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Journal of Steroid Biochemistry and Molecular Biology 69 (1999) 19–29

The Journal of Steroid Biochemistry & Molecular Biology

Congenital adrenal hyperplasia: update on prenatal diagnosis and treatment ☆

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Abstract

The diagnostic term 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 translated as autosomal recessive traits, with the enzyme deficient in more than 90% of CAH cases being 21-hydroxylase. In the classical forms of CAH (simple virilizing and salt wasting), owing to 21-hydroxylase deficiency (21-OHD), androgen excess causes external genital ambiguity in newborn females and progressive postnatal virilization in males and females. Non-classical 21-OHD (NC21OHD) 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 HLA genes. Specific mutations may be correlated with a given degree of enzymatic compromise and the clinical form of 21-OHD. NC21OHD patients are predicted to have mild mutations on both alleles or one severe and one mild mutation of the 21-OH locus (compound heterozygote). In most cases the mutation groups represent one diagnosis (e.g., Del/Del with SW CAH), however we have found several non-correlations of genotype to phenotype. Non-classical and classical patients were found within the same mutation group. Phenotypic variability within each mutation group has important implications for prenatal diagnosis and treatment.

Prenatal treatment of 21-OHD with dexamethasone has been utilized for a decade. An algorithm has been developed for prenatal diagnosis and treatment, which, when followed closely, has been safe for both the mother and the fetus, and has been effective in preventing ambiguous genitalia in the affected female newborn. This is an instance of an inborn metabolic error successfully treated prenatally.

Since 1986, prenatal diagnosis and treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21-OHD) has been carried out in 403 pregnancies in The New York Hospital–Cornell Medical Center. In 280, diagnoses were made by amniocentesis, while 123 were diagnosed using chorionic villus sampling. Of the 403 pregnancies evaluated, 84 babies were affected with classical 21-OHD. Of these, 52 were females, 36 of whom were treated prenatally with dexamethasone. Dexamethasone administered at or before 10 weeks of gestation (23 affected female fetuses) was effective in reducing virilization. Thirteen cases had affected female sibs (Prader stages 1–4); 6 of these fetuses were born with entirely normal female genitalia, while 6 were significantly less virilized (Prader stages 1–2) than their sibs, and one was Prader stage 3. Eight newborns had male sibs; 4 were born with normal genitalia, 3 were Prader stages 1–2, and 3 were born Prader stages 3–4. No significant or enduring side effects were noted in either the mothers or the fetuses, indicating that dexamethasone treatment is safe. Prenatally treated newborns did not differ in weight, length, or head circumference from untreated, unaffected newborns.

Based on our experience, proper prenatal diagnosis and treatment of 21-OHD is effective in significantly reducing or eliminating virilization in the newborn female. This spares the affected female the consequences of genital ambiguity of genital surgery, sex misassignment, and gender confusion.

☆ Proceedings of Xth International Congress on Hormonal Steroids, Quebec, Canada, 17–21 June 1998.

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0960-0760/99/$ - see front matter
PII: S0960-0760(99)00059-X

1. Introduction

Congenital adrenal hyperplasia (CAH) is a term describing a family of inherited disorders of steroidogenesis caused by an abnormality in one of five enzymatic steps necessary in the conversion of cholesterol to cortisol in the adrenal cortex. The enzyme defects are translated as autosomal recessive traits, with the enzyme deficient in more than 90% of CAH cases being 21-hydroxylase [1].

1.1. Classical CAH

In the classical form of CAH owing to 21-hydroxylase deficiency (21-OHD), androgen excess causes external genital ambiguity in newborn females (female pseudohermaphroditism), in which they may present with a urogenital sinus, scrotalization of the labia majora, labial fusion, or clitoromegaly. After birth males and females exhibit progressive postnatal virilization, which can include central precocious puberty later in childhood, progressive penile or clitoral enlargement, precocious pubic hair, hirsutism, acne, advanced somatic and epiphyseal development, reduced fertility, in women, menstrual abnormalities, and in men, small testes. There are two forms of classical steroid 21-OHD, the simple-virilizing and salt-wasting types. Three-fourths of classical cases are salt-wasting [1]. To some extent, the symptoms can be arrested or reversed by treatment with glucocorticoid, which suppresses ACTH stimulation of the adrenal cortex. Those patients with aldosterone deficiency require treatment with salt-retaining steroids as well.

1.2. Non-classical CAH

Non-classical 21-OHD (NC21-OHD) refers to the condition in which partial deficiencies of 21-hydroxylation produce late onset, less extreme hyperandrogenemia and milder or no symptoms. Females do not demonstrate genital ambiguity at birth, though males and females may manifest signs of androgen excess at any phase of postnatal development. Short stature, premature development of pubic hair, insulin resistance, acne, reduced fertility, and in women, polycystic ovaries, hirsutism, and male pattern baldness are seen in untreated patients. Non-classical patients respond to treatment with a glucocorticoid to suppress ACTH, thereby suppressing the androgens.

1.3. Clinical spectrum

There is a wide clinical presentation of classical and non-classical 21-hydroxylase deficiency, ranging from virilization with labial fusion to precocious adrenarche, to pubertal or postpubertal virilization (Fig. 1). During their lifetimes, patients may change from symptomatic to asymptomatic.

