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Minireview

21-Hydroxylase Deficiency Congenital Adrenal Hyperplasia

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Congenital adrenal hyperplasia (CAH) results from an enzymatic block at any stage in the synthesis of cortisol. All enzyme defects causing CAH are autosomal recessive traits. It is a relatively common disease, occurring in 1 in 5000 to 1 in 15,000 births in most populations. Since the isolation of the gene responsible for steroid 21-hydroxylase deficiency (involved in about 90% of the cases of CAH) in 1984, knowledge of the specific mutations that cause the different forms of CAH has grown rapidly. Defects in the encoding gene have been confirmed as the basis of endocrine disease in the case of all but one of the adrenal steroidogenic enzymes. Analysis of DNA obtained by chorionic villus sampling in early pregnancy permits prenatal diagnosis and treatment of 21-hydroxylase deficiency CAH. The correlation between the clinical expression of endocrine disease and the mutations of the primary structural gene is not absolute. Clinicians cannot accurately predict the course of the disease or make therapeutic decisions based on the genotype alone. We will review the various forms of clinical presentation of 21-hydroxylase CAH, its etiology, diagnosis, molecular genetics, and treatment.

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Synthesis of the essential glucocorticoid cortisol in the zona fasciculata of the adrenal cortex is directly regulated by pituitary pulsatile release of adrenocorticotropin hormone (ACTH). Five enzymes are required to synthesize cortisol from cholesterol: 21-hydroxylase, 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and cholesterol desmolase. Congenital adrenal hyperplasia (CAH) arises from reduced activity of any of these enzymes. Impaired synthesis of cortisol induces oversecretion of ACTH, hyperplasia of the adrenal cortical tissue, and overproduction of precursor steroids proximal to the site of the enzymatic defect.

Three classes of steroid are produced in the adrenal: glucocorticoids, mineralocorticoids, and sex steroids. Several of the enzymes active in the synthesis of cortisol also take part in the synthesis of mineralocorticoids and sex steroids, which occur in other zones of the adrenal cortex (the mineralocorticoid aldosterone in the zona glomerulosa and C_{19} androgenic steroids in the zona reticularis). A network of parallel and interconnected pathways of steroidogenesis permits shunting of cortisol precursor compounds into alternative steroidogenic pathways when synthesis of cortisol is blocked by enzyme deficiency (see Fig. 1). In the family of disorders known as CAH, abnormal patterns of glucocorticoid, mineralocorticoid, and sex steroid secretion characterize clinical syndromes of genital ambiguity and imbalance in sodium metabolism, hypertension, and hyperandrogenemia affecting somatic growth and fertility specific to the enzyme deficiency.

The molecular genetic basis of the variants of CAH is being rapidly elucidated. Mutations in the genes encoding the enzyme proteins have been found for every type of CAH enzyme deficiency except for the rare and devastating cholesterol desmolase deficiency. One result of this knowledge is that prenatal diagnosis is now possible by chorionic villi sampling by the 10th week of pregnancy.

Though restoration of the pituitary–adrenal axis to complete normality has not been achieved with current therapy, the majority of patients with CAH can conduct relatively normal lives with lifelong steroid replacement treatment. 21-Hydroxylase CAH is also the first disease in which prenatal treatment has become a therapeutic option.

