Congenital adrenal hyperplasia: Biochemical and molecular perspectives

Anurupa Maitra* & Heena Shirwalkar
National Institute for Research in Reproductive Health (ICMR), Jehangir Merwanji Street, Parel, Mumbai 400012, India

Congenital adrenal hyperplasia is a disorder occurring in both sexes and is the commonest cause of ambiguous genitalia. It is a group of autosomal recessive disorders in which, on the basis of an enzyme defect, the bulk of steroid hormone production by adrenal cortex shifts from corticosteroids to androgens. Autosomal recessive mutations in the CYP21, CYP17, CYP11B1 and 3β-HSD genes that encode steroidogenic enzymes, in addition to mutations in the gene encoding the intracellular cholesterol transport protein steriodogenic acute regulatory protein (STAR) can cause CAH. Each of the defects causes different biochemical consequences and clinical features. Deficiencies in 21-hydroxylase (21-OH) and 11β-Hydroxylase (11β-OH) are the two most frequent causes of CAH. All the biochemical defects impair cortisol secretion, resulting in compensatory hypersecretion of ACTH and consequent hyperplasia of the adrenal cortex. Research in recent years has clarified clinical, biochemical and genetic problems in diagnosis and treatment of the disorders. Expanding knowledge of the gene mutations associated with each of these disorders is providing valuable diagnostic tools in addition to the biochemical profile and phenotype. Genotyping is useful in selecting instances to provide genetic counseling and to clarify ambiguous cases.

Keywords: Androgen, Autosomal recessive disorder, Congenital adrenal hyperplasia, Corticosteroid

Congenital Adrenal Hyperplasia (CAH) is a group of disorders caused by inborn errors of steroid metabolism. It is a generic term to describe a family of adrenal disorders characterized by deficiency of enzymes crucial for synthesis of the adrenal steroid, cortisol. Depending on the position of enzyme block, the disease has been classified into various forms. In all these forms, low plasma cortisol from abnormally low rates of synthesis causes secondary elevation of ACTH and subsequent hyperplasia. Congenital adrenal hyperplasia is thus a collective term for a number of autosomal recessive disorders affecting adrenal cortex and its development, leading to defective cortisol biosynthesis.

Symptoms due to CAH can vary from mild to severe depending on the degree of enzymatic defect. In the classic form, defects in the cytochrome P450s, 21-hydroxylase (21-OH) or 11β-hydroxylase (11β-OH) cause varying degrees of genital ambiguity in females reflecting re-routing of excess cortisol precursors to androgen synthesis pathway during fetal development. Prenatal androgen excess causes virilization of female genitalia and post-natally results in advanced bone age and puberty in both females and males. Defects in androgen synthesis due to defects in 3β-hydroxysteroid dehydrogenase (3β-HSD)/Δ5-Δ4 isomerase, in 17α-hydroxylase/17,20 lyase and in the steroidogenic acute regulatory protein result in inadequate prenatal virilization of males and depressed puberty in both sexes. Less severe, non-classical forms of CAH present postnatally as signs of androgen excess.

Over the last decades, genes causing various forms of the disorder have been identified. Emerging information is helping in reshaping thinking about their pathophysiology and supplementing their clinical and biochemical diagnosis. Review presented here provides the current state of knowledge on these aspects of CAH in the order of genes involved in the adrenal steroidogenesis, from the mitochondrial entry of cholesterol to cortisol synthesis (Fig. 1). The latest syndrome to be molecularly investigated, congenital lipoid adrenal hyperplasia (CLAH) is described first followed by the other more frequently seen syndromes viz. 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α hydroxylase (P450c17), 11β hydroxylase (P450c11) and 21 hydroxylase (P450c21) deficiencies.

