4. Biochemistry, molecular biology and molecular genetics of congenital adrenal hyperplasia

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Abstract. Congenital adrenal hyperplasia (CAH) is a common form of adrenal insufficiency inherited in an autosomal recessive manner. 90 – 95% cases account for defect in 21-hydroxylase enzyme (CYP21A2) which is located on chromosomal region which is highly prone to genetic recombination events which lead to complex rearrangements such as duplications, deletions and conversions. Patients develop salt wasting (SW) where they fail to maintain sodium homeostasis. Classical CAH confers excessive exposure to androgens prenatally resulting in virilisation of external genitalia in females. Non classical (NC) CAH impart mild suffering to patients. Mainstays of treatment are glucocorticoid and mineralocorticoid replacement therapies while new therapies are being developed. World wide spectrum of mutations in CYP21A2 gene from CAH patients revealed that Ile172Asn (30%) is the most common mutation followed by i2g (27.2%), Gln319stop (22%), gene deletion (15.9%) and Pro30Leu (2.2%). In our study mutation spectrum of CYP21A2 revealed that i2g (36%) is most common mutation followed by gene deletion (25%), Pro30Leu (20%), Val281Leu (15%) and Arg356Trp (11%). Prenatal mutation detection allows families for the treatment of affected females so as
to reduce/prevent external genitalia virilisation. Screening of neonates for CAH helps in identification of the affected individuals before classical salt wasting develops so as to avoid mortality associated with the disease. Neonatal screening helps in screening newborn boys too, who might escape from diagnosis & could be mistaken for diagnosis of CAH with some other disease. Genotype-phenotype-clinical severity strongly co-relates. We elucidate biochemistry, genetics, clinical forms & features and diagnosis of CAH. Also, there exist issues with boys and girls affected by CAH either socially or mentally.

1. Introduction

The anatomy of the adrenal glands was first described by Bartolomeo Eustachius in 1563 [1]. Until the 19th century the function of the “glandulate renis incumbentes” or “the gland that sleeps with the kidney” was unknown. It has been reported that, in 1716, the academy of Bordeaux offered a prize for the answer to the question “what is the purpose of adrenal glands” and the contest produced no winners! [2]. More than a century later, in 1865, and only 16 years after Dr. Addisons report of three patients with anemia and adrenal disease on autopsy, the Neapolitan anatomist De Crecchio reported “…..un caso di apparenze virili in una donna”, [3] the first report of a patient with congenital adrenal hyperplasia [4]. CAH replaced the term “adrenogenital syndrome,” which had been used for diverse adrenocortical disorders, including congenital or childhood-onset Cushing syndrome [5].

Congenital adrenal hyperplasia is a common autosomal recessive disorder. Presence of mutations in CYP21A2 gene are responsible for the pathogenesis of the disease. Adrenal gland is involved in steroidogenesis for production of hormones. CYP21A2 gene coding for 21-hydroxylase enzyme has a pivotal role in steroidogenic pathway resulting in mineralocorticoids, glucocorticoids & sex steroids biosynthesis. Deficiency of enzyme(s) in steroidogenic pathway leads to defective hormonal synthesis and ultimately manifests as adrenal insufficiency with or without virilisation.

Newborn screening worldwide of almost 6.5 million babies has demonstrated an overall incidence of 1:15,000 live births for the classical form of 21-OH deficiency [6-8]. Earlier, it was demonstrated that the overall frequency of non classical 21-OH deficiency is surprisingly high in the population at large and even higher in certain ethnic groups [9]. It is in fact the most common human autosomal recessive disorder in humans. Here, we limit our discussion to common form of CAH i.e. CAH due to 21-hydroxylase deficiency. We elucidate biochemistry, genetics, clinical forms & features and diagnosis of CAH. Also, there exist issues with boys and girls affected by CAH either socially or mentally.
2. Biochemistry of disease

Adrenal cortex produces the glucocorticoid, (cortisol) and the mineralocorticoid, (aldosterone) under the control of regulatory systems that function independently of each other. The cortex is divided into three distinct zones—the outer zona glomerulosa, the middle zona fasciculata, and the inner zona reticularis—defined by different cellular arrangements. These zones are functionally distinct: i.e., mineralocorticoids are synthesized in the zona glomerulosa, glucocorticoids are produced by the zona fasciculata, and androgens are synthesized in the zona reticularis. Cortisol is main glucocorticoid involved in various physiological processes which acts by stimulating gluconeogenesis in the liver and proteolysis in skeletal muscle. Aldosterone, mineralocorticoid is crucial for regulating intravascular volume and blood pressure by stimulating renal retention of sodium along excretion of potassium. The sex steroids produced by adrenals are dehydroepiandrosterone (DHEA) and androstenedione. These are weaker androgens but are converted to potent testosterone, dihydrotestosterone (DHT) and estradiol which are utmost important for bone and muscle development, skeletal growth and sexual development. The steroidogenic acute regulatory (STAR) protein shuttles cholesterol to the inner mitochondrial membrane [10]. The hormonal imbalances in CAH result from the combination of impaired enzymatic activity and subsequent impaired hormonal synthesis.