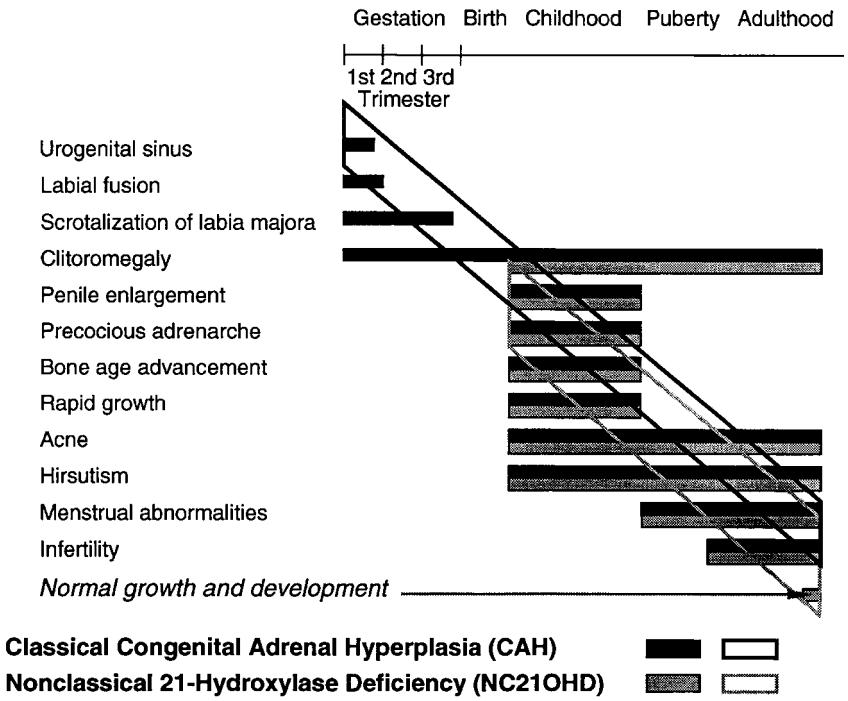

Fig. 1. Clinical spectrum of steroid 21-hydroxylase deficiency.

1.4. Frequency

Analysis of CAH incidence data from almost 6.5 million newborns screened in the general population worldwide has demonstrated an overall incidence of between 1:13,000 and 1:15,000 live births for the severe classic form of CAH [2–4]. The incidence of CAH in either homogeneous or heterogeneous general populations has been as high as one in 7500 live births (Brazil).

The overall frequency of non-classic 21-OHD is high. The study of Speiser et al. [5], assessing the population genetics of the non-classical disorder, found NC21-OHD to be much more common than the classical deficiency causing CAH, and in fact found it to be the most common human autosomal recessive disease trait. The disease frequency in the general heterogeneous population of New York City is 1/100, and 1/7 is a carrier. The highest ethnic frequency was found among Ashkenazi Jews, remarkably at 1/27, with 1/3 a carrier. Other specific ethnic groups also exhibited high disease frequency: 1/40 Hispanics, 1/50 Slavs, and 1/300 Italians. These results have also been confirmed by other reports [6,7].

2. Steroidogenesis

Aldosterone, cortisol, and testosterone are derived from cholesterol and utilize many of the same enzymes for their synthesis in the adrenal cortex (Fig. 2). Therefore, defects in any of the enzymes that are common to the synthesis pathway of these hormones can result in the loss of a combination of some or all of their production, or unchecked negative feedback loops can lead to overproduction. In the case of 21-OHD, the enzyme deficiency creates the effect of a dam behind which steroid precursors accumulate, which then overflow into biosynthetic pathways unaffected by the block, resulting in the production of excess androgens.

The production of cortisol in the adrenal cortex (in the zona fasciculata region) occurs in five steps: cleavage of the cholesterol side chain by the cholesterol desmolase enzyme, cytochrome P450scc, to yield pregnenolone; conversion of pregnenolone by 3β-dehydrogenation (with accompanying $\Delta^{5,4}$-isomerization) to progesterone by the short-chain dehydrogenase family enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD); and successive hydroxylations at the 17α, 21, and 11β

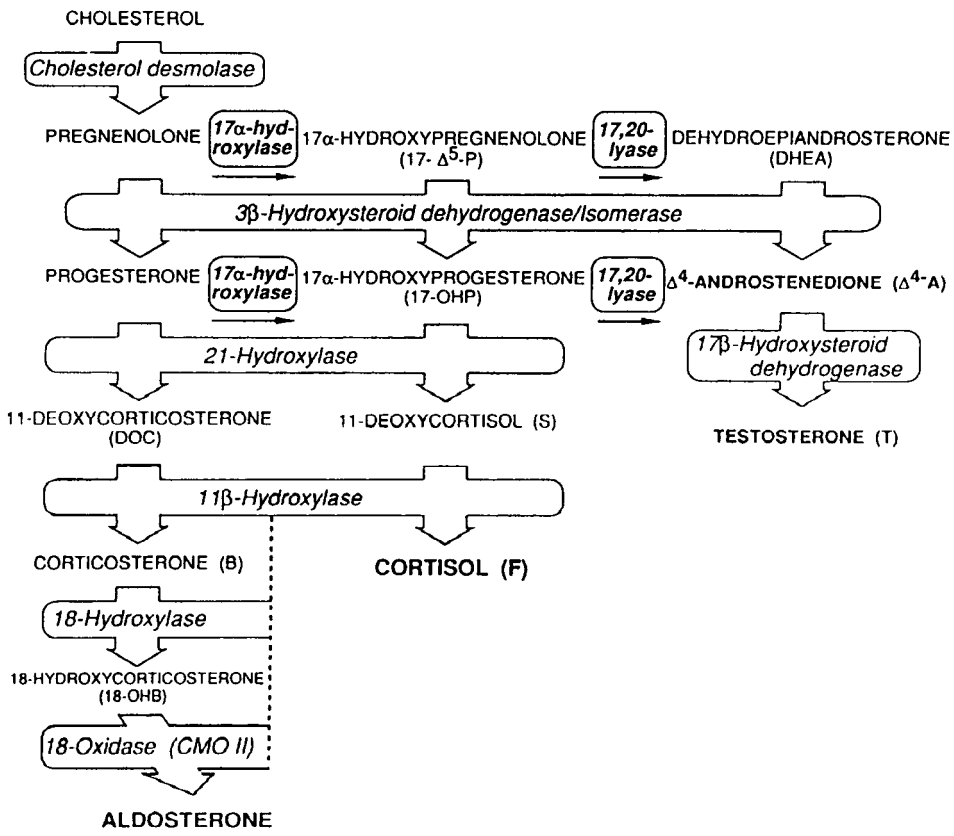

Fig. 2. Pathways of steroid biosynthesis. Enzymatic activities catalyzing each bioconversion are enclosed in boxes. For those activities mediated by specific P450 cytochromes, systematic names of the enzymes ('CYP' followed by number) are listed in parentheses. Other bioconversions (marked with an asterisk) are mediated by different enzymes in various tissues. The planar structures of cholesterol, aldosterone, cortisol, dihydrotestosterone and estradiol are placed near the corresponding labels.

positions, each mediated by a distinct cytochrome P450, resulting in cortisol (Fig. 2) [1].

