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EXTENSIVE CLINICAL EXPERIENCE

Nonclassical 21-Hydroxylase Deficiency

Maria I. New

Department of Pediatrics, Mount Sinai School of Medicine, New York, New York 10029

Context: Nonclassical congenital adrenal hyperplasia (CAH) owing to steroid 21-hydroxylase deficiency (NC21OHD) is the most frequent of all autosomal recessive genetic diseases, occurring in one in 100 persons in the heterogeneous New York City population. NC21OHD occurs with increased frequency in certain ethnic groups, such as Ashkenazi Jews, in whom one in 27 express the disease. NC21OHD is underdiagnosed in both male and female patients with hyperandrogenic symptoms because hormonal abnormalities in NC21OHD are only mild to moderate, not severe as in the classical form of CAH. Unlike classical CAH, NC21OHD is not associated with ambiguous genitalia of the newborn female.

Main Outcome Measures: The hyperandrogenic symptoms include advanced bone age, early pubic hair, precocious puberty, tall stature, and early arrest of growth in children; infertility, cystic acne, and short stature in both adult males and females; hirsutism, frontal balding, polycystic ovaries, and irregular menstrual periods in females; and testicular adrenal rest tissue in males.

Conclusions: The signs and symptoms of hyperandrogenism are reversed with dexamethasone treatment. **(*J Clin Endocrinol Metab* 91: 4205–4214, 2006)**

IN 1979 A MILD FORM of adrenal steroid 21-hydroxylase deficiency (21OHD) called nonclassical steroid 21-hydroxylase deficiency (NC21OHD) was reported (1). Although a varying clinical severity of congenital adrenal hyperplasia (CAH) had always been observed, this report defined the mild form of 21OHD CAH using specific hormonal and genetic criteria. In 1986 the gene for 21OHD was published (2), and the molecular genetic mutations specific for the nonclassical form of 21OHD were published (3–5). Over the past 20 yr, our team in New York City has followed more than 400 patients, aged 8 months to 69 yr, who presented with NC21OHD. The genotypes for probands and family members of more than 400 affected individuals have been obtained. This is by far the largest group studied with molecular genetic techniques. In this paper, the diagnosis and treatment of NC21OHD will be reviewed, and the high prevalence of this disorder will be discussed. A widely underdiagnosed disorder in children and adults, NC21OHD is a cause of a variety of hyperandrogenic symptoms that are readily treatable in both males and females.

Classical and Nonclassical 21-Hydroxylase Deficiency

CAH refers to a family of inherited disorders in which defects occur in one of five enzymatic steps required to synthesize cortisol from cholesterol in the adrenal gland. Because of the impaired cortisol secretion, ACTH levels rise via a negative feedback system and stimulate adrenal hormone secretion, resulting in hyperplasia of the adrenal cortex. In 21OHD, responsible for 90–95% of CAH cases, there is an accumulation of the precursors immediately proximal to the 21-hydroxylation step in the pathway of cortisol synthesis, and these precursors are shunted into the androgen pathway (Fig. 1).

Three forms of 21OHD CAH can be distinguished by clinical, hormonal, and molecular genetic criteria: the classical salt-wasting, classical simple-virilizing, and nonclassical forms (6). In the severe classical salt-wasting form, elevated prenatal androgens cause ambiguous genitalia in the affected female fetus, and aldosterone synthesis is impaired. In classical simple-virilizing CAH, aldosterone synthesis is not affected, but affected females present with genital ambiguity. Nonclassical patients secrete aldosterone normally, and NC21OHD-affected females do not have genital ambiguity.

The nonclassical form of 21OHD was discovered during studies of the family members of classically affected patients; some completely asymptomatic family members were discovered to have human lymphocyte antigen (HLA) genetic linkage markers, indicating the presence of mutations at the 21-hydroxylase locus. On hormonal evaluation, diminished 21OH activity was confirmed. All of these individuals later became symptomatic (7). Subsequently, the molecular genetic basis of NC21OHD was described (5) (Table 1).