The adrenal cortex appears at the 25th day of gestation as a blastema of undifferentiated cells of mesodermal origin (unlike the medulla, which is of neuroectodermal origin) [11]. During the fifth week of development, mesothelial cells located between the root of the mesentery and the developing gonad begin to proliferate and invade the underlying mesenchyme. A second wave of cells from the mesoderm then penetrates and surrounds the original cell mass by the sixth to eighth week, forming the first evidence for zonation. The latter cells are smaller than those of the first migration and form what is later going to be called the definitive zone, whereas the former mesothelial cells will give rise to the fetal zone. An additional cell type takes origin from the mesonephron and seems to arise from the region of Bowman’s capsule. Thus, adrenal cortex derives from three embryo-logically distinct mesodermal cells, two from the celomic epithelium and one from the mesonephron [12]. By the fourth month, the fetal cortex attains its maximum size and becomes actually larger than the kidneys. Between the ninth and twelfth week of embryonic development the sinusoidal vascularization of the glands forms the framework for the zonation of the adult cortex. Shortly after birth, a stringent reduction in adrenal mass
occurs as a result of rapid degeneration of the fetal cortex. The definitive zone starts differentiating into its glomerular and fascicular parts as early as the 28th embryonic week, whereas the zona reticularis does not appear until the end of the third year of life [13]. Deficiency of any of the enzymes that participate in the steroid biosynthesis affects the early development of the adrenal cortex, interfering with both differentiation and zonation [14].

2.1. Steroidogenesis and its regulation

These steroidogenic enzymes are members of the cytochrome P450 family of oxidases composed of approximately 500 amino acids and a single heme group (named as [cytochrome P450] because of their ability to absorb light at 450nm in their reduced states [15]. The process of making steroid hormones from cholesterol in adrenal glands is a complex phenomenon. Individual enzyme brings about each step (Figure 1).

This biosynthetic process is stimulated by adrenocorticotrophic hormone (ACTH) which, in turn, is inhibited by cortisol, giving rise to a negative

![Figure 1. Pathways of steroid biosynthesis in the adrenal cortex [16].](image-url)
feedback loop. The amount of cortisol produced by adrenal cortex is controlled by small gland at the base of brain called pituitary gland which is connected to the part of brain called hypothalamus. In response to more demand of cortisol, hypothalamus stimulates ACTH release from pituitary into bloodstream which then reaches adrenal cortex and regulates the production of cortisol. (Figure 2) As cortisol level rise ,the hypothalamus senses this and stop stimulating pituitary gland to produce ACTH, which inturn slows down the production of cortisol from adrenal cortex. ACTH is a trophic factor for the adrenal cortex. In absence of ACTH adrenal gland degenerate while in excess it leads to hyperplasia. Cortisol insufficiency, regardless of the underlying pathology, results in increased ACTH production.

CAH is a term used to describe a group of conditions caused by autosomal recessive defects in one of the five enzymes in the adrenal steroidogenesis pathway (Figure 1) [16]. Therefore, clinical syndromes reflect the resultant elevated levels of steroids proximal to the nonfunctioning enzymatic step and hyperstimulation of the adrenal gland by ACTH. In 21-OH deficiency, the conversion of 17α -hydroxyprogesterone (17-OHP), the main substrate of the 21-hydroxylase enzyme, to 11-deoxycortisol in the pathway of cortisol synthesis is impaired. The enzyme defect also

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**Figure 2.** Physiological regulation of hypothalamo-pituitary-adrenal axis.
impairs the conversion of progesterone to 11-deoxycorticosterone in the pathway of aldosterone synthesis. Figure 3 depicts that 21-hydroxylase catalyse the hydroxylation of the carbon atom 21 in steroids (adding an "–OH"), which is necessary for the formation of these hormones. When cortisol production is decreased, pituitary secretion of ACTH increases via the negative feedback system and further stimulates the synthesis of steroid precursors prior to enzymatic defect. These steroid precursors can serve as substrates for androgen biosynthesis and are diverted in the adrenals to androgen pathways, resulting in excess androgen secretion - dehydroepiandrosterone, Δ4-androstenedione, and testosterone.