Cortisol synthesis is regulated by a negative feedback loop in which high serum levels of cortisol inhibit the release of ACTH from the pituitary, while low serum levels of cortisol stimulate the release of ACTH. This defines the hypothalamo-pituitary-adrenal axis. The central nervous system determines the hypothalamic setpoint for the expected plasma cortisol level, so that plasma cortisol levels lower than the hypothalamic-pituitary setpoint will increase the rate and intensity of ACTH secretory pulses (net ACTH release has basal, diurnal, and stress-induced components). The adrenal enzyme deficiencies stated above, causing impaired synthesis and decreased secretion of cortisol, thus lead to chronic elevations of ACTH with overstimulation and consequent hyperplasia of the adrenal cortex. As the pathways for testosterone, dehydroepiandrosterone, and Δ^4-androstenedione precede the 21-OH step and are unblocked, the precursors are thus routed to these pathways and the androgens get oversecreted in the adrenals in utero, which is what masculinizes the female fetus.

CAH patients present with a unique hormonal profile due to their enzymatic deficiency. In our experience, the best diagnostic test for 21-OHD has proven to be the ACTH (Cortrosyn, 0.25 mg) stimulation test measuring the serum concentration of 17-hydroxyprogesterone (17-OHP). After intravenous bolus ACTH administration (preferably in the morning due to the diurnal variation of 17-OHP), 17-OHP is measured at 0 and 60 min. A logarithmic nomogram we developed (Fig. 3) provides hormonal standards for assignment of the 21-OHD type by relating baseline to ACTH-stimulated serum concentrations of 17-OHP.

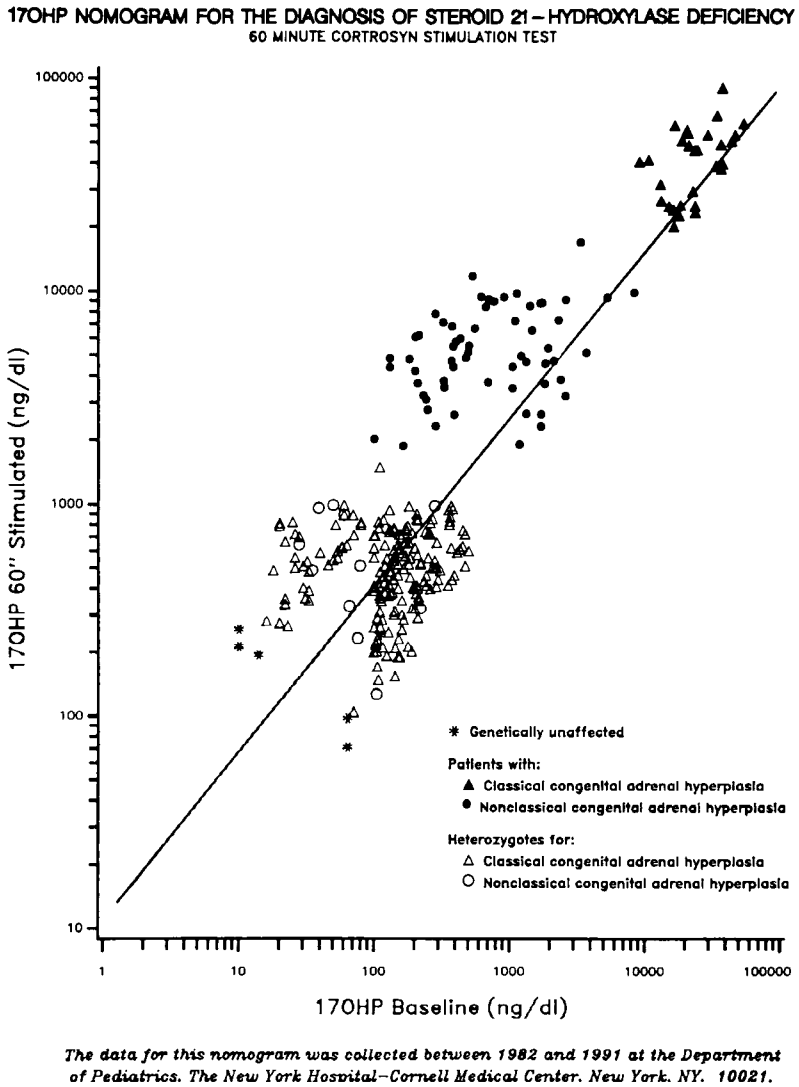

Fig. 3. Nomogram relating baseline to ACTH-stimulated serum concentrations of 17-hydroxyprogesterone (17-OHP). The scales are logarithmic. A regression line for all data points is shown.

3. Molecular genetics

The gene for adrenal 21-hydroxylase, CYP21, is located about 30 kb from an inactive cognate gene, CYP21P (P for pseudogene), on chromosome 6p in the area of the HLA genes. The high degree of sequence similarity (96–98%) between CYP21 and CYP21P apparently permits two types of recombination events: (1) unequal crossing-over during meiosis, which results in complementary deletions/duplications of CYP21 and the possible transmission of a null allele, and (2) non-correspondences between the pseudogene and the coding gene [8,9] that, if transferred by 'gene conversion', result in deleterious mutations. Deletions generally account for 20–25% of classic 21-OHD alleles, and small deletions and point mutations make up the rest.

