMENT STRATEGIES

Recent studies have shown that lowering excessive androgen production in NCAH could be achieved not only by glucocorticoid administration [125]. Other treatment options include blocking androgen action with antiandrogens or reducing ovarian androgen secretion with oral contraceptive pills (OCP) or GnRH agonists [126, 127]. A 40–60% decrease in testosterone levels has been reported after oral contraceptive pills containing the oestrogen-progestin combination, due to the inhibitory effect on androgen production in the ovaries, as well as increased synthesis of the sex hormone binding protein (SHBG) in the liver. Currently, the administration of OCP or antiandrogens in adult women with NCAH is becoming the most popular treatment strategy [128]. Among the antiandrogenic drugs that minimize the symptoms of hirsutism, cyproterone acetate and spironolactone, and flutamide and finasteride are the most commonly used. However, these drugs should not be used during pregnancy due to teratogenic effects and for the long term, especially in monotherapy, without including contraceptives due to the risk of disruption of the menstrual cycle frequency (cyproterone acetate, spironolactone) and liver damage (hence flutamide is contraindicated in women) [129, 130]. Preliminary data suggest that the prostate cancer drug (abiraterone acetate), a steroid 17a-hydroxylase inhibitor, may be a useful therapeutic agent in adult women with NCAH [131]. In mild cases of excessive hair, cosmetic treatments such as shaving, waxing, plucking, electrolysis, and laser therapy can be used to complement medical therapy [125]. This nonpharmacological approach is especially preferred in children and adolescents.

Recent studies have described new therapeutic approaches to the treatment of congenital adrenal hyperplasia including improved dosing or better mimicking of the physiological glucocorticoid levels and thereby more effective suppression of the high ACTH and androgen production in the early morning (modified-release glucocorticoid preparations and continuous subcutaneous glucocorticoid infusion), but these novel drugs are mainly introduced in the classic form of the disease, which requires higher doses of steroids [132].

TREATMENT EVALUATION

In children, parameters of growth rate, body weight, bone age, and pubertal development are used to evaluate the need for inclusion and effectiveness of glucocorticoid therapy. Therefore, children should be regularly monitored clinically for weight, height, signs of androgen excess, puberty, and bone age advancement, while in adults there are no uniform guidelines [82]. Frequent genital examinations should be avoided in girls, unless there are worrisome clinical or laboratory symptoms [112].

The Endocrine Society recommends at least an annual physical examination and determination of hormones (in the morning 17OHP and androstenedione) [82, 133, 134]. However, there are reports indicating that a single morning measurement of 17OHP levels has limited utility in adjusting the glucocorticoid dose [20]. Furthermore, normalization of 17OHP may indicate glucocorticoid overtreatment, so moderate elevation of 17OHP should be acceptable [39]. The therapeutic goal should be to maintain androstenedione and testosterone levels within the appropriate age range, and in women the testosterone to SHBG ratio at a level lower than 0.05 [124]. More recently, it has been found that the assessment of 11-oxyandrogens, such as 11-ketotestosterone, whose elevated levels have been reported in both NCAH and PCOS, may be useful in monitoring NCAH [135, 136]. However, further studies are needed to clarify the clinical utility of 11-oxyandrogens as biomarkers of adrenal and gonadal overactivity.

ADDITIONAL ASPECTS

Several studies have reported that adult patients with NCAH demonstrated an increased risk of metabolic and cardiovascular morbidities [120]. Obesity and type 2 diabetes are significantly more common in NCAH patients than in the general population, as well as cardiovascular disease (mainly stroke). These disorders might be related to prolonged use of glucocorticoids that can improve symptoms of androgen excess and fertility, but they can lead to long-term complications. Therefore, the balance of benefits and harms should always be assessed before implementing glucocorticoid therapy, and regular clinical monitoring is recommended to avoid risk factors and to improve clinical outcomes [124]. NCAH patients have also been shown to have a higher ratio of adrenal incidentalomas and psychiatric diseases such as increased psychotic disorders in males and anxiety disorders in women, which should be reflected in the proper management of patients [137, 138].

CONCLUSIONS

Nonclassical congenital adrenal hyperplasia is a difficult endocrine disorder to diagnose and treat due to the direct and indirect effects of the disease on steroidogenesis pathways and ambiguous symptoms. It may manifest in childhood, adolescence, or adulthood. The early diagnosis of the classic form of CAH is crucial for saving newborn lives, while the diagnosis and treatment of NCAH can significantly improve patient comfort and reduce the degree of complaints. NCAH is not a fatal disease, and in many cases it remains undiagnosed, but it can be a burden to many patients affecting their appearance, self-confidence, and therefore quality of life. Advances in metabolomics, genetics, and treatment strategies offer the opportunity to better understand this complex disease and choose the appropriate therapy. Determination of the 17OHP level is used primarily in the screening of 21OHD, while the gold standard of NCAH diagnosis is the ACTH stimulation test with a measurement of raised 17OHP. The introduction of more sensitive and selective analytical techniques, such as LC-MS/MS or GC-MS, can be useful to confirm the diagnosis and targeted analysis of steroid hormones. Identifying mutations within the *CYP21A2* gene is important to understand the extent of the mutation and the severity of the disease because there is a wide variation in 17OHP levels among patients. The prevalence of PCOS far exceeds that of NCAH, and despite the overlap in management (especially hirsutism and acne), infertility treatment and pre-conception considerations warrant careful diagnosis. Therapy should be tailored individually and should aim to minimize symptoms to achieve normal sexual development, fertility, and a generally understood good quality of life. Although there have been many advances in recent years, there is still much to learn about the optimal treatment and management of individuals with both classic and non-classic forms of CAH.

DISCLOSURE

The authors declare no conflict of interest.

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