Clinical Description of NC21OHD

Although NC21OHD females are not born with ambiguous genitalia, a partial deficiency of 21-hydroxylation causes postnatal androgen excess in patients of both sexes, leading to various hyperandrogenic signs that manifest from childhood to adulthood, as summarized in Fig. 2. All individuals diagnosed with NC21OHD based on molecular genetic testing develop one or more signs of hyperandrogenism over time, including those who were initially asymptomatic (7).

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Abbreviations: C4, Fourth component; CAH, congenital adrenal hyperplasia; HLA, human lymphocyte antigen; NC21OHD, nonclassical steroid 21OHD; 21OHD, 21-hydroxylase deficiency; 17-OHP, 17-hydroxyprogesterone; PCOS, polycystic ovary syndrome.

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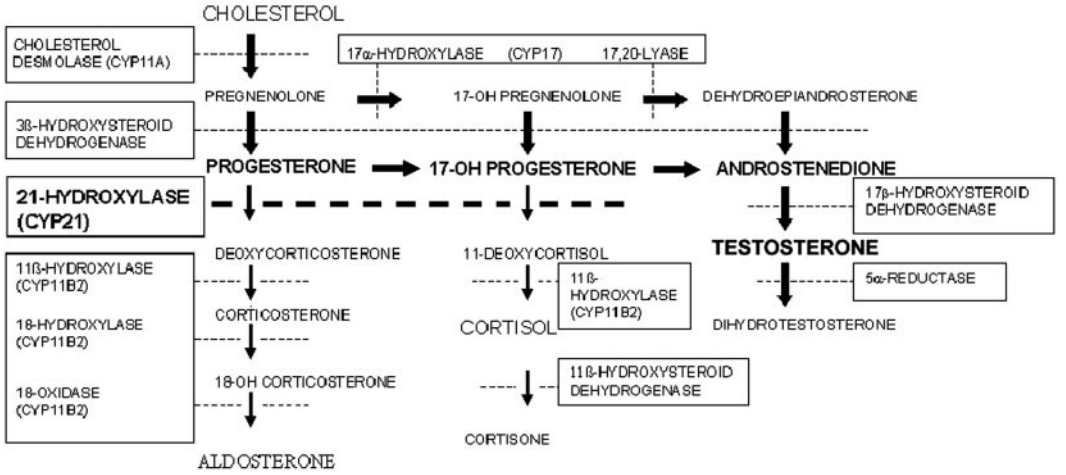

FIG. 1. Scheme of adrenal steroid synthesis.

Patients with NC21OHD have extremely variable presentations with one or more hyperandrogenic signs. There have been few large-scale studies of the disorder, partly because it was only recently described but also because there are few clinics with large patient populations, ours in New York City being the largest. The papers published to date that include patients diagnosed by DNA report on small groups of patients (3–5, 8–13).

In children, the presenting signs of NC21OHD include premature pubarche (14–17), cystic acne (Fig. 3) (18, 19), accelerated growth (20), and advanced bone age (21–24). In males and females, premature development of pubic hair may occur as early as 6 months of age (25). In one study, NC21OHD was found in 30% of children with premature pubarche (14), whereas a lower prevalence was reported by another group (26). Of 48 patients with premature pubarche screened in another study, four were found to have NC21OHD (17). Severe cystic acne refractory to oral antibiotics and retinoic acid has been associated with NC21OHD (27). Although patients with NC21OHD demonstrate excessive somatic growth and tall stature in early childhood (20), this growth is arrested because of early epiphyseal fusion, which compromises final height (21, 28, 29). Thus, patients are tall children but short adults. In our cohort, 31 of 45 (69%) children with a genetic and hormonal diagnosis of NC21OHD and bone age recorded at or before age 12 had a bone age advancement of 2 yr or more. Treatment for this problem with human GH and LHRH analog has recently been reported by Lin-Su *et al.* (56). In this report, the children with NC21OHD treated for advanced bone age with human GH and LHRH analog achieved their target height when treatment began before the bone age was 12 yr.

