Association between genotype, clinical presentation, and severity of congenital adrenal hyperplasia: a review

Abdulmoein E. Al-Agha[1], Ali H. Ocheltree[2], Masha'el D. Al-Tamimi[1]

[1]*Department of Pediatrics, King Abdul-Aziz University Hospital, Faculty of Medicine, Jeddah and* [2] *Department of Internal Medicine, North West Armed Forces Hospital, Tabuk, Kingdom of Saudi Arabia. E-mail: aagha@kau.edu.sa*

SUMMARY: Al-Agha AE, Ocheltree AH, Al-Tamimi MD. Association between genotype, clinical presentation, and severity of congenital adrenal hyperplasia: a review. Turk J Pediatr 2012; 54: 323-332.

Congenital adrenal hyperplasia (CAH) applies to a family of inherited disorders of steroidogenesis caused by an abnormality in one of the five enzymatic steps necessary in the conversion of cholesterol to cortisol. The enzyme defects are transmitted as an autosomal recessive trait. Patients with a "classical" form of CAH usually present during the neonatal and early infancy period with adrenal insufficiency, which could be associated with a salt- losing pathology. Females usually have genital ambiguity. Approximately 67% of classical CAH patients are classified as "salt-losing", while 33% have "non-salt-losing" or the "simple-virilizing" form, reflecting the degree of aldosterone deficiency. Non-classic 21-hydroxylase deficiency (NC 21-OHD) refers to the condition in which partial deficiencies of 21-hydroxylation produce less extreme hyperandrogenemia and milder symptoms. Females do not demonstrate genital ambiguity at birth. The gene for adrenal 21-hydroxylase, CYP21, is located on chromosome 6p in the area of human leukocyte antigen (HLA) genes. Specific mutations may be associated with a certain degree of enzymatic compromise and the clinical form of 21-hydroxylase deficiency (21-OHD). NC 21-OHD patients are predicted to have mild mutations on both alleles and one severe or one mild mutation of the 21-OH locus (compound heterozygote). This review aims to describe the association between the genotype and clinical presentations and severity of CAH.

Key words: congenital adrenal hyperplasia, adrenal insufficiency, genital ambiguity, genetic mutation.

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive disorders with defects in the biosynthesis of the cortisol hormone. The synthesis of other steroids, such as mineralocorticoids and adrenal/gonadal sex steroids, may also be affected (Fig. 1). The low level of cortisol stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). This chronic elevation in ACTH causes hyperplasia of the adrenal cortex, giving the characteristic enlargement of the gland. The clinical presentation of the various forms of CAH depends on the following: 1) the affected enzyme, 2) the residual enzymatic activity, 3) consequences of deficiencies of the end products, and 4) hormonal effects of the elevated precursors. The defective conversion of 17-hydroxyprogesterone to 11-deoxycortisol accounts for more than 90% of cases of CAH. This conversion is mediated by 21-hydroxylase, which is also referred to as CYP21A2.

Patients with the "classical" form present during the neonatal period and early infancy with adrenal insufficiency with or without salt-losing or as toddlers with virilization. The "classical" form is the most severe form of CAH due to CYP21A2 deficiency in particular. Females usually have genital ambiguity. Approximately 67% of classical CAH patients are classified as "salt-losing", while 33% are classified as "non-salt-losing" or "simple-virilizing", reflecting the degree of aldosterone deficiency.

"Non-classical" or late-onset CYP21A2 deficiency presents later in life with signs of androgen excess and without neonatal genital ambiguity. Clinical features in childhood may include premature pubarche and accelerated

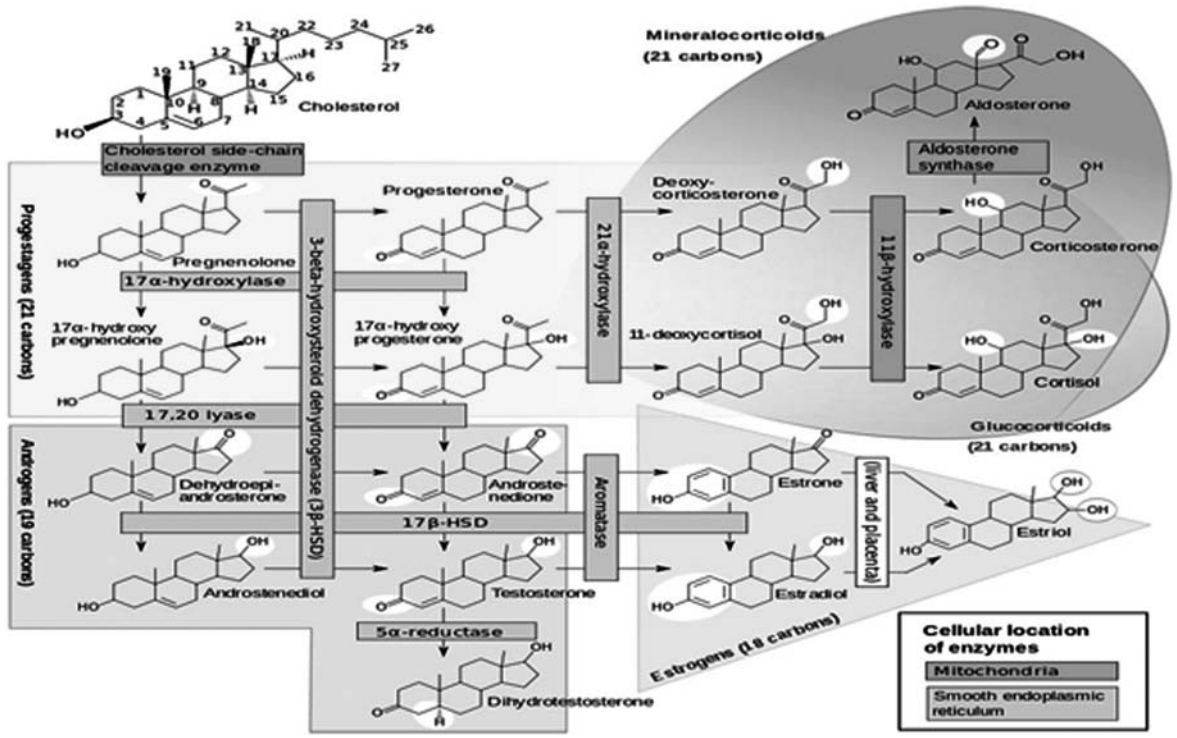

Figure 1. A scheme of the pathways of steroidogenesis, including an elaboration of the biological process by which steroids are generated from cholesterol and transformed into other steroids.