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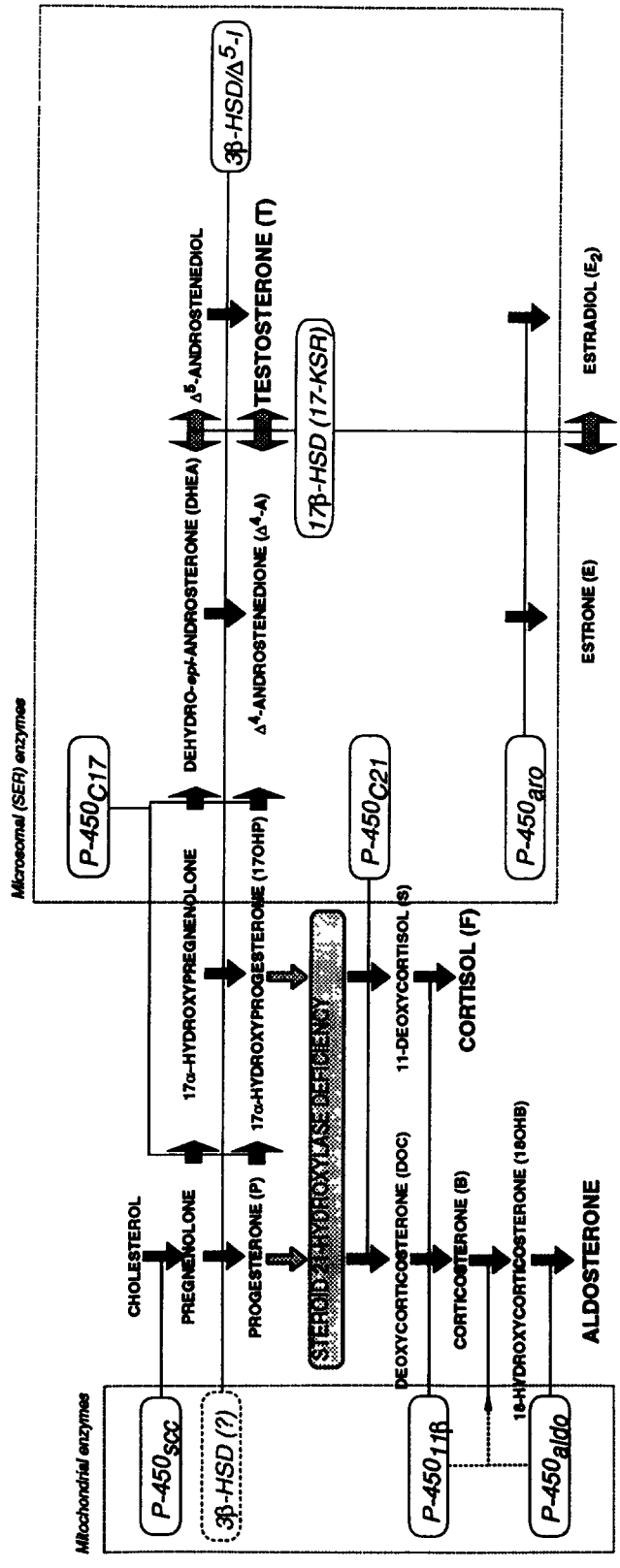

Fig. 1. Adrenal steroidogenesis. Biosynthetic pathways from cholesterol to mineralocorticoids (aldosterone), glucocorticoids (cortisol), and androgens (androstenedione) are shown and the cellular location of enzyme activities indicated. Aro = aromatase; HSD = hydroxysteroid dehydrogenase; KSR = ketosteroid reductase. (From New *et al.*, in press; reproduced with permission.)

21-HYDROXYLASE DEFICIENCY

Over 90% of cases of CAH [1] are due to mutations in the genome of the 21-hydroxylase enzyme, which mediates late steps (in parallel pathways) in the synthesis of cortisol and aldosterone in the adrenal (see Fig. 1).

There are two major forms of 21-hydroxylase deficiency. "Classical" 21-hydroxylase deficiency involves total or near-total block of enzyme activity and is clinically present at birth. "Nonclassical" 21-hydroxylase deficiency is diagnosed later in life and is generally milder than the classical form, because it involves only partial blockage of the enzyme activity.

The classical form has been found to occur at a frequency of 1 in 14,000 births [2]. The nonclassical form has been shown to have an extremely high frequency in the general white population (1/100) and increased incidence in certain ethnic groups (1/27 in Ashkenazi Jews, 1/53 in Hispanics, 1/63 in Yugoslavs, 1/333 in Italians) [3].