Specific mutations may be correlated with a given degree of enzymatic compromise and a clinical form of 21-OHD [10–14] (Fig. 4). The genotype for the classical form of CAH is predicted to be a severe mutation on both alleles at the 21-OH locus, with completely abolished enzymatic activity generally associated with salt wasting. The point mutation A (or C) to G near the end of Intron 2, which is the single most frequent mutation in classic 21-OHD, causes premature splicing of the intron and a shift in the translational reading frame [11,15]. Most patients who are homozygous for this mutation have the salt-wasting form of the disorder [16,17]. One mutation in Exon 4 (I172N), specifically associated with simple-virilizing 21-OHD [18], has been shown in in vitro cell transfection assay to result in 1% of normal enzyme activity [19]. Adrenal production of aldosterone is normally in the range of 1/100–1/1000 that of cortisol. The very low residual activity of the I172N mutation apparently is still able to allow aldosterone synthesis and thus prevent significant salt wasting in most cases of the simple-virilizing form of 21-hydroxylase deficiency.

Patients with NC21-OHD are predicted to have mild mutations on both alleles or one severe and one mild mutation of the 21-OH locus (compound heterozygote). Missense mutations in Exon 1 (P30L) and Exon 7 (V281L), which are predominantly associated with this form of the disease, reduce enzymatic activity in cultured cells to 20–50% of normal [19]. These patients do not have salt wasting.

In 1995 we published a study of genetic and clinical

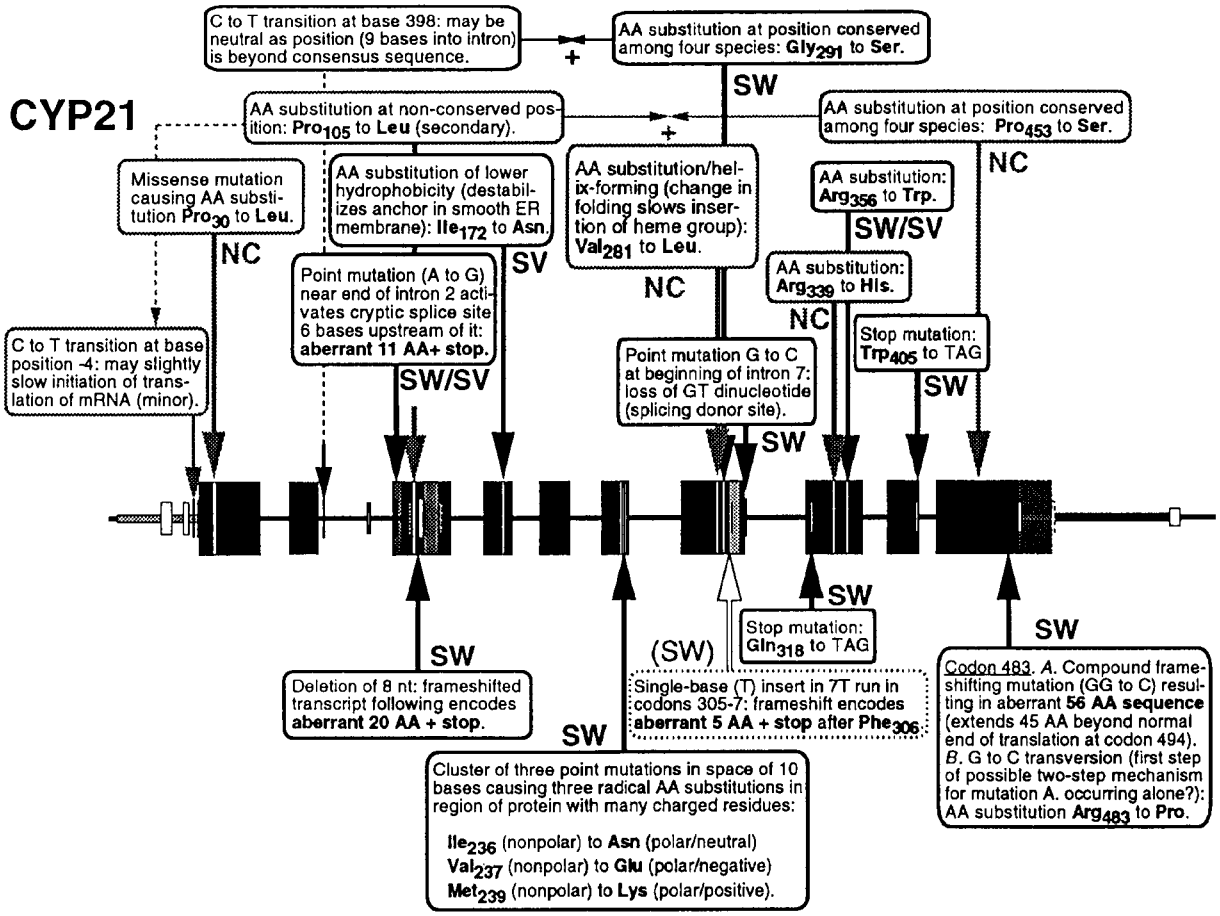

Fig. 4. Mutations in the 21-hydroxylase gene (CYP21).

findings of over 200 patients with 21-OHD [17]. We carefully assessed phenotypic characteristics by (1) genital status with respect to virilization in females, (2) ACTH stimulation tests to evaluate secretion of androgens and 17-hydroxyprogesterone, and (3) salt deprivation studies (whenever safe) to precisely describe the phenotype with respect to aldosterone deficiency and salt wasting. After dividing our patients into 26 mutation-identical groups, we found that in 11 groups, the genotype did not always predict the phenotype. One example of this nonconformity is the following: the V281L/Del genotype group consisted of 13 patients; while they had identical mutations, 11 were non-classical, 1 was a simple virilizer, and 1 was a salt waster. Another example we found of nonconcordance of genotype to phenotype is illustrated in patients with Exon1 (P30L)/Intron2 (A or C to G) mutations, as some have the salt wasting form, while the others have the non-classical form. This unexplained phenotypic variability within each mutation group has important implications for prenatal diagnosis and treatment.