bone age; adolescent and adult females may be presented with hirsutism, menstrual irregularity, infertility, and acne. Some patients with non-classic CAH remain asymptomatic[1-3].

Humans have two CYP21A genes, a non-functional pseudo-gene (CYP21A1 or CYP21P) and the active gene (CYP21A2 or CYP21), and both are located on the 35-kilobase region of chromosome 6p21.3 within the major histocompatibility locus. The pseudo-gene produces a truncated enzyme with no activity because it lacks eight bases from codons 110 to 112, resulting in a stop codon[4-7].

The two CYP21A genes are more than 90% homologous. This high degree of homology facilitates recombination events during meiosis, with consequent exchanges of segments of DNA between the two genes.

- Unequal crossover exchanges leading to deletions of large segments of the CYP21P gene or a non-functioning CYP21P/CYP21 fusion gene (macro-conversion) account for about 20% of CYP21A2 mutations described to date[1,8,9].
- Other hybrid CYP21A1/CYP21A2 gene products have decreased, not halted, enzyme activity. A patient who is heterozygous for this and with a typical large gene deletion may have non-classic CYP21A2 deficiency[10].
- Altered regions of the CYP21A1 gene can be transferred to the CYP21A2 gene through non-reciprocal gene conversion. This is a process by which a segment of genetic material is transferred to a closely related gene, altering its sequence[11,12].
- These micro-conversion events represent acquisition of smaller segments of the CYP21A1 sequence by the CYP21A2 gene and result in deleterious point mutations that reduce enzyme activity. They are present in about 70% of patients with defined genetic abnormalities[3,5,9].
- Eighteen gene conversion mutations account for nearly all affected alleles in various ethnic groups[3,13-15]. The remaining 5% of patients with defined abnormalities have one or more of the 60 point mutations[3,15-20].

Among 130 Brazilian patients, 20% did not have a known mutation, suggesting that other mutations occur. A novel missense mutation was subsequently identified in three patients with suggestion of a founder effect[15]. No mutation was detected in the entire coding region of the gene and up to 1 kb of the 5'-flanking promoter region of the gene in a Mexican and three Japanese patients, suggesting that more distant mutations may occur[21,22]. It is not always possible to predict the phenotype of these patients from the specific mutation(s) of the CYP21A2 gene, but there are general correlations between genotype and phenotype[3,6-19]. Patients with CYP21A2 mutations can be divided into groups according to the predicted effect of the mutation on 21-hydroxylase enzymatic activity, as determined by site-directed mutagenesis and expression and *in vitro* analysis of enzymatic activity[16].

The salt-losing form of the disorder is most often associated with large deletions or intron 2 mutations that affect splicing and result in no enzyme activity, while patients with the simple virilizing form have low but detectable enzyme activity (i.e., 1-2%) that supports sufficient aldosterone and glucocorticoid production. This most commonly results from point mutations leading to non-conservative amino-acid substitutions such as Ile172Asp.

Women with the non-classic form may be either compound heterozygotes (with a classic mutation and a variant allele) or heterozygotes with two variant alleles, allowing for 20-60% of normal enzymatic activity (e.g., with point mutations leading to conservative amino acid substitutions such as Val281Leu). Patients who are compound heterozygotes for two different CYP21A2 mutations usually have the phenotype associated with the less severe of the two genetic defects[13]. Heterozygotes may have mild biochemical abnormalities, but no clinically important endocrine disorder[23,24]. Despite these general correlations, the CYP21A2 deficiency phenotype does not always correlate precisely with the genotype[18,19], suggesting that other genes influence the clinical manifestations. In general, there appears to be high concordance rates between genotype and phenotype in patients with the most severe and the mildest forms of the disease, but less genotype-phenotype correlation in moderately affected patients[16-19,25,26].

Genetics Deficiencies of Uncommon Causes of CAH

1). Deficiency of 17-alpha-hydroxylase activity (CYP17):

Deficiency of 17-alpha-hydroxylase activity is a rare form of CAH. Approximately 120 cases have been reported[27,28]. However, prevalence may be higher, particularly in Brazil, where the founder effects account for over 80% of mutant alleles caused by only two mutations[29,30]. The CYP17 gene encodes an enzyme that has both 17-hydroxylase and 17, 20-lyase (desmolase, or side-chain cleavage) activities. The hydroxylation of C17 of progesterone is required for cortisol production as well as for synthesis of androgens and estrogens. Lyase activity is required for synthesis of androgens and their derivatives, the estrogenic C18 steroids. Although CYP17 has both activities *in vitro*, human CYP17 deficiency syndromes have been observed in which patients apparently lack only 17-hydroxylase deficiency or only 17, 20-lyase deficiency[31,32]. Most patients, however, have a combined defect[33].

2). 17-hydroxylase defect:

Reduction of cortisol production by the 17-hydroxylase defect results in an increased ACTH secretion. Therefore, there is an increased production of 11-deoxysteroids including corticosterone, mineralocorticoids, 11-deoxycorticosterone, and 18-hydroxy-deoxycorticosterone[34,35]. Thus, one consequence of 17-alpha-hydroxylase deficiency is mineralocorticoid excess. The ensuing volume expansion inhibits rennin release and therefore the synthesis of aldosterone[36].