![Chemical structures](image)

Figure 3. Chemical structures of substrates in steroidogenic pathway.
In simple words, 21-OH deficiency interferes with production of aldosterone and cortisol but not the production of androgens (Figure 4). The pituitary gland senses the low levels of cortisol, produces ACTH which over-stimulates adrenal cortex which causes it to increase in size i.e. hyperplasia. In classical 21-OH deficiency, the excess androgen production in early gestation causes virilization of the external genitalia in the genetic female fetus.

**Figure 4.** Effect of ACTH on steroidogenic pathway in 21-hydroxylase deficiency.

### 3. Molecular biology of disease

The gene encoding 21-hydroxylase is a microsomal cytochrome P450 termed cytochrome P450, family 21, subfamily A, polypeptide 21 (CYP21A2) (Online Mendelian Inheritance in Man OMIM # 201910). The cytochrome P450 (CYP) are heme containing monooxygenases which are located on the smooth endoplasmic reticulum of the cells throughout the body. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. Most CYPs require a protein partner to deliver one or more electrons to reduce the iron (and eventually molecular oxygen). As CYP21A2 belong to microsomal group of P450, the enzyme accepts electrons from an NADPH-dependent cytochrome P450 reductase, thus reducing molecular oxygen and hydroxylating the substrate. The P450 reductase is required because NADPH donates electrons in pairs, whereas P450s can only accept single electrons [17].
White et al cloned 21-hydroxylase gene and had described its structure in 1986. More precisely CYP21A2 spans 3.1 Kb of the genome. cDNA encoding this enzyme is 2 Kb long. The CYP21A2 gene contains 10 exons and encoded protein contains 494 amino acids residues with a molecular weight of 55 Kda [18]. Alignment of amino acid sequences of many P450s have identified strongly conserved residues that are presumed to be important for catalytic function.

CYP21A2 is located on the chromosome 6p21.3 adjacent to the human leukocyte antigen (HLA) complex (Figure 5), meaning siblings that have 21-hydroxylase deficiency are almost invariably HLA identical [17,19,20]. In addition, particular forms of 21-hydroxylase deficiency are associated with particular combinations of HLA antigens/haplotypes. This is known as genetic linkage disequilibrium [17].

The mutations in this gene cause congenital adrenal hyperplasia. A related pseudogene is located near this gene; gene conversion events involving the functional gene and the pseudogene are thought to account for many cases of steroid 21-hydroxylase deficiency. CYP21A2 and its homolog, the pseudogene CYP21A1P alternate with two genes viz C4B and C4A that encodes the two isoforms of the fourth component of the serum complement system (Figure 6) [20]. The gene for adrenal 21-hydroxylase, CYP21A2, is located 30 kb from its pseudogene (CYP21A1P), on chromosome 6p21.3 and is adjacent to the HLA class III region (Figure 5). CYP21A2 and CYP21A1P each consist of 10 exons (Figure 7) [21]. They share a high homology with a nucleotide identity of 98% in their exons and 96% in their intron sequence [22]. They had also described mutations in the CYP21A2 gene [18]. More than 100 mutations have been described including point mutations, small deletions, small insertions, and complex rearrangements of the gene [17]. These mutations result from an exchange of genetic material between the CYP21A2 gene and CYP21AP gene, nonfunctional piece of DNA called a pseudogene, which is located very close to the CYP21A2 gene on chromosome 6. This is DNA exchange called as gene conversion. The genetic material from the pseudogene contains deleterious errors which when introduced into the (CYP21A2) functional gene causes dysregulation in the translation machinery of the cell which leads to defective protein formation. Certain other mutations cause 21-hydroxylase deficiency due to change in single protein building blocks (amino acids) in the 21-hydroxylase enzyme or delete or insert pieces of DNA in the CYP21A2 gene. Approximately 20 % of alleles have 30 Kb deletions that include 3’ end of CYP21P and 5’ end of CYP21A2. This type of allele carries single non-functional chimeric gene with 5’ and 3’ ends corresponding to CYP21P and CYP21A2 respectively. This is presumably related to unequal crossing over during meiosis.
4. Molecular genetics of disease

Many mutations responsible for the disease have been described. In vitro expression analysis demonstrates that the large deletion of the CYP21A2 gene results in no 21-hydroxylase enzymatic activity, while the i2g (655A/C>G) and I172 N (999 T > A) point mutations results in 1-5 % of normal enzyme activity [23-25]. In contrast, the P30L (89 C > T) mutations demonstrate 20-60% enzyme activity in in-vitro expression analysis [25]. Table 1 indicates the grouping of mutations in CYP21A2 gene according severity.

