Chapter

Congenital Adrenal Hyperplasia

Adina Mariana Ghemigian and Nicoleta Dumitru

Abstract

Congenital adrenal hyperplasia (CAH) is a rare pathology with an estimated incidence of 1:14,000–18,000 births. It includes a group of inherited diseases with autosomal recessive transmission. The genetic defect consists of mutations of the genes encoding the enzymes involved in adrenal and eventually gonadal steroidogenesis. The most common mutation is the gene encoding 21 hydroxylase the enzyme involved in cortisol and aldosterone synthesis. However, other enzymatic defects can be identified. The excess of steroid precursors in the adrenal cortex will be directed towards adrenal androgen synthesis. Finally, the clinical picture includes a series of manifestations specific to the enzymatic deficiency, the severity depending on the degree of the genetic defect. Thus, we can meet severe deficits with clinical expression in newborns and toddlers or partial, non-classical forms with manifestation in adolescence or adulthood. Once the diagnosis of CAH is established, patients will require specific therapy and long-term monitoring.

Keywords: congenital adrenal hyperplasia, adrenal steroid synthesis, 21-hydroxylase deficiency, hyperandrogenism, dexamethasone

1. Introduction

Congenital adrenal hyperplasia (CAH) includes a heterogeneous group of autosomal recessive disorders caused by total or partial impairment of steroidogenesis, which is characterized by decreased synthesis of cortisol and, or aldosterone [1]. Cortisol deficiency causes an increase in ACTH levels by losing the effect of negative feedback on the hypothalamic-pituitary region. In turns this leads to chronic overstimulation of the adrenal cortex and consecutive adrenal hyperplasia.

From the point of view of steroid synthesis, the adrenal cortex is divided into three areas: zona glomerulosa or the outer layer, the site of mineralocorticoid synthesis, the fasciculata area, the middle largest layer, the site of glucocorticoid synthesis and the inner layer or zona reticularis involved in androgen biosynthesis [1]. Steroidogenesis involves the conversion of cholesterol to active steroid hormones and is performed under the action of many enzymes, cofactors, and accessory proteins (**Figure 1**) [1]. These enzymes are specific expressed in the three areas of the adrenal cortex, depending on the type of steroids synthesized, but some are also expressed in the gonads or placenta.

The pathophysiological mechanism of CAH consists in mutations in most of the genes encoding the enzymes involved in adrenal and eventually gonadal steroidogenesis. Impaired enzyme function in a specific step of adrenal steroid biosynthesis leads to a unique combination of elevated precursors and deficient products [2]. For this

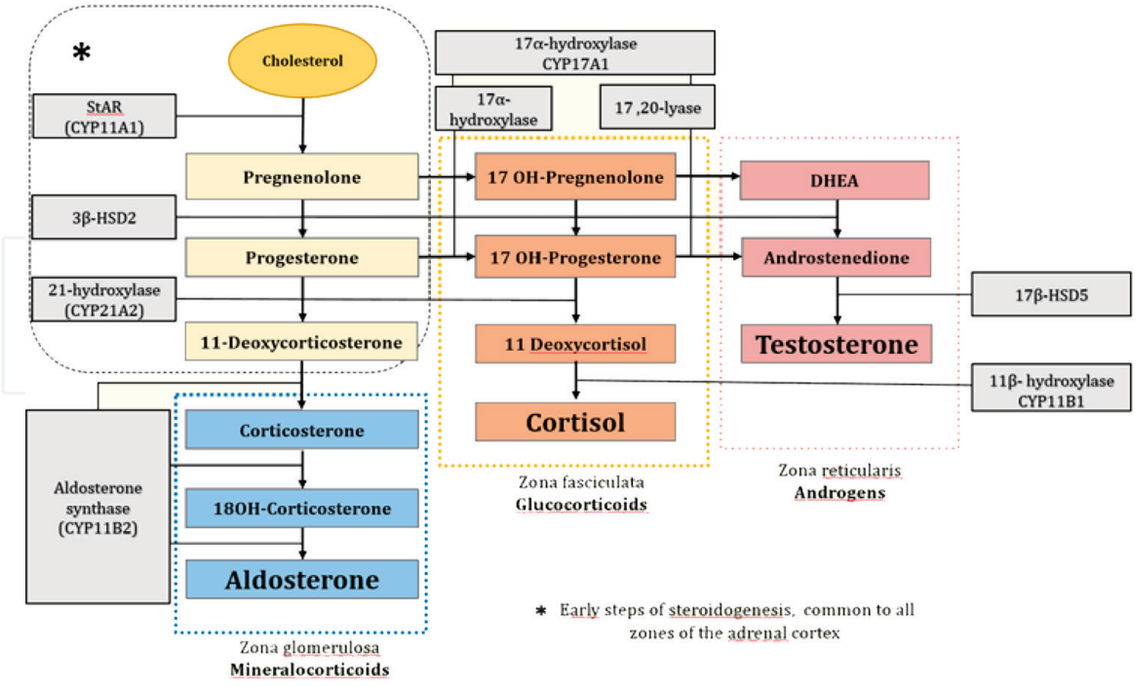

Figure 1.
Enzymatic steps of adrenal steroid synthesis.

reason, the clinical phenotype is variable and depends on the severity of the enzyme deficiency. The most common enzyme deficiency that disrupt cortisol synthesis leading to CAH is that of 21 hydroxylase [1–4].

2. 21-hydroxylase deficiency

2.1 Epidemiology

CAH due to 21-hydroxylase deficiency (21OHD) represents approximately 90–95% of CAH cases encountered in clinical practice [3, 4].

Depending on the degree of enzyme deficiency, it is classified into classical and non-classical form. Classical form occurs in 1:14,000 to 1:18,000 live births based on screening programs [1, 4, 5]. Non-classical form is more common with a prevalence of 1:500 to 1:1000 in the general white population [4]. In certain population groups like the Ashkenazi Jewish the prevalence is even higher with 1 in 27 individuals affected and 1 in 3 are carriers of the allele, making this form the most frequent autosomal recessive disorder in population [2].