4. Prenatal diagnosis and treatment

4.1. Breakthrough in prenatal diagnosis and treatment in CAH

When it was discovered that CAH-affected fetuses exhibit elevated 17-OHP and Δ^4-androstenedione in their amniotic fluid, measuring their levels by amniocentesis and hormonal assay became the first method of prenatal diagnosis for this disorder. However, to prevent prenatal virilization of an affected female, treatment must begin before 10 weeks gestation, and amniocentesis is performed in the second trimester. Because dexamethasone treatment for the developing fetus would suppress 17-OHP in amniotic fluid, this hormonal test could not be relied upon for diagnosis. When HLA was found to be linked to CAH, diagnoses were made by using HLA genetic linkage marker analysis. This method resulted in many diagnostic errors due to recombination or haplotype sharing; the method generally used at the present time is direct DNA analysis of the 21-OH gene (CYP21) with molecular genetic techniques.

4.2. Establishment of algorithm

Prenatal treatment of 21-hydroxylase deficiency with dexamethasone has now been utilized for over a decade. An algorithm has been developed for the prenatal diagnosis of 21-OHD congenital adrenal hyperplasia using direct molecular analysis of the 21-OH locus and dexamethasone treatment (Fig. 5). When properly administered, dexamethasone is effective in preventing ambiguous genitalia in the affected female, and it has been shown to be safe for both the mother and the fetus [20]. The largest human studies published so far have shown no congenital abnormalities and that the birth weight, birth length and head circumference were not different in offspring of dexamethasone-treated

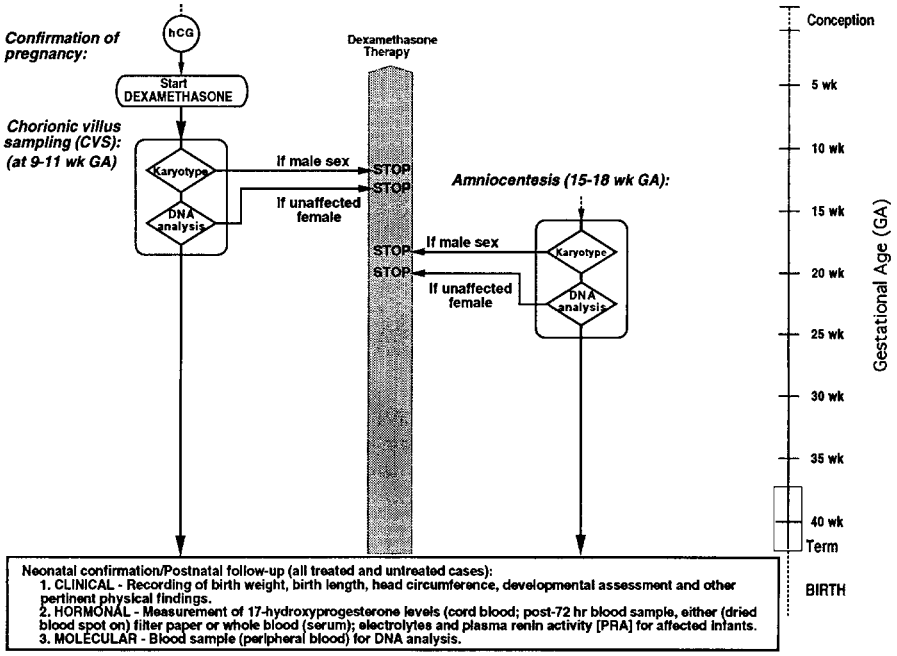

Fig. 5. Algorithm depicting prenatal management of pregnancy in families at risk for a fetus affected with 21-OHD (From Mercado et al. [20].

pregnancies from those not treated [20–22], provided patients and physicians adhered to the recommended therapeutic protocol.

Dexamethasone (20 μg/kg/day in 3 divided doses) is administered to the pregnant mother before 10 weeks gestation, blind to the affected status of the fetus, to suppress excess adrenal androgen secretion and prevent virilization should the fetus be an affected female (Fig. 5). Diagnosis by DNA analysis requires chorionic villus sampling in the eighth to tenth week gestation, or sampling of amniotic fluid cells obtained by amniocentesis in the second trimester. The fetal DNA is used for specific amplification of the CYP21 gene utilizing polymerase chain reaction (PCR) [23]. If the fetus is determined to be an unaffected female upon DNA analysis or a male upon karyotype analysis, treatment is discontinued. Otherwise, treatment is continued to term.

4.3. Update on The New York Hospital–Cornell Medical Center experience

Since 1986, prenatal examination for congenital adrenal hyperplasia due to 21-OHD has been carried out in 403 pregnancies at The New York Hospital–Cornell Medical Center. In 280, diagnoses were made by amniocentesis, while 123 were diagnosed using chorionic villus sampling (Fig. 6). The rapid allele-specific polymerase chain reaction was used for DNA analysis in some cases [23]. Of those 403 pregnancies evaluated, 84 fetuses were found to be affected with classical 21-OHD (Fig. 7). Of those, 52 were female, 36 of whom were treated prenatally with dexamethasone. Dexamethasone administered at or before 10 weeks of gestation (23 affected female fetuses) was effective in reducing virilization. Thirteen cases had affected female sibs (Prader stages 1–4); 6 of these 13 fetuses were born with entirely normal female genitalia, while 6 were significantly less virilized (Prader stages 1–2) than their sibs, and one was Prader stage 3 (Fig. 8). Among the rest, whose index cases were either cousins or male sibs, 4 were born with normal genitalia, 3 were Prader stages 1–2, and 3 were born Prader stages 3–4. The newborns who were Prader 3 and 4 were either of mothers who were extremely obese—and since we had a limit on the amount of dexamethasone we would prenatally treat with, those mothers were undertreated—or they were noncompliant and stopped treatment. Overall for affected females, the average Prader score for those treated prenatally was 1.7 (including the partially treated). In contrast, the average score for the untreated affected females was 3.9.