3). 17, 20 lyase defect:

Reduction of androgen production by impairment of 17, 20 lyase activity leads to combined androgen and estrogen deficiency because only androgens can be aromatized to form estrogens. 17-alpha-hydroxylase activity (CYP17) is also expressed in the gonads so that gonadal steroidogenesis is also decreased in patients with this disorder. CYP17 deficiency like other forms of CAH appears to be inherited as an autosomal recessive trait. Several cases were reported, and they were the product of consanguineous marriages

and obligate heterozygotes with mild defects in CYP17 activity that can be revealed by ACTH stimulation[35,37,38]. Nearly 40 different mutations in the CYP17 gene, which is located on chromosome 10, have been defined at the molecular level[30]. These include small insertions that disrupt the normal reading frame of the gene and lead to premature termination[39,40], deletions of single codons[41], deletions of several codons[42], large deletions with insertions of foreign DNA[43], and a variety of nonsense or missense mutations of CYP17 that produce stop codons, impair CYP17 enzyme activity, or alter splice sites[44-52]. Two mutations have been described in splice receptor sites[53]. There may be factors other than the CYP17 genotype that determine the phenotype of individuals with CYP17 deficiency. In a study of 24 patients from 19 families in Brazil, the majority (20/24) had one of two mutations, both of which were completely inactive *in vitro*[30]]. However, for patients with the same mutation, the phenotype was sometimes variable. As an example, of three male (XY karyotyping) patients with an R362C mutation, the first presented at birth with ambiguous genitalia, the second had female external genitalia and normokalemia, and the third had the classical presentation (hypertension, hypokalemia, and pubertal delay). A rare variant, with combined CYP21A2 and CYP17 deficiency, has been described in both boys and girls and appears to be due to mutations in the gene encoding oxidoreductase, not the CYP17 and CYP21A2 genes[54,55].

4). 3-beta-hydroxysteroid dehydrogenase deficiency:

3-beta-hydroxysteroid dehydrogenase deficiency is a rare form of CAH, in which synthesis of all steroid hormones is impaired as 3-beta-hydroxysteroid dehydrogenase (HSD3B2) catalyzes the rearrangement of the double bonds in the ring (A) of steroids and conversion of a hydroxyl group at the 3 position to a keto group. Deficiency of this enzyme results in decreased synthesis of cortisol, aldosterone, androgens, and estrogens. Cortisol deficiency leads to increased ACTH secretion and therefore accumulation of excessive amounts of steroid precursors with the delta-5, 3-hydroxy configuration (e.g., delta-5-pregnenolone, 17-alpha-hydroxypregnenolone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone-sulfate (DHEAS)[56]. The decreased enzyme activity in this disorder is caused by mutations in the 3-beta-hydroxysteroid dehydrogenase II gene. In patients with the severe, salt-wasting form, nonsense mutations introducing codons, insertion and deletion mutations causing frame shifts[57-59], and point mutations that alter enzyme function have all been described. On the other hand, all patients without salt-wasting have had missense mutations causing single amino acid substitutions that reduced the affinity of the enzyme for substrates or cofactors. The 3-beta-hydroxysteroid dehydrogenase type 1 gene, which is normally expressed in the placenta and peripheral tissues, is intact in these patients, providing an explanation for the near normal or even elevated serum concentrations of delta-4 steroids (such as 17-alpha-hydroxyprogesterone and androstenedione) in many patients. The substrates for the peripheral enzyme (delta-5-pregnenolone, 17-alpha-hydroxypregnenolone and DHEAS) are increased because of the defect in the type II enzyme in steroidogenic tissues[60].

Most patients present as neonates or in early infancy with clinical manifestations of both cortisol and aldosterone deficiencies with feeding difficulties, vomiting, volume depletion, hyponatremia, and hyperkalemia. Females may have mild virilization of their external genitalia, presumably due to excess DHEA, a little of which is converted peripherally to testosterone. Males have varying degrees of failure to normal genital development ranging from hypospadias to male pseudohermaphroditism [46 XY disorder of sex development (DSD), as new nomenclature] with near normal female external genitalia. However, 3-beta-hydroxysteroid dehydrogenase deficiency is not a common finding in patients who present with apparent idiopathic hypospadias. In one study, only 2 out of 90 boys with hypospadias had evidence of subtle molecular abnormalities in the 3-beta-hydroxysteroid dehydrogenase gene[61]. Rarely, patients with severe 3-beta-hydroxysteroid dehydrogenase deficiency have few symptoms and may not be diagnosed until they seek care for delayed puberty. Premature pubarche with exaggerated serum 17-alpha-hydroxypregnenolone responses to ACTH has been described in three girls with missense mutation of the gene. A late-onset or

the non-classic form of 3-beta-hydroxysteroid dehydrogenase deficiency that causes hirsutism and menstrual irregularity in adolescent and/or young adult women has also been described. The basis for the diagnosis was exaggerated with serum delta-5-pregnenolone responses to ACTH, but the validity of these results has been questioned.