**Congenital lipoid adrenal hyperplasia and steroidogenic acute regulatory (star) protein**

Congenital Lipoid Adrenal Hyperplasia (CLAH) is a rare autosomal recessive disorder that severely
disrupts the synthesis of all adrenal and gonadal steroids resulting in severe salt wasting and a female phenotype. It is the most severe form of CAH caused by mutations in the Steroidogenic Acute Regulatory (StAR) protein. The central role of StAR protein in causing CLAH was first described by Lin et al. in 1995 when he reported that mutation in StAR gene caused congenital lipoid adrenal hyperplasia. Key role of the protein in triggering acute steroidogenic response was evident from the finding that it promoted steroidogenesis in non steroidogenic COS 1 cells co-transfected with StAR and side chain cleavage enzyme and that mutations in the gene caused CLAH. It is now well understood that in adrenals, the chronic regulation of steroidogenesis by ACTH is at the level of gene transcription, but acute regulation, such as in response to LH surge or to infusion of ACTH is at the level of cholesterol access to P450 scc. The protein that triggers this process of acute steroidogenesis was identified as StAR and cloned and characterized by Clark et al. in 1994.

In the disease, the affected 46 XY genetic males are born with wholly female external genitalia, reflecting absence of testosterone synthesis between 6 and 12 weeks of gestation. Adrenals at birth are engorged with cholesterol ester deposits. Affected newborns have low but measurable levels of steroid hormones, but they soon die from glucocorticoid or mineralocorticoid deficiency if hormonal replacement therapy is not initiated. However survival till adulthood in some of the treated cases have been reported. Reports suggest a wide variability in the prevalence of the disease. The disease is reported to be common in Japanese, Korean and Palestinian Arab population, but is rare elsewhere. Studies on the Indian population are not available. With the

![Schematic pathway from mitochondrial entry of cholesterol to synthesis of adrenal and gonadal steroids.](image-url)
knowledge on the central role of StAR protein in regulating acute steroidogenesis, there has been a growing interest regarding mutations in the gene and its association with CAH. The gene is located on chromosome 8 and is 8kb in size with seven exons and six introns. A large number of mutations have been reported in six of the seven exons with majority of them lying in exons 5-7. Fig. 2 provides a schematic diagram of the gene and some of the major mutations observed. The mutations reported are either missense, nonsense or splice site variations causing ablation or inactivation of the protein. A preliminary attempt has been undertaken by us with this background, to amplify and analyse the coding sequences of StAR gene in a normal Indian population vis-à-vis confirmed cases of CAH. Fig. 3 demonstrates PCR amplification of the various exons of the gene from DNA extracted from peripheral blood. An insertion variant in the exon 7 within untranslated region of the gene, which has not been reported so far is indicated through our study.

Pathophysiology of Lipoid CAH has been explained through a two hit model. As per this model, although mutations in the gene ablates StAR dependent steroidogenesis, a low level of StAR independent steroidogenesis persists. This permits normal placental steroidogenesis and term gestation and also accounts for the low but detectable levels of steroid hormones in the sera of lipoid CAH patients in the first month of life. However, these concentrations are too low to suppress secretion of ACTH and other gonadotropins. These tropic hormones stimulate cellular uptake of LDL cholesterol and increased production of cholesterol from acetate. The stimulation results in the accumulation of cholesterol esters, which eventually disrupts the cell, either through physical engorgement of the cell with droplets of cholesterol esters or by a chemical action of cholesterol oxidation products or both. This second hit thus destroys the low level of StAR independent steroidogenesis, leading to the unmeasurable levels of steroid in the serum of older children with lipoid CAH.

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**Fig. 2** — Schematic diagram of steroid acute regulatory gene and major mutations reported


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**Fig. 3** — PCR amplification of various exons of steroidogenic acute regulatory gene

Lane 1: Exon 2 437 bp
Lane 2: Exon 3 246 bp
Lane 3: Exon 5 234 bp
Lane 4: DNA ladder
Lane 5: Exon 6 234 bp
Lane 6: Exon 4 200 bp
Lane 7: Exon 8 137 bp

**Exon 7**

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and the absence of circulating testosterone in affected 46 XY fetuses.

