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

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Abbreviations

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

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

Introduction

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

21-Hydroxylase Deficiency

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

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

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

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

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

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

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

Female Patients with 21-Hydroxylase Deficiency: Fertility and Pregnancy

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Male Patients with 21-Hydroxylase Deficiency

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

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

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

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

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

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

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

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

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

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

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

Stage 5 Irreversible damage of testicular parenchyma due to chronic obstruction occurs.
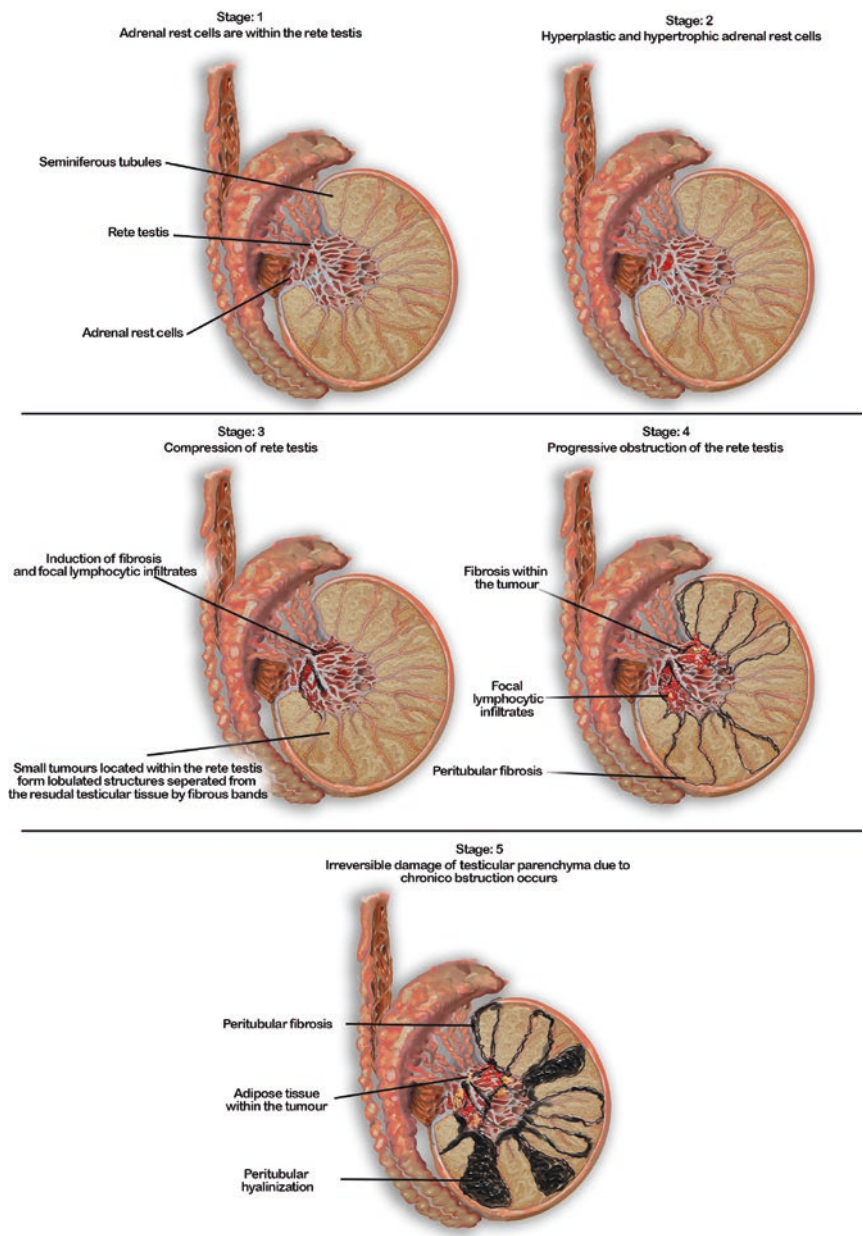
Fig. 1 Histological demonstration of different developmental stages of testicular adrenal rest tumors (TARTs) (The illustration has been drawn by Eren Arik, graphic designer)

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

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

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

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

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

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

11-Beta-Hydroxylase Deficiency

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3-Beta-Hydroxysteroid Dehydrogenase Type 2 Deficiency

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

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

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

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

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

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

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

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

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

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

P450 Oxidoreductase Deficiency

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

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

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

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

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

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

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

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

Lipoid Congenital Adrenal Hyperplasia

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

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

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

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

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

Conclusion

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

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