The result of 21-hydroxylase deficiency is that 17-hydroxyprogesterone is not converted to 11-deoxycortisol resulting in (1) deficiency of cortisol synthesis and (2), as a result of the consequent lack of negative feedback to the hypothalamus and pituitary, increased secretion of ACTH, which leads to overproduction of and accumulation of precursors proximal to the 21-hydroxylation step. The 17-hydroxylated precursors are metabolized by the 17,20-lyase activity to the androgens dehydroepiandrosterone (DHEA) and Δ^4-androstenedione. Δ^4-Androstenedione is converted peripherally to testosterone. The abnormally high levels of circulating androgens produce progressive virilism and advanced somatic development.

CAH due to 21-hydroxylase deficiency is the most common cause of ambiguous genitalia in females, who when affected with classical 21-hydroxylase deficiency manifest varying degrees of genital ambiguity at birth ranging from mild clitoral enlargement to the profound morphological anomaly of a penile urethra. The development of the internal genitalia is normal, and normal childbearing capacity exists. Postnatally, untreated children of either sex exhibit accelerated somatic growth with advanced bone maturation, early closure of the epiphyses, and final short adult stature relative to that predicted on the basis of parental heights. Other symptoms of androgen excess include premature appearance of sexual hair, acne, adult body odor, and temporal balding. Females may develop polycystic ovarian syndrome and amenorrhea or irregular menses. In pubertal males, high levels of adrenal androgens may suppress gonadotropin secretion, resulting in poor spermatogenesis.

In three-fourths of classical (i.e. presenting at birth) cases of 21-hydroxylase deficiency, there is salt-wasting from deficient aldosterone synthesis. This form of the disorder is termed "salt-wasting" disease and is best defined by hyponatremia and hyperkalemia, inappropriately high urinary sodium, and low serum and urinary aldosterone with high plasma renin activity (PRA). In severe cases, hypovolemia and shock are present at the time of diagnosis. Patients not demonstrating salt-wasting are said to have "simple virilizing" disease. The distinction between these two forms of classical disease on a molecular genetic basis is not absolute, as will be discussed below.

Nonclassical 21-hydroxylase deficiency does not produce ambiguous genitalia in the newborn female and the clinical features secondary to androgen excess are variable and may present at any age. It can result in premature development of pubic hair in children, severe cystic acne, and final adult height less than predicted on the basis of parental heights [4]. Females with irregular menses, secondary amenorrhea, and hirsutism have been diagnosed with this disease, and women with polycystic ovarian syndrome may in fact be patients with nonclassical 21-hydroxylase deficiency. Some individuals with identical biochemical abnormalities (i.e. with equally elevated serum 17-hydroxyprogesterone after an intravenous injection of ACTH) remain entirely asymptomatic. Longitudinal follow-up of these patients often shows signs of hyperandrogenism to wax and wane with time.

MOLECULAR GENETICS

21-Hydroxylase deficiency is inherited as a monogenic autosomal recessive trait that is closely linked to the HLA major histocompatibility complex (MHC) located on the short arm of chromosome 6 [5]. The HLA complex is an assembly of genes coding for cell–surface antigens that are the major barriers for allogenic transplantation. HLA-A, -B, and -C are structurally related and are referred to as "class I" antigens, while HLA-DR is the main "class II" antigen [6]. The class III genes are located between the class I and II antigens. Data on intra-HLA recombination indicated a gene locus for 21-hydroxylase between HLA-B and -DR [7, 8].

In addition to linkage of the 21-hydroxylase locus with the HLA-B and -DR antigen loci, 21-hydroxylase deficiency alleles are found in linkage disequilibrium with HLA antigen genes in configurations known as haplotypes [7]. The salt-wasting form is associated with the extended haplotype HLA-AcBw47, DR7 and the nonclassical disease is associated with the haplotype HLA-B14, DR1 [3].