With regard to females, fetal ovaries do not express the genes for steroidogenic enzymes and the 46 XX females affected with CLAHD do not receive the second hit until the onset of puberty, when LH stimulates low levels of STAR-independent steroidogenesis. Each month another follicle is recruited and stimulated by gonadotropins, producing spontaneous age-appropriate breast development in affected girls. However, gonadotropin stimulation quickly results in cholesterol engorgement in these cells (the second hit in lipid CAH), so that the later phase of ovarian steroidogenesis, the secretion of large amounts of progesterone, does not occur. Follicles that are not recruited remain unstimulated and constitute a reservoir of steroidogenic cells undamaged by the second hit of lipid CAH. Thus with each monthly cycle, a new undamaged follicle is recruited and estrogen is produced, leading to cyclic uterine estrogen withdrawal bleeding that resembles normal menses, but as there is no progesterone, these cycles are anovulatory.

The two-hit model has been further substantiated through generation of STAR knock-out mice by Caron et al. in 1997 and Hasegawa et al. in 2000.

3β-hydroxysteroid dehydrogenase deficiency and CAH

3β-Hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3βHSD) catalyses the 3β-hydroxysteroid dehydrogenation and Δ5 to Δ4 isomerization of the Δ5 steroids pregnenolone, 17-hydroxyprogrenolone, dehydroepiandrosterone and androstenediol into their respective Δ4-steroids, namely progesterone, 17α hydroxyprogesterone, Δ4-androstenedione and testosterone. Thus as will be evident from Fig. 1 this bifunctional dimeric enzyme is required for the biosynthesis of all classes of steroid hormones, namely glucocorticoids, mineralocorticoids, progesterone, androgens and estrogens. Isozymes of the 3β-HSD family have been characterized during the past decade and their critical role in the steroidogenic reactions in the adrenal cortex, gonads, placenta and a variety of peripheral target tissues have been elucidated. It is a membrane bound enzyme located in the endoplasmic reticulum and mitochondria. The type 1 3β-HSD gene was first cloned by Luu and his co-workers in 1989 followed by TypeII 3βHSD gene by Rheaume et al. in 1991. The type I gene encodes an enzyme of 372 amino acids and is predominantly expressed in the placenta and peripheral tissues. Type II gene, sharing 93% homology with Type I encodes a protein of 371 amino acids and is almost exclusively expressed in the adrenals, the ovary and testis. The genes are 7.8 kb in size consisting of four exons and are assigned to chromosome 1. All known mutations depleting 3βHSD activity are in the 3βHSDII gene.

CAH due to 3βHSD deficiency is relatively rare and is known to exist in two distinct classes viz. classical and nonclassical forms. Classical form, accounting for about 1% to 10% of CAH cases, has been shown to result from mutations in the type II 3βHSD gene. It can exist in either salt-wasting or non salt-wasting forms. The non classical 3βHSD deficiency, also referred to as late onset deficiency has been more difficult to diagnose and has not been associated with any mutations in the 3βHSD gene. The salt losing form is usually diagnosed during the first few months of life and is associated with insufficient biosynthesis of aldosterone and consequent salt loss which may be fatal if not diagnosed and treated early. In contrast in non salt-losing form of 3βHSD deficiency may be diagnosed later, often delayed until adrenarche. In classical 3βHSD deficiency, the basal plasma levels of Δ5 steroids such as pregnenolone, 17-OH pregnenolone and DHEA are elevated in affected individuals, of which the elevated plasma level of 17-OH pregnenolone after stimulation with ACTH appears to be the best indicator. Over all an elevated ratio of Δ5/Δ4 steroids is considered to be the best biological parameter for diagnosis of 3βHSD deficiency.