2.2 Pathophysiology

CYP21A2 is a microsomal enzyme belonging to the cytochrome P450 family, also known as P450c21 [1]. The major substrate of CYP21A2 is 17 hydroxyprogesterone (17OHP), which is converted to 11-deoxycortisol in zona fasciculata, an intermediate metabolite in the cortisol synthesis stage. In zona glomerulosa, CYP21A2 acts on progesterone, converting it to 11-deoxycorticosterone within the aldosterone pathway [1]. Blocking this enzymatic step will cause cortisol deficiency with a secondary increase

in ACTH secretion [2]. This in turn, will stimulate excessive synthesis of precursor molecules in both pathways blocked by the enzyme deficiency, namely 17OHP and progesterone. The precursor will be redirected to adrenal androgens synthesis via the 17,20-lyase activity of CYP17A1, the step unaffected by the enzyme deficiency [1, 2].

Intrauterine exposure to excess adrenal androgens will not significantly influence male sexual differentiation. The situation is totally different in the case of a fetus 46, XX. In this case, excess of androgens will interfere with the differentiation of the external genitalia, causing prenatal virilization. The severity of virilization is quantified using a five-point scale developed by Prader, where grade I involves minimal virilization with clitoral hypertrophy, while grade V is a typical male aspect of the external genitalia [2].

2.3 Genetics

The gene that encodes CYP21A2 is located on the short arm of the 6 chromosomes within the HLA class III region (6p21.3) arranged in tandem with its inactive pseudogene (*CYP21A1P*) [1, 3]. Both *CYP21A2* and *CYP21A1P* genes share a high nucleotide homology of about 98% and 96% in exons and introns respectively, but only *CYP21A2* gene encodes the active microsomal P450 enzyme, 21-hydroxylase (CYP21A2, P450c21) [1, 6]. The pseudogene CYP21A1P is inactive because of the presence of multiple pathogenic variants, small insertions or deletions and point pathogenic variants that prevent the synthesis of a functional protein [3, 7].

In about 70–75% of cases *CYP21A2* mutations arise from gene microconversion, namely transfer of deleterious mutations from *CYP21A1P* during meiosis, generating point mutations with consecutive reduction of the enzymatic activity of CYP21A2 [1, 6]. In 20–25% of the cases, *CYP21A2* mutations are due to gross misalignment owing to unequal crossing over during meiosis leading to gene deletions, gene duplications or the appearing of a hybrid gene or pseudogene-gene chimeras, yielding a non-functional *CYP21A2* gene [3, 6]. Less than 5% of the pathogenic variants in the CYP21A2 gene are de novo point mutations, most of these affected patients are heterozygous [3, 6].

2.4 Genotype-t correlation

Most patients with 21OHD are heterozygous compounds, with more than one mutation in one or both *CYP21A2* alleles, which is why there is a wide spectrum of phenotypes [1, 4]. In general, the phenotype reflects the residual activity of the lightest mutation [3, 7].

Scholastically, two forms of this condition are described, namely the classical and non-classical form. In turn, the classic form is subdivided into salt-wasting (SW) and simple virilizing (SV) form [1, 4].

The SW form is characterized by mutations in both alleles leading to complete loss of enzymatic activity, such as deletions, large gene conversions, nonsense mutations, frameshifts, and missense mutations [1].

The SV form occurs by mutation associated with complete loss of function on one allele and on the other allele a mutation that reduces enzymatic activity by 1–5%, like a nonconservative amino acid substitution, p.Ile172Asn (I172N) or splicing mutation on intron 2 [1, 4]. This mutation that alters splicing of intron 2 is particularly common in SV patients, but also in SW ones.

In the nonclassical (NC) form, most of the affected individuals are compound heterozygotes, with various mutations on each of the two alleles, of which at least one can allow enzymatic activity of 20–50% [6]. The genetic defect may be a point mutation in exon 7 (p.Val281Leu), or a missense mutation as p.Pro30Leu (P30L) [1, 4, 6]. However the clinical observations suggest that patients carrying the P30L allele are somewhat more symptomatic, being rather a borderline form between SV and NC [1].

Although we still use this classification of CAH forms, in practice we must consider the *CYP21A2* allelic variants with their phenotypic manifestations as a continuum clinical spectrum.

2.5 Clinical picture

The classic form of CAH due to 21OHD is identified in 1 of 14,000–18,000 newborns and includes the two variants: salt-wasting and simple virilizing form [1, 4].

2.5.1 Classic salt-wasting 21-OHD CAH

Of the two variants, the SW form is the most severe, but also the most common, representing about 75% of all cases [6]. It is characterized by null or minimal enzymatic activity (<1%) in both *CYP21A2* alleles, resulting in severe cortisol and aldosterone deficiency and elevated androgen levels [1, 3]. Clinically it is characterized by prenatal virilization of female girls by exposure to potent androgens (testosterone and Δ4-androstenedione) at critical stages of sexual development [6]. Aldosterone deficiency will be manifested soon after birth by the appearance of hypovolemia with hyperreninemia, hyponatremia and hyperkalemia, which associated with hypoglycemia due to severe cortisol deficiency, will generate an increased risk of seizures in these infants [2]. Also, these children are at high risk for adrenal crisis, that can occur at 1–6 weeks after birth, with azotemia, vascular collapse, shock, and finally death [6]. Affected males are more prone for a salt-wasting adrenal crisis at home because their normal male genitalia do not raise the suspicion for this condition, being discharged from the hospital without diagnosis [4, 6]. The episodes of salt loss in the infant period are similar in symptoms to pyloric stenosis or gastroenteritis with poor feeding, weight loss, failure to thrive, vomiting, dehydration [6]. The differentiation from the latter is achieved by the fact that children with CAH maintain diuresis.

2.5.2 Classic simple virilizing 21-OHD CAH

This second variant of classic CAH represents about 25 of the cases and is characterized by a reduced enzymatic activity of only 1–5%, which generates a clinical picture in which the exposure to excess androgens predominates. These children do not have episodes of salt loss due to the relatively normal production of aldosterone, but they do associate cortisol deficiency. In utero exposure of females to adrenal androgen excess results in external genital virilization at birth with varying degrees of clitoris enlargement, fusion of the labioscrotal folds, and formation of a urogenital sinus [6]. Excess androgens are imperceptible at birth in males. It should be noted that it is difficult to make a difference between SV and SW form based on the degree of virilization of an affected female at birth [6]. This is why all infants detected with classic CAH will be treated with glucocorticoid and mineralocorticoid treatment at least within the first year of life, because infants have a relative renal tubular resistance to the salt-retaining effects of aldosterone and a low sodium content diet [1].