Little has been published about males with NC21OHD. Typically, males with NC21OHD do not suffer from impaired gonadal function and tend to have normal sperm counts (23, 30). However, manifestations of adrenal androgen excess may include short stature or oligospermia and diminished fertility (23, 31, 32). Importantly, reversal of infertility and oligospermia upon treatment with glucocorticoids has been observed (23, 33–35).

Women with NC21OHD can suffer from gonadal dysfunction and menstrual disorders including amenorrhea, anovulation, oligomenorrhea (7, 25, 36), and infertility (10, 37). Previous studies suggest that these irregularities may be due to the conversion of excess adrenal androgens to estrogens, which then disrupt gonadotropin secretion (38, 39). In

TABLE 1. Common gene mutations of the 21-hydroxylase gene *CYP21A2* (75)

Exon/intron	Mutation type	Mutation	Phenotype	Severity of enzyme defect (% enzyme activity)	Ref.
Nonclassical mutations					
Exon 1	Missense mutation	P30L	NC	Mild (30–60%)	3
Exon 7	Missense mutation	V281L	NC	Mild (20–50%)	5
Exon 8[a]	Missense mutation	R339H[a]	NC	Mild (20–50%)	4
Exon 10[a]	Missense mutation	P453S[a]	NC	Mild (20–50%)	4
Classical mutations					
Deletion	30-kb deletion		SW	Severe (0%)	66
Intron 2	Aberrant splicing of intron 2	656 A/C-G	SW, SV	Severe (ND)	76
Exon 3	Eight-base deletion	G110 Δ8nt	SW	Severe (0%)	93
Exon 4	Missense mutation	I172N	SV	Severe (1%)	79, 80
Exon 6	Cluster	I236N, V237E, M239K	SW	Severe (0%)	79, 80
Exon 8	Nonsense mutation	Q318X	SW	Severe (0%)	87
Exon 8	Missense mutation	R356W	SW, SV	Severe (0%)	81
Exon 10[a]	Missense mutation	R483P[a]	SW	Severe (1–2%)	42

SW, Salt wasting; SV, simple virilizing; NC, nonclassical; ND, not determined.
[a] Not routinely assayed.

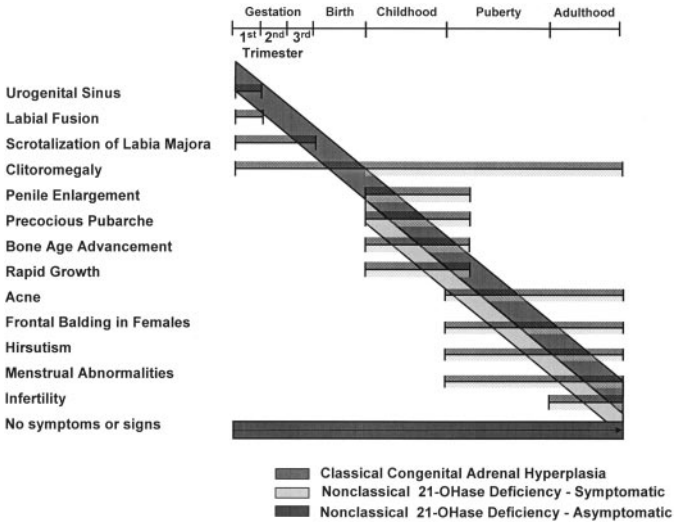

FIG. 2. Clinical spectrum of classical and nonclassical 21OHD.