No significant or enduring side effects were noted in those fetuses who were prenatally treated. Fetal wastage was approximately the same for dexamethasone-treated than for untreated (1 female at 35 weeks and 1 male at 19 weeks prenatally treated; and 2 females—13 and 36 weeks—and 1 male at 24 weeks untreated) (Fig. 9). In addition, as reported in the previous studies, prenatally treated newborns did not significantly differ in birth weight from untreated newborns (Fig. 10). Mean birth weight for dexamethasone prenatally treated fetuses was 3.4 kg, while for

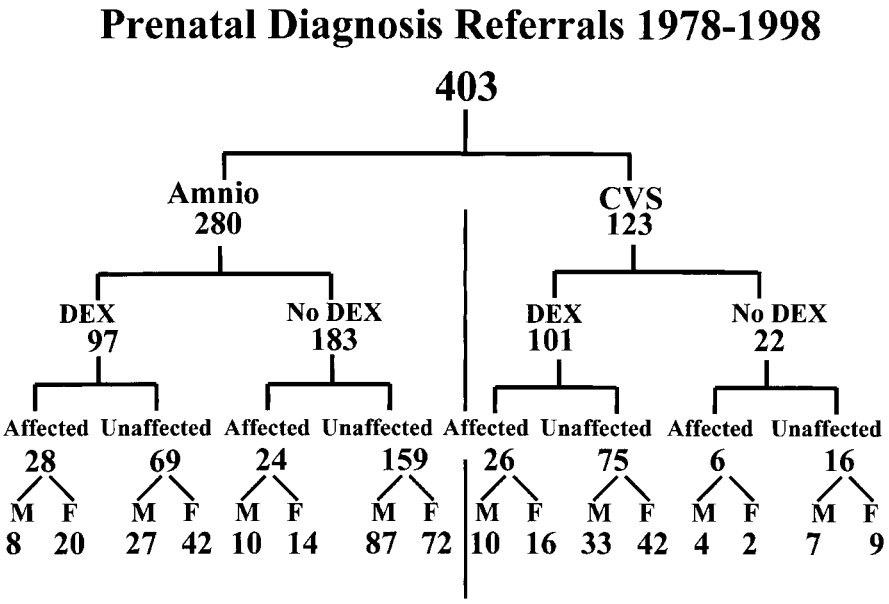

Fig. 6. Diagram depicting experience of prenatal diagnosis and dexamethasone treatment for 21-hydroxylase deficiency congenital adrenal hyperplasia at The New York Hospital–Cornell Medical Center. Dex = dexamethasone; Amnio = amniocentesis; CVS = chorionic villus sampling; F = female; M = male.

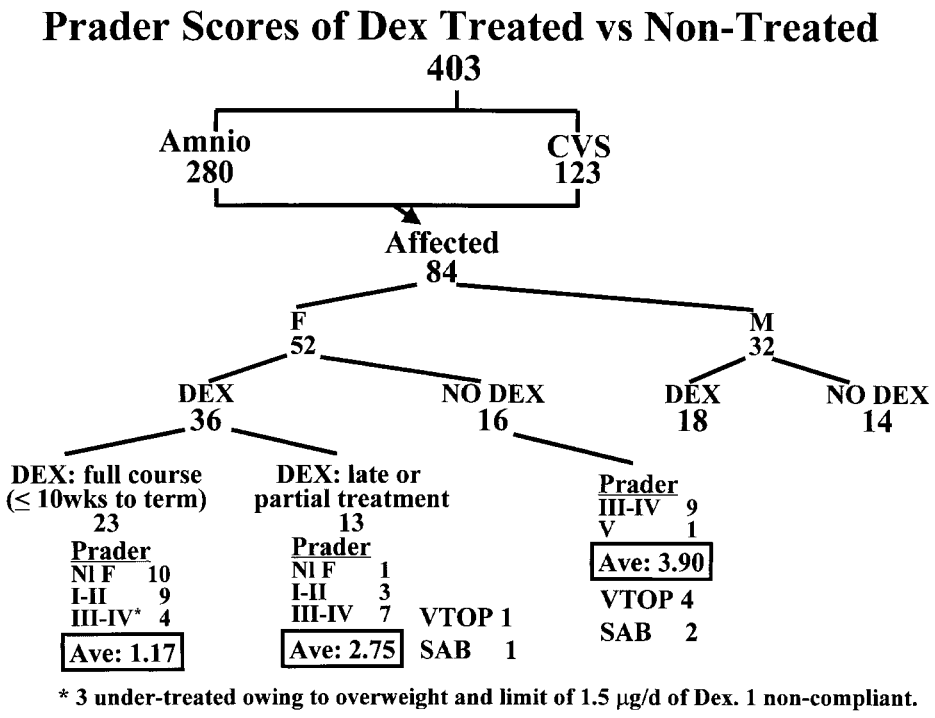

Fig. 7. Diagram depicting prenatal dexamethasone treatment outcome by Prader scores in fully and partially treated affected newborns. Dex = dexamethasone; Amnio = amniocentesis; CVS = chorionic villus sampling; F = female; M = male; Nl = normal; VTOP = voluntary termination of pregnancy; SAB = spontaneous abortion.

untreated it was 3.5 kg ($P = 0.26$) (mean for treated affected fetuses was 3.3 kg and for unaffected 3.4 kg; untreated affected had a mean of 3.6 kg and unaffected of 3.5). When we surveyed 101 mothers whose children had been prenatally diagnosed through our laboratory, they were asked the basic questions of (1) how was their child, and (2) how was their child doing in school? Of those who responded, the mothers said that 101 of 103 children were doing well, were among the top of their class, were very bright and were happy, and we did not find a difference between dexamethasone treated and untreated (Fig. 11). The exceptions