5). Congenital lipoid adrenal hyperplasia:

Congenital lipoid adrenal hyperplasia is the rarest and usually the most severe form of adrenal steroidogenesis defect. Congenital lipoid adrenal hyperplasia is characterized by deficiency of all adrenal and gonadal steroid hormones, increased ACTH secretion, and marked adrenal hyperplasia with progressive accumulation of cholesterol esters. It is transmitted as an autosomal recessive trait. It has been thought that the defect in this disorder would reside in the CYP11A1 (side chain cleavage) gene. However, CYP11A1 is required for biosynthesis of progesterone, and placental progesterone synthesis is required to maintain pregnancy. Therefore, complete CYP11A1 deficiency was thought to be lethal and no homozygous mutation has been identified in any patient with congenital lipoid adrenal hyperplasia[62,63]. Subsequently, two patients with congenital lipoid adrenal hyperplasia have been shown to have heterozygous mutations of CYP11A1[64,65]. One had apparent haploinsufficiency of CYP11A1[64] and the other was a compound heterozygote with partial inactivation of the gene[65]. The defect in the majority of patients within congenital lipoid adrenal hyperplasia resides in a gene on chromosome 8 that codes for a protein called the steroidogenic acute regulatory protein (StAR). StAR is a mitochondrial phosphoprotein that mediates the acute response to steroidogenic stimuli by increasing cholesterol transport from the outer to the inner mitochondrial membrane[66-70]. StAR is expressed in the adrenal cortex and gonads but not in the placenta, and for this reason, placental synthesis of progesterone, which requires CYP11A1, is unaffected in congenital lipoid adrenal hyperplasia[57]. The role of StAR has been studied by sequencing the gene in patients with congenital lipoid adrenal hyperplasia[71-74]. More than 35 different mutations have been described; furthermore, the mutated StAR proteins were inactive in functional assays. Most of the mutations reduce activity of the lipid transfer domain of the StAR protein. Steroidogenic cells that lacked StAR were initially capable of low levels of steroidogenesis; this explains why some steroid hormones may be secreted after puberty. Bose et al.[75] concluded that the congenital lipoid adrenal hyperplasia phenotype is the result of two separate events: 1) the initial defect in steroidogenesis due to the StAR mutation, and 2) a subsequent further defect in steroidogenesis due to cellular damage from accumulated cholesterol esters. The "two hit" hypothesis is supported by data from a girl with a homozygous StAR mutation who underwent spontaneous puberty at the age of 13[75].

In mice, with knocked out StAR and apolipoprotein A-1 genes, the lipid deposits were composed mostly of high-density lipoprotein (HDL)-derived cholesterol esters. The mitochondrial structure of StAR knockout mice is less abnormal than that of CYP11A1 knockout mice, perhaps because CYP11A1 plays an important role in determining the morphology of steroidogenic mitochondria[76]. A patient with male pseudohermaphroditism (46,XY DSD) and adrenal insufficiency who was phenotypically similar to patients with StAR deficiency had a mutation of the SF-1 gene[77].

Patients with congenital lipoid hyperplasia caused by StAR mutations typically have severe adrenal insufficiency very soon after birth, although they occasionally present later in infancy[78], with vomiting, diarrhea, volume depletion, hyponatremia, and hyperkalemia. Male infants usually have female external genitalia due to lack of testicular androgen production. In comparison, female infants are normally developed at birth and occasional patients undergo spontaneous puberty[79]. A possible explanation for the relative sparing of ovarian steroid synthesis may be the dormancy of the ovary until puberty, thereby preventing the excess cholesterol accumulation that damages the adrenal glands and testes. The same explanation may account for the detectable, if very low, serum aldosterone concentrations with high plasma rennin activity in these patients; the adrenocortical cells destined to become glomerulosa cells are minimally stimulated *in utero*[75]. This condition should be considered in a neonate with any

symptoms or signs of adrenal insufficiency and in male DSD (46,XY DSD). The diagnosis is confirmed by the absence of demonstrable steroid biosynthetic activity by either the adrenals or the gonads. The two patients with CYP11A1 deficiency presented with adrenal insufficiency at a later age and did not have enlarged adrenal glands. One was incompletely virilized at birth with an XY karyotype and low levels of all measured steroids when diagnosed at the age of 4 years[64]. The other patient had an XX karyotype and presented with adrenal insufficiency at the age of 9 months[80].

6). 11-beta-hydroxysteroid dehydrogenase type 1 (apparent cortisone reductase) deficiency:

This rare disorder is not a true CAH (i.e., does not involve a defect in cortisol biosynthesis but is associated with adrenal hyperplasia and changes in the cortisol metabolism). 11-Beta-hydroxysteroid dehydrogenase type 1 (HSD11B1) is expressed in certain tissues such as the liver, adipose tissue, brain, and adrenal gland, in which it regulates the local availability of glucocorticoids. Six women and one boy have been reported to have deficient expression of this enzyme[81-85]. However, no mutation in the coding regions of the gene was found, suggesting either that the defect lies outside the coding region or that some other abnormality results in inhibition of the function of the enzyme. The disorder may result from either an autosomal recessive or an acquired defect[81]. Additional studies in four patients demonstrated a triallelic digenic pattern of inheritance[73]. Each affected individual was homozygous or heterozygous for two mutations in intron 3 of the HSD11B1 gene; the other introns, exons and 1.5 kb of the promoter region, were normal. *In vitro*, transcriptional activation of constructs containing these mutations was 2.5 times lower than that of wild type constructs. However, since 25% of unaffected individuals were heterozygotic for these mutations and 3% were homozygous, this mutation alone does not explain the cortisone reductase deficiency. Each of the affected individuals, but none of the unaffected individuals, also had a mutation in exon 5 of the hexose-6-phosphate dehydrogenase (H6PDH) gene. Since HSD11B1 requires NADPH for activity and H6PDH generates NADPH, it is likely that the combination of mutations led to cortisone reductase deficiency[82].

Patients with 11-beta-hydroxysteroid deficiency present as adolescent or adult women with truncal obesity, facial plethora, oligomenorrhea, hirsutism, infertility, and/or acne. One patient even had a successful pregnancy[81]. Another six-year-old boy presented with gonadotropin-independent precocious puberty[83]. The adrenal glands are diffusely enlarged. Patients with deficiency in this enzyme have normal serum cortisol concentrations, high serum adrenal androgen concentrations, very high urinary excretion of 5-beta-reduced cortisone metabolites (tetrahydrocortisone and cortolones), and very low urinary excretion of cortisol metabolites (tetrahydrocortisol and cortols), especially the 5-alpha-reduced metabolites[81]. Conversion of orally administered cortisone to cortisol is impaired. Adrenal steroidogenesis is suppressed normally by administration of low-dose dexamethasone (0.5 mg every 6 hours for 48 hours).