Figure 5. CYP21A2 gene is located on the short (p) arm of chromosome 6 at position 21.3 Source: http://ghr.nlm.nih.gov/gene/CYP21A2.

Figure 6. Genomic structure of the HLA locus containing the CYP21A and CYP21P, and XB, and C4A and C4B genes.

More than 90% of CAH cases are caused by mutations of the CYP21A2 gene. Approximately 75% of the defective CYP21A2 genes are generated through intergenic recombination so that CYP21A2 carriers have one or more deleterious mutations usually found in the neighbouring CYP21A1P [26]. Apart from gene deletions and large gene conversions, there are eight mutations reported with a higher frequency in the CYP21A2 gene (Fig. 7):
**Table 1.** Common mutations in CYP21A2 gene causing 21-hydroxylase deficiency.

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Location</th>
<th>% activity of 21-hydroxylase based on <em>in vitro</em> studies</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large deletion</td>
<td></td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>P30L</td>
<td>Exon 1</td>
<td>30-60</td>
<td>Mild</td>
</tr>
<tr>
<td>t2g (655A/C&gt;G)</td>
<td>Intron 2</td>
<td>&lt;5</td>
<td>Severe</td>
</tr>
<tr>
<td>3 bp gene deletion</td>
<td>Exon 3</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>I172N</td>
<td>Exon 4</td>
<td>1</td>
<td>Moderate</td>
</tr>
<tr>
<td>V281L</td>
<td>Exon 7</td>
<td>20-50</td>
<td>Mild</td>
</tr>
<tr>
<td>Q318X</td>
<td>Exon 8</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>R356W</td>
<td>Exon 8</td>
<td>0</td>
<td>Severe</td>
</tr>
</tbody>
</table>

**Figure 7.** Common mutations in the CYP21A2 gene.

89C>T (p.P30L), 655A/C>G (t2 splicing), 707_714delGAGACTAC (p.G110_Y112delfs), 999T>A (p.I172 N), 1683G>T (p.V281L), 1994C>T (p.Q318X), 2108C>T (p.R356W) and 2578C>T (p.P453S) [13,27]. Except for the last one, all the other seven mutations are present in CYP21A1P and
are presumed to have been transferred to CYP21A2 by short gene conversions. The mutation, 2578C>T (p.P453S), has been suggested to be occasionally present in the pseudogene as a polymorphism, and transferred to CYP21A2 by gene conversion events just like the other most frequent point mutations. Study revealed that Ile 173Asn (32%) is most common mutation followed by i2g (27.2%), Gln 319 stop (22%), gene deletion (15.9%) and Pro 311 Leu (2.2%). Less numbers of patients were subjected for characterization of mutations in CYP21A2 gene. They also documented the genotype and phenotype correlation among these patients. Unequal meiotic crossover arrangement can produce duplicated CYP21A2 genes, which have been found in Dutch, Swedish, Italian and other population mainly with the presence of the severe Gln318X or i2g mutations in 1 of the CYP21A2 genes [18]. Three of these mutations are associated with the nonclassical form of 21-OH deficiency; one is typically related to simple virilising classical 21-OH deficiency, and three to salt wasting classical 21-OH deficiency. The mutation 655A/C>G has been reported both in salt-wasting and in simple virilising cases. This mutation activates cryptic splicing receptor sequences causing the incorrect processing of almost all the mRNA. These mutations are termed apparent gene conversion. 20-25% of mutations are CYP21A2 gene deletion or CYP21A1P/CYP21A2 chimeric genes formed by unequal meiotic crossover. In sperm recombinations between CYP21A1P and CYP21A2 are detected thus making CYP21 one of the most polymorphic human genes. This is presumably related to unequal crossover during meiosis. Consequently to avoid false-positive results, assessment of the CYP21A2 gene copy number is very important when a mutation is present, especially the Gln318X or i2g mutation.