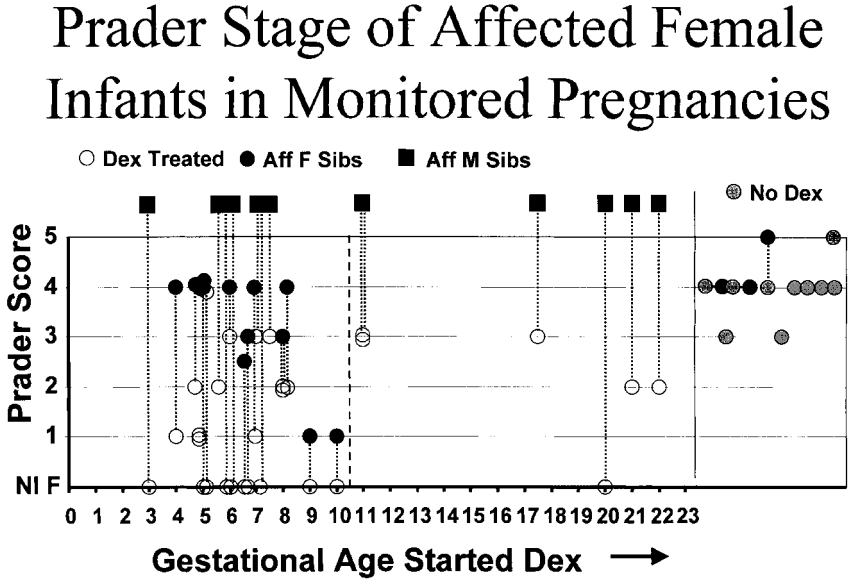

Fig. 8. Diagram depicting Prader stages of affected female infants in monitored, dexamethasone prenatally treated pregnancies, in relation to gestational age when dexamethasone was started. Affected untreated sibs are shown attached by a dotted line. The shaded circles in the right-hand column indicate the affected untreated female referrals.

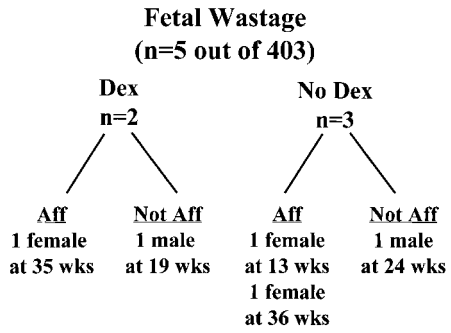

Fig. 9. Fetal wastage in dexamethasone prenatally treated CAH-affected and non-affected fetuses vs untreated. Dex = dexamethasone; Aff = affected.

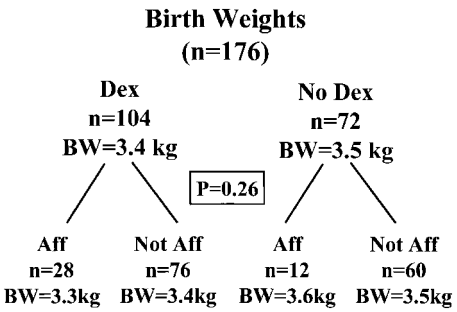

Fig. 10. Birth weights in dexamethasone prenatally treated CAH-affected and non-affected fetuses vs untreated. Dex = dexamethasone; Aff = affected.

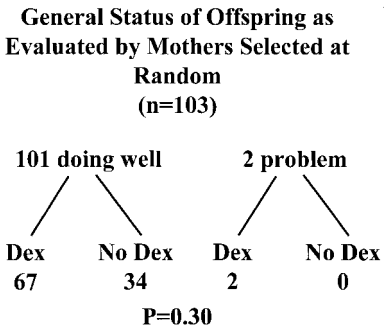

Fig. 11. In a random survey regarding 103 children with CAH, the general status of the offspring was evaluated by their mothers. They were asked how the children were doing overall, and how they were doing in school. Dex = prenatal dexamethasone treatment.

were that two dexamethasone-treated children reportedly had learning problems, one with dyslexia and one with attention deficit disorder, but this was not significant by the chi-square test. Quantitative follow-up studies are currently in progress regarding cognition, gender, temperament, and handedness (an indicator of prenatal androgen effect) in children and adults who were prenatally treated with dexamethasone.

We also did not find significant differences in side effects in the mothers who were treated with dexamethasone from the mothers who were not, which had been a concern of some investigators, except in weight gain. By report, mothers who were not treated with dexamethasone gained an average of 28.6 lb, while treated mothers gained an average of 36.8 lb, which was statistically significant ($P < 0.005$) (Fig. 12). There were no statistically significant differences found for the presence of striae ($P = 0.14$) (Fig. 13), edema ($P = 0.56$) (Fig. 14), hypertension ($P = 0.60$) (Fig. 15), or gestational diabetes ($P = 0.42$) (Fig. 16) by report, either. Furthermore, in our random survey of the mothers, 14 of them had had CAH-affected girls who were prenatally treated with dexamethasone, and of those, all 14 were satisfied with the treatment outcome. Of the 35 mothers we asked whether they would take dexamethasone again if they got pregnant, 33 said yes, 2 said they would not, and 1 said that she would abort.

4.4. Mendelian ratios and genetic frequency

With regard to the Mendelian ratio of our patients, we found 31% were homozygous affected, 48% were

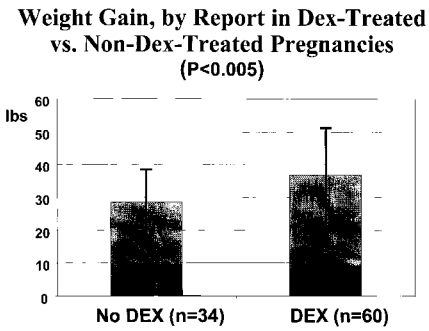

Fig. 12. Weight gain reported by mothers who underwent prenatal dexamethasone treatment for their child at risk for CAH vs non-treated at-risk pregnancies. Dex = dexamethasone.