Familial Glucocorticoid Resistance:

This is a rare syndrome that is not a true CAH, but is associated with adrenal hyperplasia and clinical features similar to those of CYP11B1 (11-beta-hydroxylase) deficiency. Familial glucocorticoid resistance is inherited as an autosomal recessive or dominant disorder characterized by mutations in the glucocorticoid receptor gene, leading to diminished cortisol action and secondary stimulation of ACTH release[86,87]. However, some patients with clinical and biochemical glucocorticoid resistance never had mutations in the glucocorticoid receptor gene, indicating the presence of other defects in the glucocorticoid action[88].

Glucocorticoid receptor defects include decreased steroid binding affinity caused by missense point mutations in the ligand binding domain, defective nuclear binding caused by a missense point mutation in the DNA-binding domain and decreased receptor number caused by a four-base splice site deletion in exon 6. Mutant receptors may impair the function of receptors coded by the normal allele by preventing normal dimerization or exerting antagonistic effects on glucocorticoid response elements (GREs), thereby exerting dominant negative effects that result in autosomal dominant expression[87-89].

Some of the mutations reduce binding of the receptor-ligand complex to transcription factors or co-activators[86]. Patients with familial glucocorticoid resistance present in childhood to adulthood with hirsutism, male pattern baldness, menstrual abnormalities and infertility in women, isosexual precocious puberty, abnormal spermatogenesis and infertility in boys, and with hypertension and hypokalemic alkalosis in both sexes. The clinical presentation varies from asymptomatic to severely symptomatic. Heterozygotes have mild glucocorticoid resistance but are asymptomatic[86,87,89]. Plasma ACTH and serum cortisol concentrations are increased in this disorder, but maintain their normal diurnal rhythm[90-92]. They are relatively resistant to suppression with dexamethasone. Serum concentrations of adrenal androgens (delta-4-androstenedione, DHEA and its sulfate [DHEAS]) and of ACTH-dependent mineralocorticoids (cortisol, corticosterone, and deoxycorticosterone) are also increased because of the chronically elevated plasma ACTH concentration. Serum aldosterone and plasma rennin activity tend to be decreased. The adrenal glands appear normal to moderately enlarged. Symptoms of adrenal insufficiency do not occur in patients with familial glucocorticoid resistance because of the compensatory increases in ACTH and cortisol secretion. The excess of androgens and mineralocorticoid can be ameliorated by the administration of dexamethasone (0.5 to 1.5 mg/day). Thiazide diuretics administered alone may cause severe hypokalemia[87-91].

REFERENCES

1. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 2000; 21: 245-291.
2. Pang SY, Wallace MA, Hofman L, et al. Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 1988; 81: 866-874.
3. Speiser PW, White PC. Congenital adrenal hyperplasia. N Engl J Med 2003; 349: 776-788.
4. Carroll MC, Campbell RD, Porter RR. Mapping of steroid 21-hydroxylase genes adjacent to complement component C4 genes in HLA, the major histocompatibility complex in man. Proc Natl Acad Sci U S A 1985; 82: 521-525.
5. White PC, Grossberger D, Onufer BJ, et al. Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. Proc Natl Acad Sci U S A 1985; 82: 1089-1093.
6. White PC, New MI, Dupont B. Structure of human steroid 21-hydroxylase genes. Proc Natl Acad Sci U S A 1986; 83: 5111-5115.
7. Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y. Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a pseudogene and a genuine gene. Proc Natl Acad Sci U S A 1986; 83: 2841-2845.
8. New MI, White PC. Genetic disorders of steroid hormone synthesis and metabolism. Baillieres Clin Endocrinol Metab 1995; 9: 525-554.
9. Miller WL. Clinical review 54: genetics, diagnosis, and management of 21-hydroxylase deficiency. J Clin Endocrinol Metab 1994; 78: 241-246.
10. L'Allemand D, Tardy V, Grüters A, Schnabel D, Krude H, Morel Y. How a patient homozygous for a 30-kb deletion of the C4-CYP 21 genomic region can have a nonclassic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab 2000; 85: 4562-4567.
11. Miller WL. Gene conversions, deletions, and polymorphisms in congenital adrenal hyperplasia. Am J Hum Genet 1988; 42: 4-7.
12. Cooper DN, Ball EV, Krawczak M. The human gene mutation database. Nucleic Acids Res 1998; 26: 285-287.
13. Lajić S, Clauin S, Robins T, et al. Novel mutations in CYP21 detected in individuals with hyperandrogenism. J Clin Endocrinol Metab 2002; 87: 2824-2829.
14. Stikkelbroeck NM, Hoefsloot LH, de Wijs IJ, Otten BJ, Hermus AR, Sistermans EA. CYP21 gene mutation analysis in 198 patients with 21-hydroxylase deficiency in The Netherlands: six novel mutations and a specific cluster of four mutations. J Clin Endocrinol Metab 2003; 88: 3852-3859.
15. Billerbeck AE, Mendonca BB, Pinto EM, Madureira G, Arnhold IJ, Bachega TA. Three novel mutations in CYP21 gene in Brazilian patients with the classical form of 21-hydroxylase deficiency due to a founder effect. J Clin Endocrinol Metab 2002; 87: 4314-4317.
16. Speiser PW, Dupont J, Zhu D, et al. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Invest 1992; 90: 584-595.
17. Wedell A, Thilén A, Ritzén EM, Stengler B, Luthman H. Mutational spectrum of the steroid 21-hydroxylase gene in Sweden: implications for genetic diagnosis and association with disease manifestation. J Clin Endocrinol Metab 1994; 78: 1145-1152.
18. Wilson RC, Mercado AB, Cheng KC, New MI. Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. J Clin Endocrinol Metab 1995; 80: 2322-2329.