4.1. Heterozygous carriers

Heterozygous carriers of 21-OH deficiency carry one severe or one mild mutation and one normal gene and usually are asymptomatic. They are found in family studies of affected individuals, where slightly increased ACTH-stimulated 17-OHP levels place the suspicion and finally the diagnosis is confirmed by DNA analysis. Although strategies with higher sensitivity for heterozygote detection have been published using ACTH stimulated 21-deoxycortisol levels, ACTH stimulated 17-OHP levels are not diagnostic in these individuals because there is an overlap with unsuspected subjects in about one-third to one half of heterozygotes [16,28].

Indian scenario of CYP21A2 mutation spectrum is given in Table 2. Various groups had identified mutations in CYP21A2 gene. Study from AIIMS, New Delhi had reported that i2g, 655A/C.G (40 %) is the most
common mutation. It is followed by P30L, I173N, Q318X, gene deletion and R356W. Results from our study revealed that i2g (36 %) is most common mutation followed by gene deletion (25 %), Pro30Leu (20 %), Val281Leu (15 %) and Arg356Trp (11 %). Work related to diagnosis and effect of concentration of dose of drug for determination of best effective dose had been studied on a cohort of patients. Also, issues related to beliefs and social pressure had been reported.

5. Clinical forms of disease

The disease is classified into the following forms: The classical form with the most prominent feature being the virilisation of external genitalia and/or the body with renal salt wasting as defined by hyponatremia, hyperkalemia, inappropriate natriuresis and low serum and urinary aldosterone levels (salt-wasting form of CAH) (Table 3). The non-classical form is characterised by virilisation, menstrual disturbances, acne and/or
seborrhea, obesity, oily skin, hirsutism and sterility. Gonadal dysfunction, oligospermia, subfertility, subtle hyper-pigmentation and penile enlargement due to increased adrenal androgen have been reported in men with 21-OH deficiency in both classical and/or non-classical form [27,29,30].

5.1. Classical form

As previously reported, in the classical CAH with complete or severe deficiency of the enzyme, (enzyme activity 0–3% of the normal) a possible manifestation is virilisation of the external genitalia of female fetus at birth, because these girls are exposed to high systemic levels of adrenal androgens from approximately sixth week of gestation (CAH due to 21-OH deficiency is the most common cause of 46XX disorder of sexual development (DSD) in the newborn resulting in female pseudohermaphrodite [27,30]. About one-third of patients with 21-OH deficiency have the simple virilising form [29,30]. If untreated or inadequately treated, the female with CAH may develop signs of progressive virilisation. Pubic hair will appear early by age 2–4, followed by axillary hair. Bone age is advanced by the age of 2, and due to early epiphyseal closure, increased growth rate in childhood is achieved with a final short stature in adulthood. Progressive masculinisation continues with the development of a male habitus, acne, deepened voice, amenorrhea and infertility, which may develop in adolescent and in girls with any form of 21-OH deficiency. However, about 80% of women with simple virilising form (SV) and 60% of those with salt wasting form (SW) are fertile [31]. An electrolyte imbalance of the salt-losing type is usually apparent within a few days of birth (this crisis occurs usually in the third week of life) and occurs in approximately one-third of patients with the virilising form. The infant with this disorder goes on to an Addisonian-like crisis (salt-loosing crisis) with hyponatremia, hyperkalemia and acidosis.

Table 3. Clinical features in individuals with classical & non-classical 21OH deficiency CAH.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Classical 21 OH deficiency CAH</th>
<th>Non Classical 21 OH deficiency CAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal virilization</td>
<td>Present in females</td>
<td>Absent</td>
</tr>
<tr>
<td>Postnatal virilization</td>
<td>Males and females</td>
<td>Variable</td>
</tr>
<tr>
<td>Salt wasting</td>
<td>75% of all individuals</td>
<td>Absent</td>
</tr>
<tr>
<td>Cortisol deficiency</td>
<td>100%</td>
<td>Rare</td>
</tr>
</tbody>
</table>
5.2. Non-classical form

Non-classical form of 21-OH deficiency (enzyme deficiency about 30–50% of the normal) is a disease of extreme phenotypic diversity. Individuals with the disease, while normal at birth, may present with pre-, peri- or post-pubertally symptoms such as precocious adrenarche, acne, hirsutism, menstrual irregularity, clitoromegaly, amenorrhea, short adult height and infertility. The phenotype and clinical symptoms of women as reported are manifested either before or after puberty and may be not different from those shown in patients with PCOS, idiopathic hirsutism. PCOS is similar to a group of women with non classical CAH due to 21-OH deficiency. Non-classical and cryptic 21-OH deficiency may exist in siblings of families who are biochemically and genotypically identical.