The structural gene encoding the adrenal cytochrome *P*450 specific for steroid 21-hydroxylation (*P*450c21) is named CYP21 or CYP21B and contains 10 exons. This gene and a 98% identical pseudogene (CYP21P or CYP21A) [9] are located in close proximity (30 kb) in the HLA complex adjacent to and alternating with the C4B and C4A genes encoding the fourth component of serum complement [10, 11]. The pseudogene CYP21P does not produce a detectable mRNA or a protein due to several deleterious mutations.

A pair of genes of unknown function has been found on the strand opposite the strand containing the CYP21 and C4 genes [12]. These "X" genes overlap the distal portion of each of the CYP21 genes. There are few mutations reported in these "protected" regions of the CYP21 gene. Remarkably, a second pair of genes of unknown function, so called "Y" genes, has now been reported in this locus [13], these overlap the CYP21P and the CYP21 genes on the sense strand and the active use of the CYP21P promoter by the YA gene suggests that the so called pseudogene may have an as yet unknown role in the adrenal cortex.

Mutations in CYP21 appear to be generated by either of two types of recombination mechanisms. Misalignment of the tandem C4A-CYP21P-C4B-CYP21 arrangement during meiosis could lead to unequal crossing over, resulting in, in one gamete, a deletion of a DNA segment including the C4B and CYP21, and, in the other gamete, an additional duplication of this segment, as have been found. Alternatively, small deleterious mutations could be transferred from CYP21P to CYP21 in gene conversion events.

Gene deletions have been carefully studied employing multiple informative restriction digests (such as TaqI and BglIII), oligonucleotide hybridizations, and rare-cutting endonucleases such as BssHII [14]. The frequency of gene deletions in different ethnic groups ranges from 11 to 35%, and many of these are found in association with the haplotype HLA-B47;DR7 [15, 16].

Gene conversion is responsible for the 65 to 90% of the disease haplotypes in which deletional mutations were not identified [17]. Nine mutations are the result of transferral of normal sequences of the CYP21P pseudogene into the active CYP21 gene; they have adverse effects at any of the stages of gene expression (Fig. 2).

One affected gene had a nonsense mutation in codon 318 (C → T substitution). Any encoded translation product would be truncated and completely inactive. This mutation was seen in 4 to 7% of salt-wasting classical 21-hydroxylase deficiency haplotypes [18].

Other converted genes carried the 8-bp deletion in exon 3 normally found in CYP21P [19]. This shifts the reading frame and encodes a completely inactive enzyme. This mutation is found in 3 to 10% of haplotypes of persons with salt-wasting disease [17]. A cluster of mutations, Ile-Val-Glu-Met-235-238 → Asn-Glu-Glu-Lys and a single substitution, Arg-356 → Trp [20] have also been described in patients with the salt-wasting disease. A point mutation (A → G