As regards molecular diagnosis, a total of 34 mutations in the typeII 3βHSD gene have been reported so far (Fig. 4). This includes 5 frameshift, 4 nonsense, 1 in-frame deletion, 1 splicing and 23 missense mutations. The heterogeneous clinical presentation seen in the disorder has been attributed to this wide distribution of the gene mutations. Functional characterization of most of the mutant genes has also been carried out by transient expression of the mutant proteins using intact human 293 cells. The studies have provided valuable information on the genotype phenotype relationship of the mutant proteins with severity of the disease.

17α-hydroxylase deficiency and CAH

17α Hydroxylase (P450 c17) is the enzyme that brings about 17-hydroxylation of pregnenolone and progesterone to 17α-hydroxyprogrenolone and 17α-
hydroxyprogesterone respectively. This 17α-hydroxy-
pregnenolone may further undergo scission of the
C17, 20 carbon bond to yield dehydroepiandrosterone.

Defects in P450c17 lead to male pseudo-
hermaphroditism with various degrees of ambiguous
genitalia. Patients with the deficiency have decreased
cortisol synthesis, overproduction of ACTH and
stimulation of steps proximal to P450c17 (Fig. 1). These
patients may have mild symptoms of glucocorticoid
deficiency, but this is not life-threatening as the lack of
P450c17 results in the overproduction of corticosterone,
which also has glucocorticoid activity. Affected females
are phenotypically normal but fail to undergo adrenarche
and puberty.

Gene encoding the enzyme complex CYP17 is
located on chromosome 10 that is structurally related
to the gene for P450c21 (11-hydroxylase). Several
different mutations in the gene have been reported
leading to either a complete or partial form of the
disease18.

**11 β-hydroxylase deficiency and CAH**

11β-hydroxylase (P450c11) is the enzyme that
catalyses the terminal step in biosynthesis of cortisol
viz. 11β-hydroxylation of 11-deoxycortisol to cortisol.
Deficiency of the enzyme is the second most common
cause of CAH, next to 21-hydroxylase deficiency. It
is encoded by the gene CYP11B1, which is induced
by ACTH and is suppressed by glucocorticoids such
as dexamethasone. Mutations in the gene lead to
deficient adrenal 11β-hydroxylase activity and
inefficient conversion of 11-deoxycortisol to cortisol.
Decreased cortisol production leads via poor feedback
control to increased ACTH secretion. This leads to

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**Fig. 4** — Schematic representation of the mutations identified in the *HSD3B2* gene in individuals suffering from classical 3β-HSD deficiency. (A) The missense mutations that have been shown to retain a certain amount of 3β-HSD activity. Also highlighted are the cofactor binding domain (CBD), the two putative substrate binding domains (SBD), and the two membrane-spanning domains (MSD). (B) The missense mutations that have been shown to abolish enzyme activity (top), and the nonsense, frameshift and in-frame deletion
mutations (bottom). Mutant recombinant proteins with apparent instability are shown in bold black boxes. (Adapted from Simard, Moisan
overproduction by adrenal cells of the steroid precursors prior to the block. The precursors are thus shunted into androgen pathway resulting in marked androgen excess and virilization. 11β-hydroxylase deficiency is an autosomal recessive disorder and is estimated to be prevalent in about 5% of patients with CAH. Overall prevalence in the general population is estimated to be approximately 1 in 250,000. Females with classic 11β-hydroxylase deficiency are born with masculinization of their external genitalia, caused by secretion of adrenal androgens during embryonic and fetal development. In non-classic 11β-hydroxylase deficiency, the patients are born with normal genitalia and present with signs of androgen excess as children. Adult women may present with hirsuitism and amenorrhea. However only a small percentage of women with hirsuitism and amenorrhea have non-classic 11β-hydroxylase deficiency.

The gene encoding P450c11, CYP11B1 is 7 kb in size located in chromosome 8 and contains 9 exons. In classic 11β-hydroxylase deficiency more than 30 different mutations in the functional regions of the gene have been reported so far (Fig. 5). The mutations reported are missense, nonsense and splice site mutations. Functional analysis of these mutations show an abolition of the enzyme activity.