Postnatal, both females and male's infants with SV CAH develop signs of androgen excess including precocious development of pubic and axillary hair, acne, seborrhea, comedones, acceleration of linear growth, advanced bone maturation leading to short final stature, with a consecutive catecholamine deficient secretion [6]. The pubertal development of untreated children consists:

- isosexual early pseudo-puberty in boys with disharmony between testicular development (prepubertal type) and penile enlargement (pubertal type)
- heterosexual early pseudo-puberty in girls

Persistence of glucocorticoid deficiency in adulthood may lead to impaired vascular tone and cardiac function, as a result of impaired development and function of adrenal medulla [6].

2.5.3 Non-classic 21-OHD CAH

As mentioned above, the genetic defect associated with NC form of CAH retains an enzymatic activity of 20–50% for CYP21A2. Thus, cortisol production is sufficient for a normal lifestyle, but insufficient to adequately suppress ACTH synthesis, and so the excessive androgen secretion is maintained. External genitalia are normal at birth, as are 17-hydroxyprogesterone levels, which is why these cases are not detected in neonatal screening [2, 7]. This form is usually diagnosed later during childhood or adolescence or even in adulthood, therefore in the past it was called the "late-onset" form [6, 7].

The clinical features of androgen excess are related to age at diagnosis [7]. In the case of prepubertal children, both sexes show accelerated growth, with advanced skeletal maturation compared to chronological age, premature development of pubic hair with a prevalence up to 30%, described even at the age of 6 months [2, 7]. Girls may have clitoromegaly, and boys may have enlarged penis with prepubertal testicles, similar to classic SV form [6]. In 30–60% of cases the ACTH 1–24 test reveals a slight cortisol deficiency, requiring replacement therapy during periods of stress.

In adolescents and adult women, the signs of hyperandrogenism predominate with treatment-resistant nodulo-cystic acne, seborrhea, androgenic alopecia, hirsutism, risk of infertility, increased rate of miscarriages [2, 6, 7]. Clinically they may associate a profile like polycystic ovary syndrome (PCOS) [7]. Up to 30% of women with PCOS have undiagnosed NCCAH [6].

Affected men are often asymptomatic. In their case, the diagnosis is made either from an affected female member or in the context of evaluation for peripubertal gynecomastia, short stature, infertility, oligospermia, or adrenal incidentalomas [2]. Up to 3% of adult men with bilateral adrenal events may have undiagnosed NCCAH [6].

2.6 Diagnosis

Diagnosis of CAH must be suspected in infants born with ambiguous genitalia [2]. CAH is a life-threatening disorder for this reason it is important to establish the diagnosis as early as possible.

2.6.1 Hormonal evaluation

Diagnosis of 21-OHD CAH can be confirmed biochemically by identifying high values of 17-OHP in a blood sample. Such hormonal evaluation is the basis of the

newborn-screening program developed to identify affected patients with classical form of CAH [2]. This neonatal program screening is currently being used in more than 30 countries around the world to prevent morbidity and mortality from adrenal crisis [1, 4]. The screening procedure implies collection of blood samples by heel puncture at more than 24 h after birth, but not later than 1 week [8]. Clinical guidelines recommend as first-tier screens the use of immunoassays to measure 17OHP in dried blood spots, on the same filter paper cards used for other newborn screening tests [4].

A value of 17OHP on the 3rd day of life less than 30 nmol/L or 10 ng/mL is considered normal. However, there are no universally accepted standards for stratifying infants, most laboratories use a series of birth weight-adjusted cut-offs [4]. It should be noted that neonatal screening:

- has a risk of false positive results, ranging from 0.02 to 1.2%. This situation includes the samples collected less than 36 h after birth, premature, sick or stressed infants, a late maturation of 11 hydroxylase or in case of cross-reaction with sulphated metabolites and immature adrenal precursors [4, 8].

- In case of borderline first-tier test results some screening programs recommend repeating screening, reevaluating samples with a second-tier test by liquid chromatography–tandem mass spectrometry (LC-MS/MS) or perform molecular testing to identify the pathogenic variants in *CYP21A2* [3, 4, 6].

- 17OHP assay does not identify children with NC form of 21-OHD

- Direct biochemical analysis of 17OHP or other steroids using LC-MS/MS improve the positive predictive value of CAH screening. According to the current guidelines, the improvement of the screening prognosis can also be achieved through: [4].

- Measuring additional analytes: 21-deoxycortisol, produced by 11b-hydroxylation of 17OHP, is normally low even in preterm infants. Finding an elevated levels is highly specific for 21OHD.

- Measuring ratios of analytes: the sum of 17OHP and 21-deoxycortisol levels divided by the cortisol level detect all affected children with no false positives results and with a positive predictive value of 100%

- Measuring urinary metabolites: pregnantriol and 17b-hydroxypregnanolone, the 17OHP metabolites or pregnanetriolone, the 21-deoxycortisol metabolite; these metabolites are increased in CAH and have a good specificity, even in preterm infants. 17-hydroxypregnanolone is an intermediate product in the alternative or "backdoor" pathway of dihydrotestosterone synthesis [1]. This alternative pathway is a major contributor to fetal female virilization in 21OHD [1].

For infants with positive newborn screening for CAH the guidelines recommend referral to pediatric endocrinologists and evaluation by cosyntropin stimulation test [4].