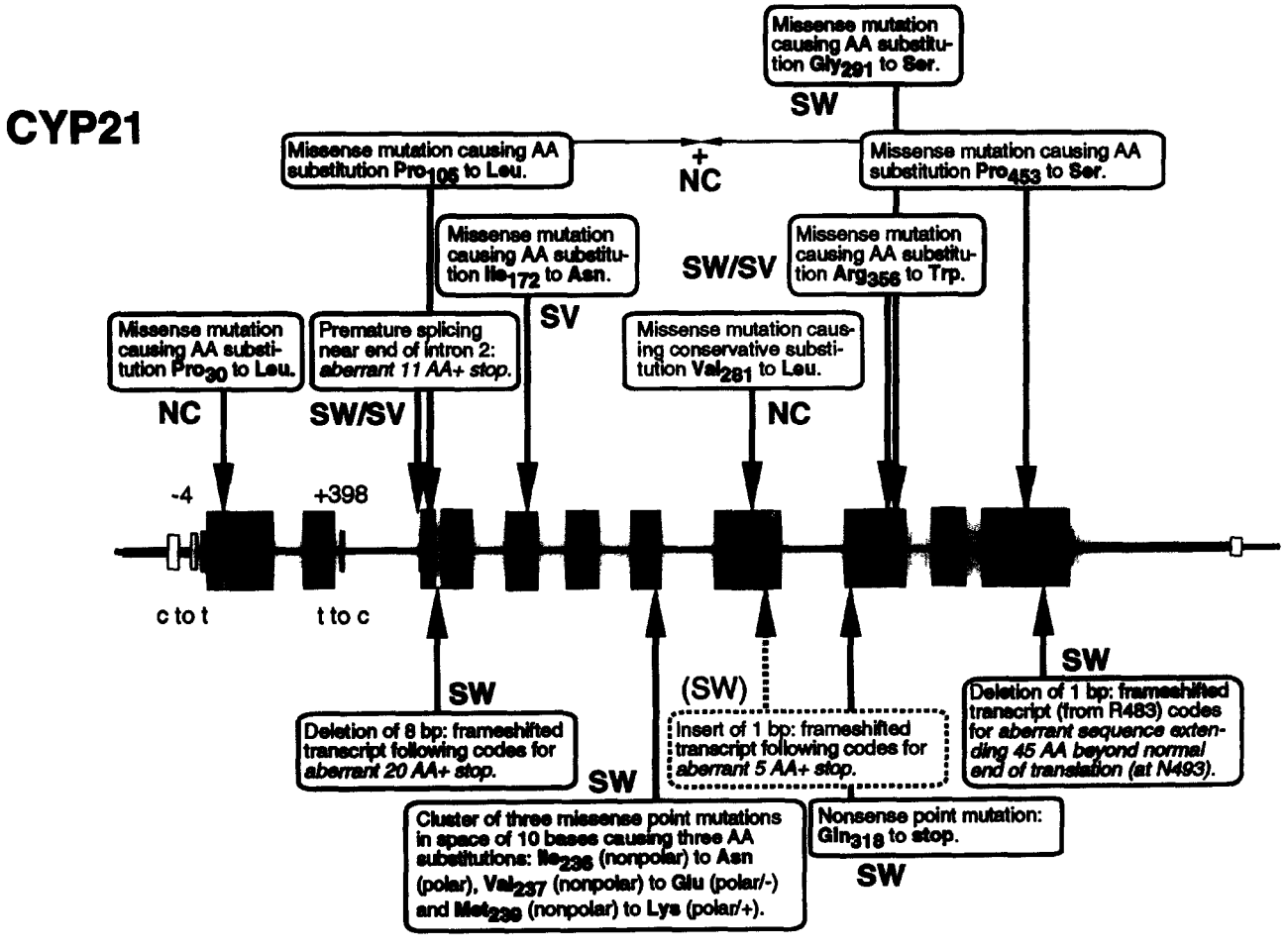

Fig. 2. Frameshift/point mutations arising from 21-hydroxylase gene conversion events. (From New *et al.*, in press; reproduced with permission.)

substitution) in the second intron introduces a new acceptor site to be recognized by the intron splicing mechanism [21].

Mutation A → G in intron 2 constitutes the single most frequent allele causing classical 21-hydroxylase deficiency. This mutation causes the intron to end prematurely so that 19 nucleotides normally spliced out of mRNA are retained. This leads to a shift in the translational reading frame, preventing synthesis of an active protein. Almost all of the mRNA is aberrantly spliced, but *in vitro* a small amount of normally spliced mRNA can be detected. If no other mutations are present, a small amount of normal enzyme might thus be synthesized.

The presence of the aberrantly spliced mRNA in small amounts has been demonstrated *in vitro* after transfection of COS cells with the functional CYP21 gene. It is possible that abnormal protein can be produced in the adrenal tissue of normal individuals. We do not know what proportion of mRNA is normally spliced in the adrenal glands of patients with this mutation. Most (but not all) patients who are homozygous or hemizygous for this mutation have the salt-wasting form of the disorder, indicating that they have insufficient enzymatic activity to permit adequate aldosterone synthesis.