19. Jääskeläinen J, Levo A, Voutilainen R, Partanen J. Population-wide evaluation of disease manifestation in relation to molecular genotype in steroid 21-hydroxylase (CYP21) deficiency: good correlation in a well defined population. J Clin Endocrinol Metab 1997; 82: 3293-3297.

20. Ordoñez-Sánchez ML, Ramírez-Jiménez S, López-Gutierrez AU, et al. Molecular genetic analysis of patients carrying steroid 21-hydroxylase deficiency in the Mexican population: identification of possible new mutations and high prevalence of apparent germ-line mutations. Hum Genet 1998; 102: 170-177.

21. Nimkarn S, Cerame BI, Wei JQ, et al. Congenital adrenal hyperplasia (21-hydroxylase deficiency) without demonstrable genetic mutations. J Clin Endocrinol Metab 1999; 84: 378-381.

22. Koyama S, Toyoura T, Saisho S, Shimozawa K, Yata J. Genetic analysis of Japanese patients with 21-hydroxylase deficiency: identification of a patient with a new mutation of a homozygous deletion of adenine at codon 246 and patients without demonstrable mutations within the structural gene for CYP21. J Clin Endocrinol Metab 2002; 87: 2668-2673.

23. Gutai JP, Kowarski AA, Migeon CJ. The detection of the heterozygous carrier for congenital virilizing adrenal hyperplasia. J Pediatr 1977; 90: 924-929.

24. Charmandari E, Merke DP, Negro PJ, et al. Endocrinologic and psychologic evaluation of 21-hydroxylase deficiency carriers and matched normal subjects: evidence for physical and/or psychologic vulnerability to stress. J Clin Endocrinol Metab 2004; 89: 2228-2236.

25. Krone N, Braun A, Roscher AA, Knorr D, Schwarz HP. Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany. J Clin Endocrinol Metab 2000; 85: 1059-1065.

26. Deneux C, Tardy V, Dib A, et al. Phenotype-genotype correlation in 56 women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 2001; 86: 207-213.

27. Yanase T, Simpson ER, Waterman MR. 17 alpha-hydroxylase/17,20-lyase deficiency: from clinical investigation to molecular definition. Endocr Rev 1991; 12: 91-108.

28. Biglieri EG. 17 alpha-Hydroxylase deficiency: 1963-1966. J Clin Endocrinol Metab 1997; 82: 48-50.

29. Kater CE, Biglieri EG. Disorders of steroid 17 alpha-hydroxylase deficiency. Endocrinol Metab Clin North Am 1994; 23: 341-357.

30. Costa-Santos M, Kater CE, Auchus RJ; Brazilian Congenital Adrenal Hyperplasia Multicenter Study Group. Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. J Clin Endocrinol Metab 2004; 89: 49-60.

31. Zachmann M, Völlmin JA, Hamilton W, Prader A. Steroid 17,20-desmolase deficiency: a new cause of male pseudohermaphroditism. Clin Endocrinol (Oxf) 1972; 1: 369-385.

32. Zachmann M, Werder EA, Prader A. Two types of male pseudohermaphroditism due to 17, 20-desmolase deficiency. J Clin Endocrinol Metab 1982; 55: 487-490.

33. Winter JS, Couch RM, Muller J, et al. Combined 17-hydroxylase and 17,20-desmolase deficiencies: evidence for synthesis of a defective cytochrome P450c17. J Clin Endocrinol Metab 1989; 68: 309-316.

34. Kater CE, Biglieri EG, Brust N, Chang B, Hirai J. The unique patterns of plasma aldosterone and 18-hydroxycorticosterone concentrations in the 17 alpha-hydroxylase deficiency syndrome. J Clin Endocrinol Metab 1982; 55: 295-302.

35. Griffing GT, Wilson TE, Holbrook MM, et al. Plasma and urinary 19-nor-deoxycorticosterone in 17 alpha-hydroxylase deficiency syndrome. J Clin Endocrinol Metab 1984; 59: 1011-1015.

36. Rovner DR, Conn JW, Cohen EL, Berlinger FG, Kem DC, Gordon DL. 17 alpha-Hydroxylase deficiency. A combination of hydroxylation defect and reversible blockade in aldosterone biosynthesis. Acta Endocrinol (Copenh) 1979; 90: 490-504.

37. D'Armiento M, Reda G, Kater C, Shackleton CH, Biglieri EG. 17 alpha-hydroxylase deficiency: mineralocorticoid hormone profiles in an affected family. J Clin Endocrinol Metab 1983; 56: 697-701.

38. Wit JM, van Roermund HP, Oostdijk W, et al. Heterozygotes for 17 alpha-hydroxylase deficiency can be detected with a short ACTH test. Clin Endocrinol (Oxf) 1988; 28: 657-664.

39. Kagimoto M, Winter JS, Kagimoto K, Simpson ER, Waterman MR. Structural characterization of normal and mutant human steroid 17 alpha-hydroxylase genes: molecular basis of one example of combined 17 alpha-hydroxylase/17,20 lyase deficiency. Mol Endocrinol 1988; 2: 564-570.

40. Yanase T, Sanders D, Shibata A, Matsui N, Simpson ER, Waterman MR. Combined 17 alpha-hydroxylase/17,20-lyase deficiency due to a 7-basepair duplication in the N-terminal region of the cytochrome P45017 alpha (CYP17) gene. J Clin Endocrinol Metab 1990; 70: 1325-1329.

41. Yanase T, Kagimoto M, Suzuki S, Hashiba K, Simpson ER, Waterman MR. Deletion of a phenylalanine in the N-terminal region of human cytochrome P-450(17 alpha) results in partial combined 17 alpha-hydroxylase/17,20-lyase deficiency. J Biol Chem 1989; 264: 18076-18082.