When CAH is suspected later in childhood or in an adult, diagnosis confirmation is based on an early morning 17OHP [7]. After the newborn period, a morning 17OHP value below 2.5 nmol/L (or 0.8 ng/mL) in children, respectively below 6 nmol/L (or 2 ng/mL) in adults excludes the diagnosis of CAH (**Table 1**) [7]. It should be noted

17OHP values	Classic CAH	Non-classic CAH	Non-CAH
Basal	>300 nmol/L (>100 ng/mL)	>15 nmol/L (>5 ng/mL)	<6 nmol/L (<2 ng/mL)
ACTH-stimulated	>300 nmol/L (>100 ng/mL)	>30 nmol/L (>10 ng/mL)	<30 nmol/L (<10 ng/mL)

Table 1.
The basal and ACTH stimulated 17OHP levels.

that 17OHP has a pulsating secretion so, the values obtained mid-morning or in the afternoon may be normal. For this reason, it is recommended that blood be collected in the morning, 7.30–8.30 AM. In addition, in women it should be measured in the follicular phase of the menstrual cycle or in amenorrhea [7]. It should be noted that a simple determination of 17OHP may be accompanied by a false negative result, estimated at 2–11%, especially in adults with NC form [7].

The same as in the case of classic form, improving screening for NC CAH form can be achieved through 17OHP measurement by LC-MS/MS. This method provides increased analytical specificity, the ability for multiplex analysis (simultaneously measure multiple analytes like androstenedione, testosterone, 21-deoxycortisol), and the advantage of using minute specimen volumes [4].

2.6.2 Additional tests

If the clinical suspicion remains despite the use of LC-MS/MS, the next diagnostic step is the ACTH stimulation test, which is the golden standard for the diagnosis [2, 6, 7]. The ACTH stimulation test is performed in the morning, 8.00–9.00 AM. It involves administration of an intravenous injection of 250 μg of synthetic ACTH (Cosyntropinum, Cortrosyn), or 36 μg/kg in children under 1 year, with measuring the levels of 17OHP, androstenedione and cortisol at baseline and 60 min after the injection (**Table 1**).

Given the fact that in girls and women with non-classical form of CAH, the features of hyperandrogenism predominate in the clinical picture, it is important to be able to differentiate the etiology of this virilization syndrome. Thus, we can use the long dexamethasone test, with administration of dexamethasone for 4 days at a dose of:

- 1 mg/sqm/day divided into 4 doses (at 6 hours)—in children

- 2 mg/day divided into 4 doses (every 6 hours)—for adults

In CAH, in contrast to PCOS or Cushing syndrome there will be a suppression of androgen values (DHEAS, 17 (OH) P, testosterone) with minimum of 50 (75%) of the basic values, and also for cortisol level.

2.6.3 Molecular genetic testing

Molecular testing can detect CYP21A2 mutations and should be carried out in equivocal cases to support the diagnosis, for genetic counseling and for better prognostic and treatment guidance [2, 4, 7]. There are commercial kits that detect the most common 10–12 mutations. Testing should be performed in parents to confirm

the parental origin of each mutation, to rule out the coexistence of two mutations on the same allele (cis), to determine compound heterozygosity, distinguish hemizygosity from homozygosity in the index case, and estimate the recurrence risk [1]. Once the *CYP21A2* pathogenic variants have been identified in an affected family member, molecular genetic prenatal testing for 21-OHD CAH can be performed [6]. Most often prenatal testing is considered when the parents have a previous child with 21OHD [1].

There are two methods used: chorionic villus biopsy and amniocentesis, implying invasive sampling [1]. By chorionic villus biopsy is obtained fetal DNA at gestational week 9–11. Amniocentesis was the initial method available for prenatal diagnosis [1]. It allows analysis of fetal hormones in amniotic fluid and can be performed at gestational week 12–14 [1]. Both methods imply an increased risk of fetal loss and do not totally allow avoiding prenatal treatment in male fetuses as they cannot be performed earlier than week 9 of gestation [1, 3].

However, noninvasive prenatal diagnostic method has been developed, which prevent prenatal treatment of males and unaffected females [5]. This method is based on PCR amplification of cell-free fetal DNA that can be isolated from maternal plasma as early as week 6–9 [1]. Protocols must include screening for Y chromosomal DNA in maternal blood, by detecting SRY gene [3]. Sequencing of cell-free fetal DNA can ascertain *CYP21A2* mutations, but not suitable in clinical settings [1, 3]. Instead, it can identify single nucleotide polymorphisms flanking CYP21A2 that are specific for the mother, father, and proband, determining the maternal and paternal alleles inherited by the fetus [1].

2.7 Management of 21OHD CAH forms

Treatment goals [1, 4]:

- preventing prenatal virilization of external genitalia in girls
- prevention of adrenal crisis and virilization
- ensuring normal growth and development
- ensuring normal pubertal maturation from birth to adolescence,
- prevention of long-term complications
- Therapeutic options:

2.7.1 Prenatal treatment

The prenatal treatment involves the use of dexamethasone in dose of 20 μg/kg/day, to maximum 1.5 mg/day given in three doses to pregnant women with a fetus at risk for classic CAH [1]. Dexamethasone has increased half-life, crosses the barrier placenta and is not degraded by placental 11βhydroxysteroid dehydrogenase type 2 (HSD11B2). It can reduce the fetal ACTH and subsequently the androgen levels, with the aim of preventing prenatal virilization of external genitalia in affected girls. The effectiveness of prenatal therapy with dexamethasone depends on when treatment is started, optimum by gestational week 6–7. The benefit is reduced to 15% in case of late initiation, early discontinuation, or reduced compliance with therapy, thus

allowing slight virilization of the fetus. The treatment is controversial due to safety concerns related to exposure to dexamethasone in this stage of embryonic development that may impact much more than the hypothalamic-pituitary-adrenal axis [1].

2.7.2 Treatment of classic 21OHD CAH forms

In SW form:

- Dietary supplementation with NaCl, especially in the first months of life. The dose in breast-fed infants is 10–12 mmol/KgC/day [5].
- Mineralocorticoid replacement with fludrocortisone typically 100–200 μg/day divided in 1 or 2 oral doses, of which one must be in the morning [2, 5].

Due to relative mineralocorticoid resistance at this age and secondary to the antimineralocorticoid effects of elevated 17OHP, neonates and young infants require higher fludrocortisone doses [1]. However, the need for fludrocortisone diminishes with time. The monitoring will be done by electrolytes, plasma renin, and blood pressure measurement [2].