5.3. Clinical presentation and features

5.3.1 External genitalia

Adrenocortical function begins around the seventh week of gestation; thus, exposure of adrenal androgens to female fetus with classical CAH at the critical time of sexual differentiation (approximately 9 to 15 weeks gestational age) is more. In classical CAH patients, the degree of genital virilization may range from mild clitoral enlargement alone to, in rare cases, a penile urethra. Degrees of genital virilization are classified into five Prader stages (Fig. 8) [32].

Stage I  : clitoromegaly without labial fusion  
Stage II : clitoromegaly and posterior labial fusion  
Stage III: greater degree of clitoromegaly, single perineal urogenital orifice, and almost complete labial fusion  
Stage IV : increasingly phallic clitoris, urethra-like urogenital sinus at base of clitoris, and complete labial fusion  
Stage V  : penile clitoris, urethral meatus at tip of phallus, and scrotum-like labia (appear like males without palpable gonads) [33].

5.3.2 Internal genitalia

As the androgens interact with the receptors on genital skin, they induce changes in the developing external female genitalia such as clitoral enlargement, fusion of the labial folds, and rostral migration of the urethral/vaginal perineal orifice. However, internal female genitalia (uterus,
fallopian tubes, and ovaries) are normal, as females cannot produce Mullerian-inhibiting hormone because they do not have testicular Sertoli cells. Therefore the Mullerian ducts do not regress, and the internal female internal genitalia develop normally [33].

![Image](image.png)

**Figure 8.** Different degrees of virilization according to the scale developed by Prader [33].

### 6. Molecular diagnosis

#### 6.1. Hormonal diagnosis

Biochemical diagnosis of 21-OH deficiency can be confirmed by hormonal evaluation. In timed blood sample, a very high concentration of 17-hydroxyprogesterone (17-OHP), the precursor of the defective enzyme action, is diagnostic of classical 21 OH deficiency. The best standard to establish hormonal diagnosis for non classical 21 OH deficiency and for certain other cases is the corticotropin stimulation test (250 mg cosyntropin intravenously), measuring levels of 17-OHP and Δ 4 -androstenedione at baseline and 60 minutes. The 17-OHP values can then be plotted in the published nomogram to ascertain disease severity (Fig. 9) [34]. The corticotropin stimulation test is crucial in establishing hormonal diagnosis of the non classical form of the disease, as even early-morning values of 17-OHP may not be sufficiently elevated to allow accurate diagnosis. For example, a non classical patient may have a normal baseline 17-OHP of 100ng/dL, yet stimulate to greater than 2000 ng/dL, and that would be diagnostic for non classical CAH. Patients with classical CAH typically have stimulated 17-OHP levels of 20,000 to 100,000ng/dL. The corticotropin stimulation test may also be helpful in males for distinguishing between the NC and SV forms, as males with 21 OHD have normal genitalia [33].
6.2. Mutation analysis

Hormonally and clinically defined forms of 21 OH deficiency CAH are associated with distinct genotypes characterized by varying enzyme activity demonstrated through in vitro expression studies. In recessive disorders, the less severe mutation of the two alleles typically dictates phenotype. Classical 21 OH deficiency is most often caused by two alleles with severe mutations. In contrast to the classical form, patients with non classical 21 OH deficiency are predicted to have mild mutations on both alleles or one severe and one mild mutation (compound heterozygosity) of CYP21A2 [33]. In order to determine the presence of normal gene for 21-hydroxylase enzyme, HLA testing and DNA analysis can be done. It can also help in detecting carriers. Several strategies to detect deleterious mutations on basis of PCR with allele specific oligonucleotides to CYP21A2 gene had been developed. Reactions involving nested PCR and single stranded conformation polymorphism are considered efficient & accurate in diagnosis of 21-hydroxylase deficiency. However, direct sequencing of exons could be part of another strategy to detect mutations. Also, promoter region of gene could also be sequenced for identification of mutations.