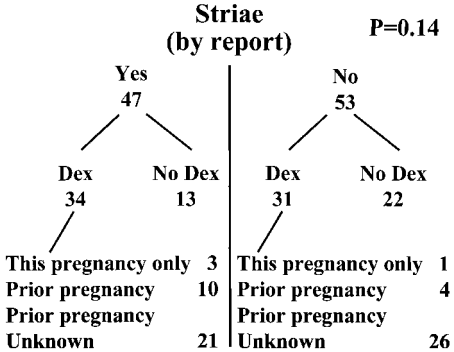

Fig. 13. Striae reported by mothers who underwent prenatal dexamethasone treatment for their child at risk for CAH vs non-treated at-risk pregnancies. Dex = dexamethasone.

heterozygous, and 21% were homozygous normal (Fig. 17). This does not differ from the expected Mendelian ratio of 1:2:1. However, it is interesting to note that of the 51 affected babies, 66% were female: this is a much greater proportion of females and a significant difference from the 1:1 expected ratio (Fig. 17). A possible explanation for this is that genital ambiguity, which only occurs in females, is more likely to result in a referral for further investigation at our Center.

The frequency of mutations is the same as we described in our last report in 1995 [17]. The Intron2 mutation remains the most common, and deletions and variations among Intron2 and Exon3 are the second most common (Fig. 18). Because of this variability, there is no shared mutation that distinctly identifies a CAH diagnosis.

Based on our experience, proper prenatal diagnosis and treatment of 21-OHD is safe and is effective in significantly reducing or eliminating virilization in the affected female. This spares the newborn female the consequences of genital ambiguity of genital surgery, sex misassignment, and gender confusion. Of the monogenic disorders, steroid 21-OHD is one of the few in which prenatal treatment is effective and influences postnatal life.

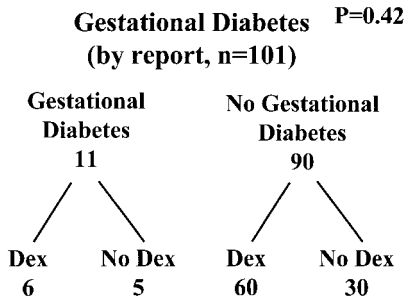

Fig. 16. Gestational diabetes reported by mothers who underwent prenatal dexamethasone treatment for their child at risk for CAH vs non-treated at-risk pregnancies. Dex = dexamethasone.

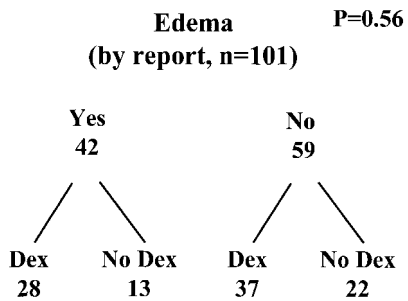

Fig. 14. Edema reported by mothers who underwent prenatal dexamethasone treatment for their child at risk for CAH vs non-treated

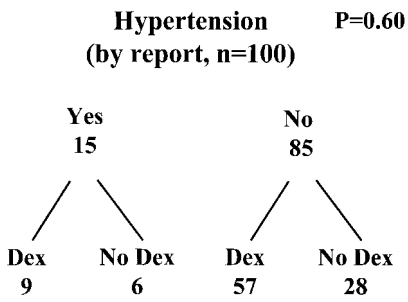

Fig. 15. Hypertension reported by mothers who underwent prenatal dexamethasone treatment for their child at risk for CAH vs non-treated at-risk pregnancies. Dex = dexamethasone.

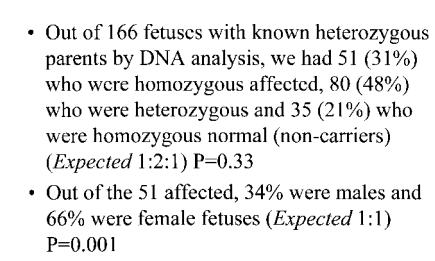

Fig. 17. Concordance of our mutational findings with Mendelian inheritance expectations; and nonconcordance in terms of male to female ratio, which may be explained by the lack of genital ambiguity in male fetuses, resulting in less referrals.

MUTATION GROUPS IN PRENATAL REFERRALS (N=72)

Mutation Grp	N	Mutation Grp	N
Int2/Int2	10	Ex1 Int2 Ex3/ND*	1
Del/Ex1 Int2 Ex3	7	Del/Ex7v Ex7T	
Del/Int2	6	Ex8318 Ex8356	1
Ex1 Int2 Ex3/Int2	5	Ex1 Int2 Ex3/Ex7v	1
Ex4/Int2	4	Ex1 Int2 Ex3/Ex8318	1
Del/Ex7v	4	Del/Del	1
Del/Ex4	4	Ex3/Ex3	1
Del/Ex8318	3	Ex3/Ex6	1
Ex6/Ex6	2	Ex3/Int2	1
Ex8318/Int2	2	Ex4/ND*	1
Ex1 Int2 Ex3/ Ex1 Int2 Ex3	2	Ex4/Ex8318	1
Ex8356/Int2 Ex7v	2	Ex4/Ex8356	1
Int2/ND*	2	Ex6/Int2	1
Del/Int2 Ex3	2	Ex7v/Ex8356	1
Del/Ex3	1	Ex8318/ND*	1
Del/Ex8356	1	Ex1 Int2 Ex3/Ex8356	1

*ND= No mutation detected.

Fig. 18. Mutation groups identified in the CYP21 locus in the prenatal 21-hydroxylase deficiency CAH referrals. Del = gene deletion; E × 1 = Exon 1 (P30L); Ex3 = Exon 3 (8 base pair deletion); Ex4 = Exon 4 (172N), Ex6 = Exon 6 (cluster: I236N, V237Q, M239 K); Ex7v = Exon 7 (V281L); Ex7 T = Exon 7 (T306 insertion); Ex8318 = Exon 8 (Q318X); Ex8356 = Exon 8 (R356W); Int2 = Intron 2 (A or C to G).

Acknowledgements

Significant sections of the work on which the data reported herein were supported by USPHS Grant HD00072 and GCRC Grant No. 06020.

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