- Glucocorticoid replacement with hydrocortisone is the preferred option, because of its shorter half-life that minimizes the adverse side effects [1].

The hydrocortisone tablets are crushed and mixed into food or suspended and given in a dose of 10–15 mg/sqm daily, in 3–4 divided doses [1]. Treatment will be monitored every 3 months in infant younger than 18 months and every 4–6 months in older children, or more frequently after a change in dosing [1, 4]. Serum levels of 17OHP and androstenedione will be monitored. The suggested target for 17OHP level is 2–3 times the upper normal level, but not higher than 10 ng/mL (or 30 nmol/L) when measured in the early morning before medication [1]. It is recommended to avoid the normalization of the 17OHP level because of the risk of glucocorticoid overdosing causing iatrogenic Cushing syndrome [1, 2, 4].

The hydrocortisone dose may need to be dubled in case of minor intercurrences with fever below 39 degrees or tripled in case of fever above 39 degrees. Raising the dose is not recommended for everyday mental and emotional stress, minor illness, or before routine exercise [1, 4].

As growth cartilage closes, teens and later adults may receive slow-release corticosteroids, such as prednisone 5–7.5 mg/day or dexamethasone 0.25–0.5 mg/day at bedtime. However, in adults hydrocortisone remains the preferred option for glucocorticoid treatment due to its better metabolic, cardiovascular and bone mineral density outcome compare to dexamethasone or prednisone [1, 4].

Adolescents and women with clinical and ultrasound criteria for PCOS may benefit from treatment with oral contraceptives that may be associated, or not with antiandrogens (spironolactone) [4].

In SV form:

Treatment includes hydrocortisone at a dose of 8–15 mg/sqm, doses being increased in conditions of overload, except for intense physical effort [1, 4].

Replacement with mineralocorticoids is done only during infancy to allow the use of minimum doses of glucocorticoids. After this period the child will receive mineralocorticoids only if the renin values are increased [1].

A multidisciplinary team is needed in approaching the individual with genital ambiguity consisting of specialists in pediatric endocrinology, pediatric urology/surgery, clinical genetics, and psychology is essential for the best diagnosis and management [6].

Genitoplasty surgery in girls involves clitoroplasty, opening of the vaginal introitus and labioplasty [1]. Surgical techniques are intended for erectile tissue removal, keeping the clitoris with sensitive innervation, ensuring a proper vaginal opening, preventing recurrent urinary tract infections [1].

2.7.3 Treatment of non-classic 21OHD CAH form

Decisions about starting treatment should be individualized and based on clinical symptoms [2]. The guidelines do not recommend routine treatment with glucocorticoid in asymptomatic individuals [1, 4]. The main goal in children with NC CAH is to maintain normal growth and pubertal development. This childrens will be regularly monitored clinically for height, weight, signs of androgen excess, puberty, and bone age advancement [7].

Hydrocortisone treatment will be recommended in case of:

- ACTH stimulation test highlights maximum cortisol values below 18 μg/dL (or < 500 nmol/L) [4].

- Symptomatic hyperandrogenism present in the prepubertal period (early and rapidly evolving pubarche, accelerated bone maturation)—low doses of hydrocortisone (6–10 mg/sqm).

- Alternative treatment options in adolescent and young adult females includes:

- Oral contraceptives containing progestins with low androgenic activity such as desogestrel to induce menstrual cycles and improve acne and hirsutism [4, 7].

- Antiandrogens for patient-important hirsutism that persists despite oral contraceptives [1].

- Eflornithine hydrochloride cream can be used as topical therapy for facial hirsutism. Its effect is of inhibiting the anagen phase of hair production. It is most effective when combined with physical means of hair removal, such as topical lasers [1].

2.7.4 Management of adrenal emergency in CAH

Adrenal crisis is estimated to be responsible for up to 42% of deaths in patients with CAH [1, 5]. Patients with SW are the most exposed to this risk, but not only them. The most common precipitants of this medical emergency are infectious illnesses, gastrointestinal and upper respiratory tract infections, for all ages [1, 5].

Prevention of adrenal crisis is accomplished through patient education on stress dosing (2–3 times usual doses) of glucocorticoid [1, 5]. But there may be situations when oral stress doses will not prevent the progression to adrenal crisis [1]. Each family should have a glucocorticoid injection kit for emergency use at home if oral medication is not tolerated during episodes of major stress [1, 2, 4]. All family members should be trained for its intramuscular administration, especially for patients living

far from medical facilities. The injectable dose of hydrocortisone in an emergency is 25 mg for infants, 50 mg for children under 40 kg, and 100 mg for children over 40 kg and for adults [2].

Management of adrenal crisis involves giving an immediate bolus of hydrocortisone 50–100 mg/sqm intravenously or intramuscularly, followed by hydrocortisone 50–100 mg/sqm/day as either continuous infusion or divided at every 6 h [2]. Glucocorticoid doses will be adjusted according to clinical status (state of consciousness, pulse, blood pressure), blood glucose level and serum ionogram. Next day, parenteral administration will be reduced by 1/3 of the dose starting on the day the digestive tolerance reappears and, in parallel with the reduction of parenteral therapy, oral therapy is gradually reintroduced, returning to pre-decompensation doses.

In combination with glucocorticoid therapy, the patient is rehydrated by rapid infusion of intravenous fluids: 1000 mL of 0.9% sodium chloride during the first 60 minutes, or 20 mL/kg up to 60 mL/kg normal saline in children, continuation of therapy will be guided by level of dehydration [1, 2].

If hypoglycemia is present may require dextrose bolus of 0.5–1 g/kg can be given intravenously at 2–3 ml per minute [2].

If the patient has also hyperkalemia, it requires cardiac monitoring for EKG changes and should be treated using insulin with glucose infusion [2].

All patients with CAH should wear medical alert identification tags (Medical Alert bracelet or medallion) indicating the diagnosis of adrenal insufficiency, for prompt therapy initiation in case of emergencies [1, 2, 4].

2.8 Comorbidities asociated with 210HD

2.8.1 Effect on growth and final adult height

As mentioned earlier, exposure to excessive androgens determines advanced somatic and epiphyseal development accompanied by premature epiphyseal maturation and closure, resulting in a lower final adult height that expected from parental heights. An early diagnosis with the initiation of appropriate treatment could improve the final adult height. However, it is necessary to use an optimal dose of glucocorticoid, avoiding overdose, which could have a negative impact on growth [2, 5].