**Figure 9.** Nomogram of 17 OHP [34]. Patients with: * - genetically unaffected, ----- classical CAH, ----- non classical CAH, ----- heterozygotes for Classical CAH, ----- Heterozygotes for non classical CAH
7. Treatment

7.1. Hormone replacement

The goal of therapy in CAH is to both correct the deficiency in cortisol secretion and suppress ACTH overproduction. Optimal treatment with glucocorticoid reduces stimulation of the androgen pathway, thus preventing further virilization and allowing normal growth and development. The usual requirement of hydrocortisone (or its equivalent) for the treatment of classical CAH is about 10 to 15 mg/m²/day divided into 2 or 3 doses per day. Dosage requirements for patients with non classical CAH may be less. Adults may be treated with the longer-acting dexamethasone or prednisone. A small dose of dexamethasone a bedtime (0.25 to 0.5 mg) is usually adequate for androgen suppression in non classical patients. Ultimate goal of corticosteroid therapy is to give the lowest dose required for optimal control. Anti-androgen treatment may be useful as adjunctive therapy in adult women who continue to have hyperandrogenic signs despite good adrenal suppression. Titration of the dose should be aimed at maintaining androgen levels at age and sex-appropriate levels and 17-OHP levels of less than 1000 ng/dL to normalize growth in growing children. Patients with SW-CAH have elevated plasma renin activity (PRA) in response to the sodium-deficient state, and they require treatment with the salt retaining steroid 9α-fludrocortisone acetate. It has not been customary to supplement conventional glucocorticoid replacement therapy with the administration of salt-retaining steroids in the SV and non classical forms of CAH, although there has been some suggestion that adding fludrocortisones to patients with elevated PRA may improve hormonal control of the disease [35]. Monitoring of glucocorticoid/mineralocorticoid replacement therapy is recommended every 3 to 4 months while children are actively growing, and less often thereafter; and monitoring for TART (Testicular adrenal rest tumor) in males every 3 to 5 years after onset of puberty. In adulthood, long-term follow-up in the following areas is recommended: overweight/obesity, bone mineral density, fertility, and cardiovascular risks [33].

7.2. Surgery

In the past, it was routine to recommend early corrective surgery for neonates born with ambiguous genitalia. In recent years, the implementation of early corrective surgery has become increasingly controversial because of lack of data on long-term functional outcome. Thus, the role of the parents in sex assignment becomes crucial in all aspects of the decision-making process,
and all possible therapeutic options for the intersex child, particularly early versus delayed surgery. The aim of surgical repair in females with ambiguous genitalia caused by CAH, when the decision is made by parents or patients themselves, is generally to remove the redundant erectile tissue, preserve the sexually sensitive glans clitoris, and provide a normal vaginal orifice that functions adequately for menstruation and delivery. The extent of surgery depends on the degree of genital virilization. For female patients with greater degrees of virilization (an enlarged phallus, fused labia, and a single perineal opening), surgical procedures may include reduction of the enlarged clitoris, separation of the labia, and opening of the lower vagina. Further procedures may be required in adolescence and adulthood to provide an adequate introitus for menstruation.

7.3. Bone mineral density

It is affected by the simultaneous competing actions of androgen excess (from undertreatment) and glucocorticoid excess (from overtreatment), which can both occur at the same time in a patient. To adequately suppress androgen production in patients with CAH, the usual requirement of hydrocortisone is generally higher than the endogenous secretory rate of cortisol. Chronic therapy with glucocorticoids at supraphysiologic levels can result in diminished bone accrual and lead to osteopenia and osteoporosis. The discrepancies may be attributable to steroid 21-OH deficiency in age and gender, as well as varying treatment regimens. The increased adrenal androgens, which are converted to estrogens, may counteract the detrimental effects of glucocorticoids on bone mass. This may explain why older CAH women, particularly those who are postmenopausal, are at higher risk for osteoporosis than younger CAH patients [33].

8. Prenatal diagnosis & treatment

Prenatal diagnosis is appropriate in families where a previous family member has been affected. Pre-natal treatment with dexamethasone suppresses the formation of androgens by the fetal adrenal gland and can prevent or minimize the ambiguity of the female genitalia, thus precluding an incorrect sex assignment, and the ensuing psychiatric problem of gender confusion in affected females. Prenatal diagnosis has now been utilized for over a decade in the prenatal treatment of 21-OH deficiency (approximately 780 at-risk pregnancies were referred to our hospital). Dexamethasone was chosen because it crosses the placenta, crossing from the maternal to the fetal circulation. It enters the fetus to suppress ACTH because it is not bound to high-affinity transport proteins in the blood and because it cannot be
metabolized by the placental 11β-hydroxysteroid dehydrogenase. Dexamethasone (20µg/kg/day in three divided doses) is administered to the pregnant mother beginning before 10 weeks gestation, blind to whether the fetus is female or is affected, to suppress excess adrenal androgen secretion and to prevent virilization should the fetus be an affected female (Fig. 10) [36]. From the timetable of sexual differentiation, it is evident that the urogenital sinus has already begun to be formed by the ninth week of gestation, and thus treatment must begin before this to prevent virilization of the genitalia. Diagnosis by DNA analysis can be made after chorionic villus sampling in the eighth to tenth week gestation or by sampling amniotic fluid cells (amniocentesis) in the second trimester. Treatment is discontinued if the fetus is shown to be an unaffected female on DNA analysis or a male on karyotype analysis. Currently, prenatal treatment for prevention of external virilisation is not recommended as risk-benefit ratio is not supportive.

