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

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

- 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.

- 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.

- 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.

- Eighteen gene conversion mutations account for nearly all affected alleles in various ethnic groups. The remaining 5% of patients with defined abnormalities have one or more of the 60 point mutations.
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\textsuperscript{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\textsuperscript{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\textsuperscript{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 \textit{in vitro} analysis of enzymatic activity\textsuperscript{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\textsuperscript{13}. Heterozygotes may have mild biochemical abnormalities, but no clinically important endocrine disorder\textsuperscript{23,24}. Despite these general correlations, the CYP21A2 deficiency phenotype does not always correlate precisely with the genotype\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 \textit{in vitro}, human CYP17 deficiency syndromes have been observed in which patients apparently lack only 17-hydroxylase deficiency or only 17, 20-lyase deficiency\textsuperscript{31,32}. Most patients, however, have a combined defect\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{35,37,38}. Nearly 40 different mutations in the CYP17 gene, which is located on chromosome 10, have been defined at the molecular level\textsuperscript{30}. These include small insertions that disrupt the normal reading frame of the gene and lead to premature termination\textsuperscript{39,40}, deletions of single codons\textsuperscript{41}, deletions of several codons\textsuperscript{42}, large deletions of foreign DNA\textsuperscript{43}, and a variety of nonsense or missense mutations of CYP17 that produce stop codons, impair CYP17 enzyme activity, or alter splice sites\textsuperscript{44-52}. Two mutations have been described in splice receptor sites\textsuperscript{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 \textit{in vitro}\textsuperscript{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\textsuperscript{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. 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\textsuperscript{54,55}.

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\textsuperscript{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. Subsequently, two patients with congenital lipoid adrenal hyperplasia have been shown to have heterozygous mutations of CYP11A1. One had apparent haploinsufficiency of CYP11A1 and the other was a compound heterozygote with partial inactivation of the gene. 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. 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. The role of StAR has been studied by sequencing the gene in patients with congenital lipoid adrenal hyperplasia. 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. 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.

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. 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. 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, 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. 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. 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. The other patient had an XX karyotype and presented with adrenal insufficiency at the age of 9 months.

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. 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. Additional studies in four patients demonstrated a triallelic digenic pattern of inheritance. 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 heterozygous 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.

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. Another six-year-old boy presented with gonadotropin-independent precocious puberty. 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. 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. 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.

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.
Some of the mutations reduce binding of the receptor-ligand complex to transcription factors or co-activators\(^8\). Patients with familial glucocorticoid resistance present in childhood to adulthood with hirsutism, male pattern baldness, menstrual abnormalities and infertility in women, pseudoprecocious 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\(^8\). Plasma ACTH and serum cortisol concentrations are increased in this disorder, but maintain their normal diurnal rhythm\(^9\). 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 mineralocorticoids can be ameliorated by the administration of dexamethasone (0.5 to 1.5 mg/day). Thiazide diuretics administered alone may cause severe hypokalemia\(^8\).\(^9\)

**REFERENCES**