2.8.2 Impaired bone mineral density (BMD)

Is another consequence of long-term use of supraphysiological doses of glucocorticoid, dexamethasone having the most deleterious effects on BMD compared to hydrocortisone or other intermediate-acting glucocorticoids [1, 5]. In terms of bone metabolism, adrenal androgens including DHEAS can increase BMD, mainly cortical bone [1, 7]. Thus, regarding bone mass late diagnosis and or a poor hormonal control may improve BMD by exposure to higher androgen levels [1]. Similar to the general population, these patients may also benefit from adequate vitamin D intake, a high-calcium diet, and physical activity to prevent bone loss.

2.8.3 Tumor risk

It is estimated that 20–30% of adult patients with CAH have adrenal masses: benign adrenal tumors (29%) or myelolipomas (8.6%). They generally occur in

patients with a poor hormonal control, suggesting a role of ACTH stimulation in pathogenesis [5]. There is no evidence that adrenocortical carcinoma is more prevalent in CAH patients [1].

A common and important complication in individuals male with CAH is development of testicular tumors of adrenal-like tissue or testicular adrenal rest tumors (TARTs) [5]. TARTs are bilateral benign testicular tumors, mostly painless, centrally located in the rete testis and easily identified by ultrasound [1, 5]. The central location of TARTs can compress the seminiferous tubules, leading to irreversible damage to the surrounding testicular tissue with gonadal dysfunction and infertility [5]. According to the guidelines, boys with classic CAH should have a testicular ultrasound upon completion of puberty and regular examination for TARTs every 2–5 years [1, 4]. TARTs are rare in NC form, so routine ultrasound is not recommended in NC CAH males [4].

It should be noted that TARTs tumors may also occur in other adrenal enzyme deficiencies like 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase type 2 deficiencies [1].

Due to its strong adrenal-suppressive effect, dexamethasone is preferred in the treatment of TARTs. Testis sparing surgery may be an option, but usually does not improve gonadal function, so this patients may be recommended cryopreservation of sperm before surgery [1].

2.8.4 Fertility

Is affected in both men and women with CAH, especially those with a classic form.

In males fertility is impaired due to hyper- or hypogonadotrophic hypogonadism or through TARTs [2, 7].

In women, elevated androgen and 17OHP levels result in menstrual irregularities and anovulatory cycles [7]. Adverse effects of elevated progesterone on the uterine lining, combined with secondary development of PCOS with oligo-amenorrhea, increases the risk of sub-fertility or infertility [5]. In general, there is an association between the severity of the CAH phenotype and the level of gonadal dysfunction and fertility, mostly reported in women with the salt-wasting subtype [1, 5]. A major cause for low child rates in women with CAH is considered to be a lowest interest in motherhood, especially with the SW phenotype [1]. This may be caused by prenatal androgen exposure that influence gender role behavior, associated with a lack of a partner, dissatisfaction with genital appearance, decreased sexual satisfaction and sexual dysfunction as a result of corrective surgery [1].

2.8.5 Cardiovascular and metabolic disease

Patients with CAH have increased cardiometabolic morbidity [1]. A Swedish population-based study found an increased prevalence of obesity, type 2 diabetes mellitus, obstructive sleep apnoea, hypertension, elevated lipids, atrial fibrillation and venous thromboembolism in CAH individuals compared with control [1, 5]. Regular follow-up with lifestyle interventions to prevent obesity, and screening for diabetes, especially gestational diabetes, and dyslipidemia may improve cardiometabolic outcome [1, 5].

3. 11β-hydroxylase deficiency

CAH owing to 11β-hydroxylase deficiency (11β-OHD) is the second cause of CAH, accounting for 5–8% of all cases [2].

Steroid 11-hydroxylase (CYP11B1, P450c11β) and aldosterone synthase (CYP11B2, P450c11AS, P450aldo) are closely related enzymes, encoded by duplicated genes, and catalyze the final steps in the synthesis of glucocorticoids and mineralocorticoids [1]. CYP11B1 is expressed abundantly in the zona fasciculata, where it converts 11-deoxycortisol to cortisol and deoxycorticosterone (DOC) to corticosterone (**Figure 1**), and also in the zona reticularis, where it initiates the 11-oxo-pathway [1]. CYP11B2 expression is less abundant and confined to the zona glomerulosa where it catalyzes the 11 β-hydroxylase, 18-hydroxylase, and 18-methyloxidase activities which lead to conversion of DOC to aldosterone [1]. This steroid 11-hydroxylase are [1].

11β-OHD occurs as a result of mutations in *CYP11B1*, the gene encoding 11β-hydroxylase, with occurrence of deficient cortisol, and increased DOC and adrenal sex steroids [1]. *CYP11B1* gene is located on the long arm of chromosome 8 (8q24), about 40 kb from the homologous *CYP11B2* gene encoding aldosterone synthase [2]. It consists of 9 exons and 8 introns. Mutations in this gene tend to be grouped into exons 2, 6, 7 and 8. Several types of mutations affecting *CYP11B1* have been described, including missense mutations, splicing, small deletions, small insertions, regulatory deletions, large deletions, or complex rearrangements [9].

The general incidence of this form is estimated to be 1 in 100,000 individuals, and in contrast to 21 hydroxylase deficiency CAH, the disorder is more prevalent in the Middle East and North Africa (1:5000–1:7000 individuals). A mild non-classical form of 11β-OHD CAH has been reported, with an unknown frequency [9].

3.1 Clinical picture

The prominent clinical features of 11β-OHD are female virilization, similar to classical 21OHD and low renin hypertension [9]. Clinically we will encounter virilization in females, childhood gynecomastia, early isosexual/contrasexual pseudopuberty, accelerated bone maturation with reduced adult final height.