42. Fardella CE, Zhang LH, Mahachoklertwattana P, Lin D, Miller WL. Deletion of amino acids Asp487-Ser488-Phe489 in human cytochrome P450c17 causes severe 17 alpha-hydroxylase deficiency. J Clin Endocrinol Metab 1993; 77: 489-493.

43. Biason A, Mantero F, Scaroni C, Simpson ER, Waterman MR. Deletion within the CYP17 gene together with insertion of foreign DNA is the cause of combined complete 17 alpha-hydroxylase/17,20-lyase deficiency in an Italian patient. Mol Endocrinol 1991; 5: 2037-2045.

44. Yanase T, Kagimoto M, Matsui N, Simpson ER, Waterman MR. Combined 17 alpha-hydroxylase/17,20-lyase deficiency due to a stop codon in the N-terminal region of 17 alpha-hydroxylase cytochrome P-450. Mol Cell Endocrinol 1988; 59: 249-253.

45. Rumsby G, Skinner C, Lee HA, Honour JW. Combined 17 alpha-hydroxylase/17,20-lyase deficiency caused by heterozygous stop codons in the cytochrome P450 17 alpha-hydroxylase gene. Clin Endocrinol (Oxf) 1993; 39: 483-485.

46. Monno S, Ogawa H, Date T, Fujioka M, Miller WL, Kobayashi M. Mutation of histidine 373 to leucine in cytochrome P450c17 causes 17 alpha-hydroxylase deficiency. J Biol Chem 1993; 268: 25811-25817.

47. Lin D, Harikrishna JA, Moore CC, Jones KL, Miller WL. Missense mutation serine106----proline causes 17 alpha-hydroxylase deficiency. J Biol Chem 1991; 266: 15992-15998.

48. Yanase T, Waterman MR, Zachmann M, Winter JS, Simpson ER, Kagimoto M. Molecular basis of apparent isolated 17,20-lyase deficiency: compound heterozygous mutations in the C-terminal region (Arg(496)----Cys, Gln(461)----Stop) actually cause combined 17 alpha-hydroxylase/17,20-lyase deficiency. Biochim Biophys Acta 1992; 1139: 275-279.

49. Ahlgren R, Yanase T, Simpson ER, Winter JS, Waterman MR. Compound heterozygous mutations (Arg 239----stop, Pro 342----Thr) in the CYP17 (P45017 alpha) gene lead to ambiguous external genitalia in a male patient with partial combined 17 alpha-hydroxylase/17,20-lyasedeficiency. J Clin Endocrinol Metab 1992; 74: 667-672.

50. Fardella CE, Hum DW, Homoki J, Miller WL. Point mutation of Arg440 to His in cytochrome P450c17 causes severe 17 alpha-hydroxylase deficiency. J Clin Endocrinol Metab 1994; 79: 160-164.

51. Yamaguchi H, Nakazato M, Miyazato M, Kangawa K, Matsukura S. A 5'-splice site mutation in the cytochrome P450 steroid 17alpha-hydroxylase gene in 17alpha-hydroxylase deficiency. J Clin Endocrinol Metab 1997; 82: 1934-1938.

52. Brooke AM, Taylor NF, Shepherd JH, et al. A novel point mutation in P450c17 (CYP17) causing combined 17alpha-hydroxylase/17,20-lyase deficiency. J Clin Endocrinol Metab 2006; 91: 2428-2431.

53. Costa-Santos M, Kater CE, Dias EP, Auchus RJ. Two intronic mutations cause 17-hydroxylase deficiency by disrupting splice acceptor sites: direct demonstration of aberrant splicing and absent enzyme activity by expression of the entire CYP17 gene in HEK-293 cells. J Clin Endocrinol Metab 2004; 89: 43-48.

54. Arlt W, Walker EA, Draper N, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 2004; 363: 2128-2135.

55. Flück CE, Tajima T, Pandey AV, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. Nat Genet 2004; 36: 228-230.

56. Bongiovanni AM, Root AW. The adrenogenital syndrome. N Engl J Med 1963; 268: 1283-1289.

57. Rhéaume E, Simard J, Morel Y, et al. Congenital adrenal hyperplasia due to point mutations in the type II 3 beta-hydroxysteroid dehydrogenase gene. Nat Genet 1992; 1: 239-245.

58. Zhang L, Sakkal-Alkaddour H, Chang YT, Yang X, Pang S. A new compound heterozygous frameshift mutation in the type II 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD) gene causes salt-wasting 3 beta-HSD deficiency congenital adrenal hyperplasia. J Clin Endocrinol Metab 1996; 81: 291-295.

59. Simard J, Rhéaume E, Leblanc JF, et al. Congenital adrenal hyperplasia caused by a novel homozygous frameshift mutation 273 delta AA in type II 3 beta-hydroxysteroid dehydrogenase gene (HSD3B2) in three male patients of Afghan/Pakistani origin. Hum Mol Genet 1994; 3: 327-330.

60. Simard J, Rheaume E, Mebarki F, et al. Molecular basis of human 3 beta-hydroxysteroid dehydrogenase deficiency. J Steroid Biochem Mol Biol 1995; 53: 127-138.

61. Codner E, Okuma C, Iñiguez G, et al. Molecular study of the 3 beta-hydroxysteroid dehydrogenase gene type II in patients with hypospadias. J Clin Endocrinol Metab 2004; 89: 957-964.

62. Sakai Y, Yanase T, Okabe Y, et al. No mutation in cytochrome P450 side chain cleavage in a patient with congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metab 1994; 79: 1198-1201.

63. Lin D, Gitelman SE, Saenger P, Miller WL. Normal genes for the cholesterol side chain cleavage enzyme, P450scc, in congenital lipoid adrenal hyperplasia. J Clin Invest 1991; 88: 1955-1962.

64. Tajima T, Fujieda K, Kouda N, Nakae J, Miller WL. Heterozygous mutation in the cholesterol side chain cleavage enzyme (p450scc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. J Clin Endocrinol Metab 2001; 86: 3820-3825.