The single base change (T → A) in exon 4 isoleucine-172 to asparagine (polar to nonpolar substitution) disrupts the important hydrophobic interaction of the enzyme with the endoplasmic reticulum and essentially abolishes enzyme activity (about 1% of normal activity as measured by the first-order rate constant, V_{max}/K_m). This mutation has been associated with the simple virilizing phenotype [20].

Associated with the HLA-B14;DR1 haplotype (the haplotype seen in 78% of nonclassically affected patients) is a change in codon 281 of CYP21 from GTG, valine, to TTG, leucine. This is a conservative amino acid substitution which nonetheless leads to a change in conformation of the protein, reducing the enzyme activity [22].

Recently a missense mutation at residue 30 in exon 1, changing proline to leucine, was revealed in 16% of haplotypes in patients with nonclassical disease [23].

One gene conversion that has not yet been identified in isolation in any affected allele is a single-base (T) insertion in the seventh exon, which, by causing a frameshift that results in a truncated protein, would be expected to produce the severe, salt-wasting phenotype.

CORRELATION OF GENOTYPE WITH PHENOTYPE

In general mutant *P*450c21 enzymes carrying specific amino acid substitutions identified in patients with 21-hydroxylase deficiency display activities that correspond roughly to the clinical severity of the disease and to the associated biochemical abnormalities [23–25].

Homozygous deletion, or deletion in *trans* with a stop mutation, or the cluster of mutations at exon 6, all

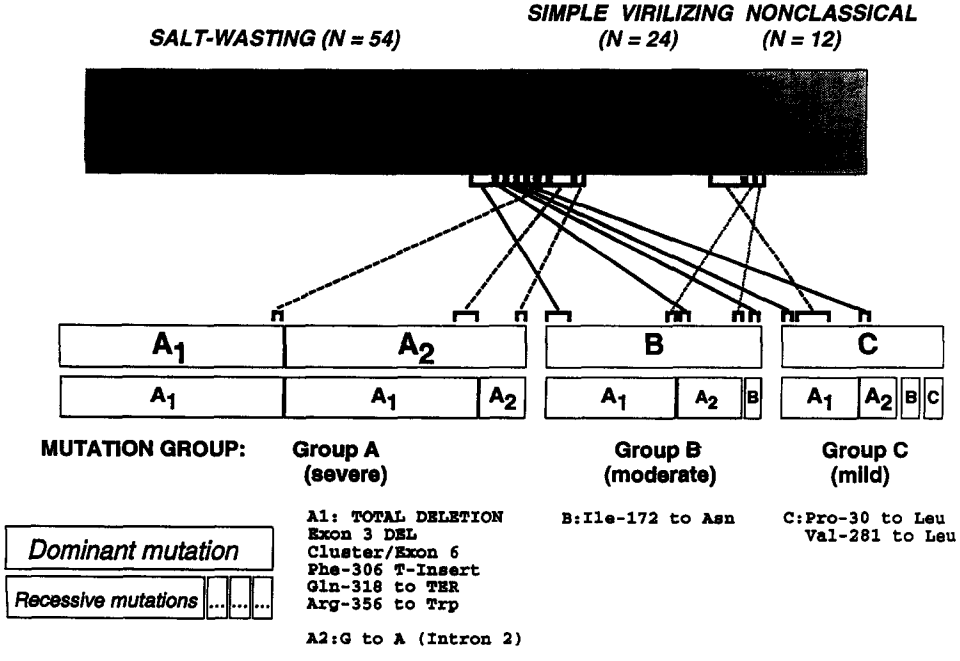

Fig. 3. Correlation of clinical phenotype with genotype determined by molecular analysis in 90 patients. The expectation is that mutations of group A (severe) will be found in patients manifesting salt-wasting disease, group B (moderate) in patients with simple virilizing classical disease, and group C (mild) in patients with nonclassical disease. Oblique lines indicate noncorrelations and the width of bracket the number of cases in which each was observed. The number of noncorrelations (20%) shows the importance of careful clinical monitoring even of those cases in which the molecular DNA defect seems straightforward. (From New *et al.*, in press; reproduced with permission.)

of which confer zero enzyme activity *in vitro*, would be predicted to result in 0% overall 21-hydroxylase activity *in vivo* and the severe salt-wasting type.