With regard to non-classic form of the disease, reports are scarce. The study by Joehrer et al. in these cases, reported nonsense as well as missense mutations that caused reduction in the enzyme activity. A wide variability in the phenotype with regard to mutations has also been reported in both classic and non-classic forms of the disease. Based on these mutational analysis of DNA obtained by amniocentesis or chorionic villus biopsies, attempts have been made for pre-natal diagnosis of the disease. However, it has been of limited value so far, mainly due to the wide distribution of mutations within the gene.

**21-hydroxylase deficiency and CAH**

The deficiency of enzyme 21-hydroxylase (21-OHD) is the most common cause of CAH with a frequency of about 1 per 14,000 live births in the general population. In an Indian study among cases of ambiguous genitalia, 21-hydroxylase deficiency was found to be the cause of CAH in more than 90% of cases detected. It is the key enzyme in adrenal cortex bringing about conversion of progesterone and 17-hydroxyprogesterone to deoxycorticosterone and 11-deoxycortisol respectively (Fig. 1). Disorders in the gene encoding the enzyme viz. CYP21 cause about 95% of all cases of CAH. Both classical and non-classical forms of CAH due to 21-hydroxylase deficiency have been recognised. The classical disorder, occurring in about 1:13,000 to 1:15,000 births world wide, has two forms, simple virilizing and salt wasting. Patients with simple virilizing 21-hydroxylase deficiency manifest virilization owing to excess androgen secretion. Salt wasting 21-OHD (in

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**Fig. 5** — Schematic representation of the genomic structure of the human CYP11B1 gene and positions of mutations reported. Exons are represented by boxes; black boxes demarcate the coding regions, and open boxes represent the non-coding regions (Δ, deletion; ins, insertion; nt, nucleotide). (Adapted from Peter M. Semi Reprod Med, 2002)
addition to hyperandrogenism), which involves a deficiency in aldosterone production as well as in cortisol, occurs in about three-fourths of classical cases. The non-classical form, which involves a milder enzymatic defect, occurs in about 1:100 births in the general population.

As has been described earlier, the deficient cortisol production in these cases is followed by overproduction of ACTH by the pituitary due to poor negative feedback, resulting in adrenocortical hyperplasia. Precursor steroids proximal to the 21-hydroxylase enzyme block and/or those for which 21-hydroxylation is not required (progesterone, 17-hydroxyprogesterone, A4-androstenedione) are over-secreted and shunted into pathway of androgen synthesis. Classically affected females who do not undergo treatment are thus virilized “in utero” and are born with ambiguous genitalia. Postnaturally in both classical and non classical forms, males and females can present at any time with signs of androgen excess.

Most patients with classic CAH-21OHID also have inadequate aldosterone and therefore cannot maintain sodium balance. About 25% have sufficient aldosterone levels and no salt wasting, yet show prenatal virilization and/or markedly increased production of hormonal precursors of 21-hydroxylase (e.g. 17 OHP). These individuals are referred to as “Simple virilizers”. Patients with the mild, non-classic form of 21-hydroxylase deficiency may have any of the signs of post natal androgen excess but affected females may have varying degrees of ambiguous external genitalia. Adrenal steroid precursors of 21-hydroxylase are only mildly elevated. 17-OH progesterone levels are raised, particularly in response to an intravenous bolus of ACTH. The severity of signs and symptoms of mild androgen excess varies widely, and probably many affected individuals are asymptomatic. Severe cystic acne, hirsutism and oligomenorrhea in young women are commonly presenting symptoms. Male patients are largely asymptomatic, but may present with acne or infertility.

Newborn males with 21-hydroxylase deficiency usually show increased phallic size, pigmented scrotum and bilateral testes. Newborn females with classic virilizing CAH, manifest variable degrees of genital ambiguity caused by high systemic levels of adrenal androgens beginning at about 7th week of gestation. Females with the milder non-classic form of the disorder are distinguished by little virilization at birth.