65. Katsumata N, Ohtake M, Hojo T, et al. Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. J Clin Endocrinol Metab 2002; 87: 3808-3813.

66. Sugawara T, Holt JA, Driscoll D, et al. Human steroidogenic acute regulatory protein: functional activity in COS-1 cells, tissue-specific expression, and mapping of the structural gene to 8p11.2 and a pseudogene to chromosome 13. Proc Natl Acad Sci U S A 1995; 92: 4778-4782.

67. Lin D, Sugawara T, Strauss JF 3rd, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 1995; 267: 1828-1831.

68. Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 1994; 269: 28314-28322.

69. Stocco DM, Ascoli M. The use of genetic manipulation

of MA-10 Leydig tumor cells to demonstrate the role of mitochondrial proteins in the acute regulation of steroidogenesis. Endocrinology 1993; 132: 959-967.

70. Stocco DM, Sodeman TC. The 30-kDa mitochondrial proteins induced by hormone stimulation in MA-10 mouse Leydig tumor cells are processed from larger precursors. J Biol Chem 1991; 266: 19731-19738.
71. Bose HS, Sugawara T, Strauss JF 3rd, Miller WL; International Congenital Lipoid Adrenal Hyperplasia Consortium. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med 1996; 335: 1870-1878.
72. Katsumata N, Kawada Y, Yamamoto Y, et al. A novel compound heterozygous mutation in the steroidogenic acute regulatory protein gene in a patient with congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metab 1999; 84: 3983-3987.
73. González AA, Reyes ML, Carvajal CA, et al. Congenital lipoid adrenal hyperplasia caused by a novel splicing mutation in the gene for the steroidogenic acute regulatory protein. J Clin Endocrinol Metab 2004; 89: 946-951.
74. Fujieda K, Okuhara K, Abe S, Tajima T, Mukai T, Nakae J. Molecular pathogenesis of lipoid adrenal hyperplasia and adrenal hypoplasia congenita. J Steroid Biochem Mol Biol 2003; 85: 483-489.
75. Bose HS, Pescovitz OH, Miller WL. Spontaneous feminization in a 46,XX female patient with congenital lipoid adrenal hyperplasia due to a homozygous frameshift mutation in the steroidogenic acute regulatory protein. J Clin Endocrinol Metab 1997; 82: 1511-1515.
76. Ishii T, Hasegawa T, Pai CI, et al. The roles of circulating high-density lipoproteins and trophic hormones in the phenotype of knockout mice lacking the steroidogenic acute regulatory protein. Mol Endocrinol 2002; 16: 2297-2309.
77. Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet 1999; 22: 125-126.
78. Gassner HL, Toppari J, Quinteiro González S, Miller WL. Near-miss apparent SIDS from adrenal crisis. J Pediatr 2004; 145: 178-183.
79. Fujieda K, Tajima T, Nakae J, et al. Spontaneous puberty in 46,XX subjects with congenital lipoid adrenal hyperplasia. Ovarian steroidogenesis is spared to some extent despite inactivating mutations in the steroidogenic acute regulatory protein (StAR) gene. J Clin Invest 1997; 99: 1265-1271.
80. Pang S, Carbunaru G, Haider A, et al. Carriers for type II 3beta-hydroxysteroid dehydrogenase (HSD3B2) deficiency can only be identified by HSD3B2 genotype study and not by hormone test. Clin Endocrinol (Oxf) 2003; 58: 323-331.
81. Jamieson A, Wallace AM, Andrew R, et al. Apparent cortisone reductase deficiency: a functional defect in 11beta-hydroxysteroid dehydrogenase type 1. J Clin Endocrinol Metab 1999; 84: 3570-3574.
82. Draper N, Walker EA, Bujalska IJ, et al. Mutations in the genes encoding 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. Nat Genet 2003; 34: 434-439.
83. Małunowicz EM, Romer TE, Urban M, Bossowski A. 11beta-hydroxysteroid dehydrogenase type 1 deficiency ('apparent cortisone reductase deficiency') in a 6-year-old boy. Horm Res 2003; 59: 205-210.
84. Biason-Lauber A, Suter SL, Shackleton CH, Zachmann M. Apparent cortisone reductase deficiency: a rare cause of hyperandrogenemia and hypercortisolism. Horm Res 2000; 53: 260-266.
85. Phillipov G, Palermo M, Shackleton CH. Apparent cortisone reductase deficiency: a unique form of hypercortisolism. J Clin Endocrinol Metab 1996; 81: 3855-3860.
86. Charmandari E, Kino T, Chrousos GP. Familial/sporadic glucocorticoid resistance: clinical phenotype and molecular mechanisms. Ann N Y Acad Sci 2004; 1024: 168-181.
87. Lamberts SW, Koper JW, Biemond P, den Holder FH, de Jong FH. Cortisol receptor resistance: the variability of its clinical presentation and response to treatment. J Clin Endocrinol Metab 1992; 74: 313-321.
88. Huizenga NA, de Lange P, Koper JW, et al. Five patients with biochemical and/or clinical generalized glucocorticoid resistance without alterations in the glucocorticoid receptor gene. J Clin Endocrinol Metab 2000; 85: 2076-2081.
89. Lamberts SW. The glucocorticoid insensitivity syndrome. Horm Res 1996; 45: 2-4.
90. Chrousos GP, Vingerhoeds A, Brandon D, et al. Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease. J Clin Invest 1982; 69: 1261-1269.
91. Lamberts SW, Poldermans D, Zweens M, de Jong FH. Familial cortisol resistance: differential diagnostic and therapeutic aspects. J Clin Endocrinol Metab 1986; 63: 1328-1333.
92. Hurley DM, Accili D, Stratakis CA, et al. Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. J Clin Invest 1991; 87: 680-686.