Homozygosity for the mutation Ile-172 → Asn, which confers ∝2% of normal activity on the gene product, usually results in the simple virilizing phenotype.

However, the distinction between the two forms of the classical 21-hydroxylase deficiency is not absolute. There are reports of HLA-identical sib pairs in which one sib demonstrates salt-wasting whereas in the other there is adequate aldosterone synthesis [26]. One patient with the Ile-172 → Asn mutation has been shown to have mild salt-wasting, and patients with identical mutations are reported to have variable amelioration of aldosterone deficiency with age [27].

As we mentioned above, homozygotes for milder mutations such as Val-281 → Leu and Pro-30 → Leu, which have about 50% of wild type enzyme activity *in vitro*, usually manifest the phenotype of the nonclassical form of the disease [17, 24].

Recently, Speiser *et al.* [28] classified 90 patients into 3 mutation groups based on the degree of predicted enzymatic compromise. Mutation group A (no enzymatic activity) consisted primarily of salt-wasting patients, group B (2% activity) of simple virilizing patients, and group C (10–20% activity) of nonclassical patients. Mutation groups were correlated with clinical diagnosis, but each group contained patients with phenotypes either more or less severe than predicted. The phenotype was accurately predicted in 85% (53/62) of group A, 72% (16/22) of group B, and 62.5% of group C (Fig. 3).

It is conceivable that individuals with phenotypes more severe than predicted or discordant from siblings have acquired additional, as yet unidentified, mutations within the CYP21 gene. It is also plausible that at least some differences in clinical disease expression are governed by factors remote from the CYP21 locus.