Elevated blood pressure is secondary to overproduction of DOC which causes salt retention and hypertension despite the fact that it is a less potent mineralocorticoid than aldosterone [9]. High blood pressure may or may not be associated with hypokalemic metabolic alkalosis and occurs in approximately 2/3 of patients, usually later in childhood or in adolescence [9]. Although the excess of DOC is incriminated, the etiology of hypertension is not fully elucidated. There are other factors involved considering that the decrease in the level of DOC after the administration of dexamethasone is not always associated with BP normalization. Likewise, clinical signs of mineralocorticoid excess and the degree of virilization are not well correlated. Some severely virilized females are normotensive, whereas mildly virilized patients may experience severe hypertension [9].

There have been reported cases of salt loss in neonates with 11β-OHD, incompletely explained pathophysiological, probably due to the natriuretic effect of pregnenolone and progesterone [9]. These episodes could be precipitated by the initiation of glucocorticoid therapy due to a sudden decrease in DOC or by conditions of infectious intercurrences.

Along with the classic form of 11β-OHD there has been described a rare form of non-classical 11β-OH. It presents with excess androgens but without hypertension and has been diagnosed in normotensive children with mild virilization or precocious pubarche and in adults with signs of hyperandrogenemia [9].

3.2 Diagnosis

It is established based on the hormonal profile, that is characterized by:

- Elevated baseline or ACTH-stimulated values of DOC and 11 deoxycortisol
- Elevated urinary metabolites (tetrahydro-11-deoxycortisol, tetrahydro-deoxicorticosterone)
- Moderately elevated 17 OHP levels, that can lead to misdiagnosis of 21OHD at newborn screening [1]
- Elevated androgen levels: androstenedione, testosterone, DHEAS.

4. 3-β hydroxysteroid dehydrogenase deficiency type II

There are 2 human 3β-hydroxysteroid dehydrogenase (HSD3B) genes: type I and type II [1, 9].

HSD3B1 encodes an isozyme found in the placenta, brain, liver, skin. HSD3B2 encodes an isoenzyme found mainly in the adrenals and gonads [1, 9]. Both of these isozymes can convert delta 5 (Δ5) to delta 4 (Δ4) steroids (**Figure 2**) [1, 9]. Mutations in HSD3B2 cause a rare form of CAH, characterized by elevated ratios of Δ5/Δ4 steroids, notably 17OHPreg/17OHP, that are >8 SD above normal [1]. About 45 mutations in HSD3B2 have been described, of which 37 are missense, nonsense, major deletion, or complex rearrangements.

Classical form of HSD3B2 deficiency causes genital ambiguity in both sexes: genetic females are mildly virilized because some fetal adrenal DHEA is converted to testosterone by HSD3B1, that can act on low concentrations of steroids in the circulation; genetic males synthesize some androgens by peripheral conversion of DHEA, but these are insufficient for complete male genital development [1].

Also, hepatic HSD3B1 permits conversion of some of the elevated 17OHPreg to 17OHP, engendering false positives in newborn screening for 21OHD [1].

Diagnosis of HSD3B2 deficiency is based on identification of elevated serum levels of Δ5 steroids and their urinary metabolites (pregnentriol and 16-pregnenetriol) and by elevated ratios of Δ5/Δ4 steroids.

It is worth mentioning that newborns show a physiological increase in steroid levels Δ5. In this case, the diagnosis requires evaluation by the ACTH stimulation

Figure 2.
Enzymatic conversion mediated by HSD3B.

test. Increases of more than 2 standard deviations of 17 OH pregnenolone, DHEA, Δ5 17OHPregnenolone/17 OH progesterone ratio and Δ5 17OHPregnenolone/cortisol ratio are considered diagnostic for HSD3B2 deficiency.

Nonclasic form of HSD3B2 deficiency manifest itself in form of precocious development of pubic and axillary hair at both sexes and virilization signs in females.

5. 17α-hydroxylase/17,20 lyase deficiency

17α-hydroxylase (CYP17A1, P450c17) is a double-acting enzyme, both 17α-hydroxylase and 17.20-lyase. The 17α-hydroxylase activity mediate conversion of pregnenolone to 17OHPreg and progesterone to 17OHP, and the 17,20-lyase activity convert 17OH-Preg to DHEA, and to a lesser extent 17OHP to androstenedione [1].

CYP17A1 is absent in zona glomerulosa, has only 17α-hydroxylase activity in zona fasciculata, and is fully expressed, with both activities in the zona reticularis [1].

This enzyme is encoded by the *CYP17A1* gene, located on the long arm of chromosome 10 (10q24.3). Mutations in the CYP17A1 gene with 17-hydroxylase deficiency (17OHD) are rare, account for approximately 1% of all CAH cases, more common in Brazil or China [1, 9]. At least 100 mutations affecting CYP17A1 have been described so far, such as missense/nonsense, splicing, small/large deletions, small insertions, but also complex rearrangements. 17OHD affects steroid synthesis in both the adrenals and gonads [9].

Lack of CYP17A1 blocks sex steroid biosynthesis, thus we will encounter undervirilized genitalia, hypertension, absence of pubertal sexualization and gynecomastia in males, respectively normal aspect of the genitals, hypertension, absence of pubertal sexualization (absence of telarche, primary amenorrhea), minimal body hair in females [1, 9].

Hypertension is caused by overproduction of DOC in the zona fasciculata. 17OHD is accompanied by a lack of cortisol synthesis, with low serum levels of potassium, renine and aldosterone, but very high values of corticosterone substitutes for glucocorticoid requirements [1].

Clinically, the suspicion of 17OHD should be considered in hypertensive patients with hypokalemia and low renin and aldosterone levels, in patients with delayed puberty or stopped developing puberty, in children with karyotype 46, XY with inguinal hernia or female external genitalia. It can also be considered in women with unexplained infertility, who may associate a partial deficit with normal sexualization.

Hormone evaluation will identify elevated values of DOC, corticosterone and 18 OH-corticosterone and gonadotropins, associated with low values of 17 OH pregnenolone and 17 OH-progesterone, renin, and aldosterone [9].

Cases of isolated 17.20 lyase deficiency have been reported [1, 9]. In this situation the conversion of pregnenolone to sex steroid precursors with 19 carbon atoms is blocked, without affecting cortisol synthesis. Clinically we will meet external female genitals at birth, regardless of genetic sex.