Figure 10. Algorithm depicting prenatal management of pregnancy in families at risk for a fetus with 21-OH deficiency.

9. Transitioning pediatric patient to adulthood

Genetic counseling plays a pivotal role in dealing with progression of the CAH patients. Transitioning the pediatric patient to adulthood is a very important aspect of caring for CAH patients [33]. There are several issues to
consider for a smooth transition. During the paediatric period, children with CAH are typically brought to doctor by their parents and are monitored very frequently. Once patients reach adulthood, however, they must assume responsibility for understanding their medical condition and for compliance. When medical visits tend to decrease in frequency, patients are at risk for poor follow-up. Another issue to take into account is that the goals of treatment are different in children compared with adults. Once growth and development are completed, however, the goals of treatment shift toward preventing symptoms of hyperandrogenism in women, preserving fertility, and satisfactory sexual function. The dose and type of glucocorticoid may require adjustment once growth is completed, as growth suppression is no longer a concern. Mineralocorticoid requirements tend to decrease with age. Although some endocrinologists are trained in both pediatric and adult endocrinology, allowing them to continue caring for their pediatric patients into adulthood, in this program, there is an outpatient clinic staffed by both a pediatric and an adult endocrinologist. The transition usually takes place around 17 to 18 years of age, at which time the CAH patient sees both endocrinologists, and the pediatric endocrinologist provides the adult endocrinologist with an extensive summary of treatment up to that time. Similar programs of transitioning may help to optimize the care of CAH patients into adulthood [33].

10. Special issues for girls with CAH

10.1. Newborn

The adrenals start synthesizing androgens before birth. These hormones virilize the genitalia towards male pattern. As a result external genitalia is ambiguous and present as clitoromegaly with patial or complete labio-scrotal fusion. Girls with CAH have healthy ovaries and uterus but sometimes lower part of vagina is not fully formed. But this condition can be treated with medical management and may not require surgical correction at same time.

10.2. Infancy

Recently health professionals believe that surgery made the genitals look normal. Surgery to reduce the size of clitoris is called clitoral reduction. Surgery to open fused labia is called vulvoplasty. Surgery to create larger vagina is called vaginoplasty. These surgeries need not to be done on same time.
10.3. Childhood

Sense of herself as a sexual person start in these years. Parent response to daughter is the key way she will learn about her body. Progressing with age, each time add some more information to her. Reassure her that she will grow into a woman.

10.4. Adolescence

Important physical change is menstruation. Vagina needs to be large enough so that mensural blood can flow out. If vaginal development is not complete, girl needs to see gynaecologist. Doctor or health care team or parents should have full and honest discussion about role of hormones in body, simultaneously role of medication in correcting body dysfunction and congenital adrenal hyperplasia.

11. Special issues for boys with CAH

Boys with CAH are likely to stop growing sooner than others as they present with gonadotropin independent precocious puberty (GIPP). The final height is slightly less than other boys in the family. So, parents should persuade their son to value other things rather than height.

12. Future perspectives

Intense research over the past time in human endocrinology has produced a plethora of discoveries spanning identification of genes coding enzyme to different forms of CAH. In addition, it has been possible to offer genetic counseling to couples at risk of having an affected baby with most severe form of CAH. However there is incomplete understanding of gene expression regulation which is dependent on multiple transcription factors where some of which are still unidentified. New technologies like proteomics, tandem mass spectometry, microchip arrays and ultra rapid DNA genotyping and sequencing will contribute to identification of new disease associated genes and new biomarkers. After elucidating the physiological functions using functional studies and animal models, it is expected that results will help to exclude false positive results that are obtained with immunoassays used in neonatal screening programs and contribute to better genetic counselling and patient treatment.

Future CAH investigations include: (1) Gene therapy, (2) Treatment & non invasive prenatal diagnosis, (3) Advance understanding of
psychoendocrinological factors. All these subjects are currently under research so that future experts can evaluate multiple physiological systems of each patient and select individualized, curative therapeutics.

References

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