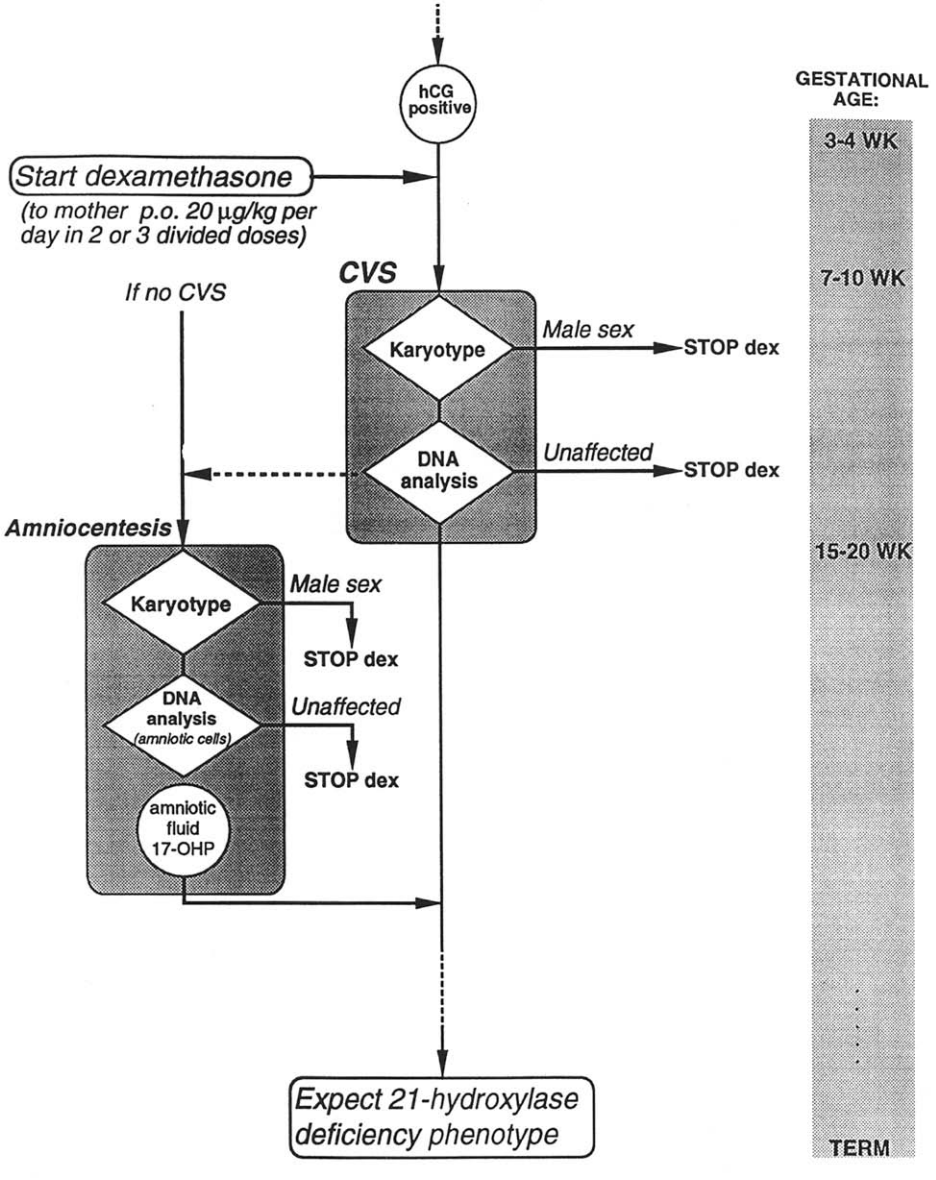

Fig. 4. Schedule for prenatal diagnosis and treatment. (From [30] pp. 1662–1667).

Finally, one might postulate that phenotypic severity is influenced by parental imprinting or by negative allelic complementation, i.e. exaggerated gene dosage effect.

THERAPY AND PRENATAL DIAGNOSIS

Glucocorticoid administration has been the keynote of treatment for this disorder. It both replaces the deficient cortisol and reduces the ACTH overproduction and overstimulation of the adrenal cortex. Therefore it suppresses the excessive adrenal androgen production. When the mineralocorticoid synthesis is impaired, a salt-retaining steroid in the replacement therapy is required. PRA has been found to be a good measure of degree of adrenal hormonal control. Genital ambiguity in females is generally corrected under the age of 2 in order to enhance formation of a strong gender identity.

Prenatal diagnosis has been used for two decades in pregnancies known to be at risk. Hormonal diagnosis has been made since 1975 during the second trimester. Currently cultured fetal cells from either the amniotic fluid or after chorionic villus sampling (CVS) provide DNA and allow early (at 8 to 10 weeks of gestation using CVS) genetic diagnosis and possible intervention. The concept of prenatal treatment using dexamethasone, a Δ^1-steroid which, when administered to a pregnant woman crosses the placental barrier to suppress the fetal adrenal, has been introduced with initial results favorable for treatment [29–31].

Treatment should be initiated during the first trimester in conjunction with diagnosis by CVS/molecular genetic techniques (Fig. 4). When pregnancy is first confirmed by an hCG test in the woman of a couple at genetic risk for having affected offspring, hormonal treatment of the fetus is begun—blind to its affected status—by administering dexamethasone orally to the mother. The CVS is used for sex karyotyping and DNA analysis. If the fetus is male, the dexamethasone can be stopped immediately. If the fetus is female, treatment continues until the results of the DNA analysis are available, when the dexamethasone is then either stopped, or, in the case of an affected female, continued to term.

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