Classic 21-hydroxylase deficiency is diagnosed based on a markedly elevated serum level of 17 OHP, the main substrate for the enzyme. The diagnosis can be further refined by performing ACTH stimulation. Androstenedione levels along with 17 OHP provides the method for monitoring efficacy of therapy in females and testosterone along with 17-OHP in case of males.

Reproductive problems for women with the disease usually become apparent in adolescence. The average age at which menarche occurs in inadequately treated girls is late compared with healthy peers. Such patients often have a clinical picture similar to polycystic ovary syndrome with sonographic evidence of multiple cysts, anovulation, irregular bleeding and hyperandrogenic symptoms. In non-classic CAH, a significant reduction in insulin sensitivity is also seen among non-obese young women as compared with controls of similar age and weight. However a review carried out in 1992, found that up to about 80% of simple virilizers and 60% of salt-wasters can bear children. In males with classic form of the disease, impaired gonadal function is less frequent compared to females. Most males are able to father children, or at least have adequate sperm counts. Among simple virilizers, testicular integrity may be normal even in the absence of treatment.

Testicular tumors of adrenogenital syndrome may occur in CAH males, especially if they are inadequately treated salt-wasters. All patients with classic 21-hydroxylase deficiency and symptomatic patients with non classic disease require glucocorticoid treatment. Infants with the salt washing form of 21-OHID require mineralocorticoid and sodium chloride supplements in addition to glucocorticoid treatment.

The molecular genetic basis of 21-OHID has been studied extensively and has important implications for prenatal diagnosis and counseling. As depicted in Fig. 6 the gene for CYP21 encoding the 21-hydroxylase enzyme is located on chromosome 6 and lies in the middle of the major histocompatibility locus. Thus, disorders of adrenal 21-hydroxylation are linked to specific human leukocyte antigen (HLA) types. The gene contains 10 exons spaced over 3.1 kb and bears close resemblance to the inactive pseudogene CYP21P. It is also reported to have the highest rates of single nucleotide polymorphisms among over 100 human genes tested. Studies have also revealed as many as 56 mutations in the gene. Approximately 40-50% of these are either deletions or a single point mutation altering splicing between
second and third exons. The mutations have been largely shown to correlate with one of three main clinical categories: classical salt-washing, classical simple virilizing and non classical or late onset form of CAH.

Deleterious mutations in the gene include an A→G substitution, 13 nucleotides before the end of intron 2 that results in aberrant splicing of pre-mRNA, an 8-nmt deletion in exon 3 and a 1 nt insertion in exon 7, each of which shifts the reading frame of translation, and a nonsense mutation in codon 318 of exon 8. Seven additional missense mutations have also been reported in patients with 21-hydroxylase deficiency. Functional effects of these missense mutations have been assessed "in-vitro" using different expression systems and show an alteration in enzyme activity.

With the gain in knowledge regarding the DNA mutations, tools have been established for pre-natal molecular diagnosis of the disease. Laboratories have developed strategies for rapid detection of the CYP21 gene mutations that account for large majority of cases. Direct analysis of DNA extracted from chorionic villus samples has led to strengthening of pre-natal diagnosis of the disease, that has been based on second trimester amniotic fluid 17-OH progesterone measurements and HLA haplotyping. However, a limiting factor in this application is the variability in the genotype phenotype association reported with these mutations.

CAH being an inherited metabolic defect, attempts have been made to explore the prospects of gene therapy for amelioration of the disease. Sporadic reports have been available in recent years suggesting feasibility of the approach. In a recent report, mice with 21-hydroxylase deficiency have been rescued by transgenesis with a murine CYP21 gene. However, the prospect of using the approach in humans is still not promising. Adequate suppression of adrenal androgens along with normal biosynthesis of cortisol, are seen to be major difficult goals to achieve. Also, maintaining the levels of expression indefinitely seems unlikely to be achieved in foreseeable future and medical therapy, although not perfect, will continue to be therapeutic options for the disease.

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