6. P450 oxidoreductase deficiency

P450 oxidoreductase (POR) is a flavoprotein that transfers electrons from NADPH to all microsomal cytochrome P450 (CYP) enzymes, including CYP17A1, CYP21A2, CYP19A1 (aromatase, P450aro) [1].

POR mutations will be associated in varying degrees with concomitant decrease in the activity of 17α-hydroxylase/17.20 lyase, 21 α-hydroxylase and aromatase. It is a very rare disease [1]. Splicing, missense, or nonsense mutations, small or large deletions, and small gene insertions affecting the gene located on the long arm of chromosome 7 (7q11.23) cause POR deficiency [9].

Clinical findings are various:

- Possible virilization of the mother associated with low serum estriol levels, which thus becomes a biological marker of disease, highlighted in the triple test performed in the second pregnancy trimester
- Girls with severe degrees of virilization (Prader III or IV)
- Boys with normal external genitalia or sexual infantilism
- During adolescence and adulthood: various degrees of virilization in women, the mildest form being similar to PCOS; various degrees of sub virilization in men, the mildest form presenting only with infertility.

Patients with POR deficiency typically has normal electrolytes and mineralocorticoid function, nearly normal cortisol levels that respond poorly to ACTH stimulation, increased levels of 17OHP, but lower than in the isolated 21OHD, and low levels of sex steroids [1].

7. Congenital lipoid adrenal hyperplasia

To initiate steroidogenesis, cholesterol from cytoplasmic storage depots is transported to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR), a transporter protein. At this level CYP11A1 (P450scc) will converted it to pregnenolone [1].

Mutations in StAR cause a rare form of CAH called congenital lipoid adrenal hyperplasia. In this disorder we have hormone deficiency on all 3 lines: mineralocorticoid, glucocorticoid, and sex steroids. Furthermore, lipoid CAH is typically associated with very large adrenals secondary to the accumulation of cholesterol esters in the adrenal glands [1].

From a clinical point of view, children have normal length and weight at birth; male newborns have a completely female phenotype but lack the mullerian derivatives; adrenal insufficiency is manifest from birth, with severe salt loss and dehydration; in 2/3 of cases, we find skin hyperpigmentation and in 1/4 cases hypoglycemia during infancy [9].

Attenuated forms are also described, in which the salt loss becomes manifest later, after the age of 1 month. Female patients with a minor deficiency may have a normal phenotype in childhood, but with the absence of puberty or spontaneous puberty, but stopped in evolution.

These patients will have electrolyte imbalances with severe hyponatremia and hyperkalemia, very low levels of cortisol, aldosterone, DHEA, but also their precursors with much higher levels of ACTH, plasma renin and gonadotropins, the latter even in middle childhood.

The treatment of this condition is common with the classic forms of CAH with adequate glucocorticoid and mineralocorticoid substitution.

8. Conclusions

Under the name of congenital adrenal hyperplasia, there is a heterogeneous spectrum of diseases whose phenotype depends on the type and degree of enzyme deficiency. The diagnosis has an important impact on both the patient and his family. Given that 21 hydroxylase deficiency is the most common etiopathogenesis of the disease, it often becomes synonymous with congenital adrenal hyperplasia. However, we must also consider the possibility of the existence of other enzymatic defects. In conclusion, we are talking about a group of complex diseases whose diagnosis and management require a multidisciplinary approach with the formation of a team of experts in neonatology, endocrinology, genetics, surgery, psychology, these patients requiring regular long-term evaluation.

Acknowledgements

The authors would like to thank to their teachers, professor doctor Constantin Dumitrache and professor doctor Mihail Coculescu, from which they inherited the passion for the secrets of endocrinology.

Conflict of interest

The authors declare no conflict of interest.

Author details

Adina Mariana Ghemigian* and Nicoleta Dumitru
C.I. Parhon National Institute of Endocrinology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

*Address all correspondence to: adinaghemi@yahoo.com

References

[1] Claahsen-van der Grinten HL, Speiser PW, Ahmed SF, Arlt W, Auchus RJ, Falhammar H, et al. Congenital adrenal hyperplasia-current insights in pathophysiology, diagnostics, and management. Endocrine Reviews. 2022;**43**(1):91-159. DOI: 10.1210/endrev/bnab016

[2] Yau M, Gujral J, New MI. Congenital adrenal hyperplasia: Diagnosis and emergency treatment. 2019 Apr 16. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [Accessed: May 16, 2022]

[3] Arriba M, Ezquieta B. Molecular diagnosis of steroid 21-hydroxylase deficiency: A practical approach. Frontiers in Endocrinology. 2022;**29**(13):834549. DOI: 10.3389/fendo.2022.834549

[4] Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An endocrine society clinical practice guideline. Journal of Endocrinology and Metabolism. 2018;**103**(11):4043-4088. DOI: 10.1210/jc.2018-01865. [Erratum in: The Journal of Clinical Endocrinology and Metabolism 2019;**104**(1):39-40]

[5] Mallappa A, Merke DP. Management challenges and therapeutic advances in congenital adrenal hyperplasia. Nature Reviews Endocrinology. 2022;**18**(6): 337-352. DOI: 10.1038/s41574-022-00655-w. Epub 2022 Apr 11

[6] Nimkarn S, Gangishetti PK, Yau M, et al. 21-Hydroxylase-deficient congenital adrenal hyperplasia. 2002 Feb 26 [Updated 2016 Feb 4]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1171/. [Accessed: June 02, 2022]

[7] Nordenström A, Falhammar H. Management of endocrine disease: Diagnosis and management of the patient with non-classic CAH due to 21-hydroxylase deficiency. European Journal of Endocrinology. 2019;**180**(3):R127-R145. DOI: 10.1530/EJE-18-0712

[8] van der Kamp HJ, Wit JM. Neonatal screening for congenital adrenal hyperplasia. European Journal of Endocrinology. 2004;**151**(Suppl. 3): U71-U75. DOI: 10.1530/eje.0.151u071

[9] New M, Yau M, Lekarev O, et al. Congenital adrenal hyperplasia. [Updated 2017 Mar 15]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278953/. [Accessed: June 03, 2022]