one multicenter study by Speiser *et al.* (40), 26 women carrying one severe and one mild *CYP21A2* mutation were said to be infertile. However, some of the women did not attempt to conceive and some may have conceived after treatment. In a recent study of 18 Croatian patients (10 males, eight females) with NC21OHD, all four women of reproductive age were fertile and all had conceived before treatment (41). Molecular genetic analysis of these women did not show correlation of fertility with genotype, although 50% were homozygous for the mild exon 7 mutation. Another study by Rumsby *et al.* (10) found that of six genetically characterized NC21OHD women, two had primary infertility, and four eventually succeeded in giving birth after experiences of miscarriage and other complications. In this small sample of six women, individuals with the exon 7 mutation in conjunction with a severe mutation presented with oligomenorrhea, whereas those homozygous for the exon 7 mutation had regular cycles and one was fertile. Thus, data from studies of small groups of patients with NC21OHD have been variable.

Some women with NC21OHD present with polycystic

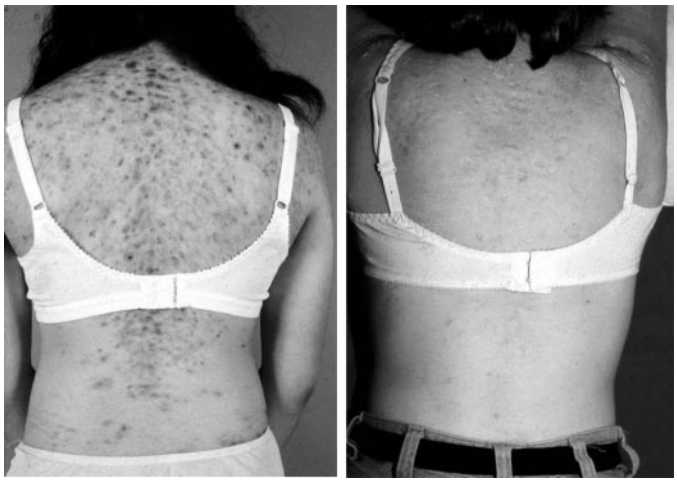

FIG. 3. Before and after treatment with dexamethasone (0.25 mg at the hour of sleep) for 3 months.

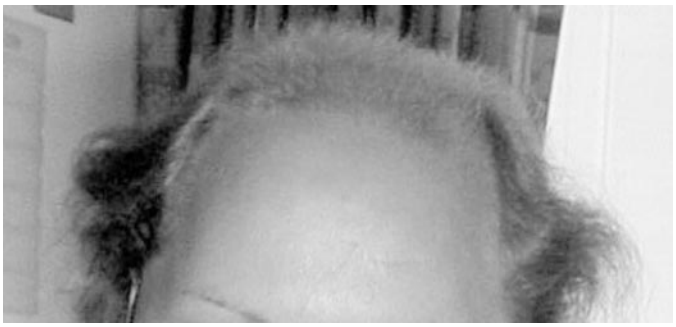

FIG. 4. Male-pattern baldness typical of females affected with NC21OHD.

ovary syndrome (PCOS) (43–45). In these cases, adrenal sex steroid excess disrupts the cyclicity of gonadotropin release or directly affects the ovary, leading to the formation of ovarian cysts. These cysts may then autonomously produce androgens to compromise fertility. A 2003 consensus statement defined the diagnostic criteria of PCOS as the presence of two of the following three findings: 1) polycystic ovaries by sonography, 2) clinical or biochemical evidence of androgen excess, and 3) chronic menstrual abnormalities or anovulation, in addition to exclusion of other known disorders (46). NC21OHD also presents with hyperandrogenemia and anovulation, and thus should be ruled out in patients with PCOS. The frequency of NC21OHD in PCOS has not been determined in a large series. A study by Stikkelbroeck *et al.* (47) of 13 patients with CAH found that polycystic ovaries were no more frequent in female CAH patients than the general population; however, all patients had been treated since the diagnosis of CAH was made (11 of 13 had DNA confirmation of the diagnosis). The treatment may have resulted in a reversal of signs of PCOS. On the other hand, several studies have shown that CAH patients may exhibit ovarian adrenal rest tumors (48, 49) which, on ultra-

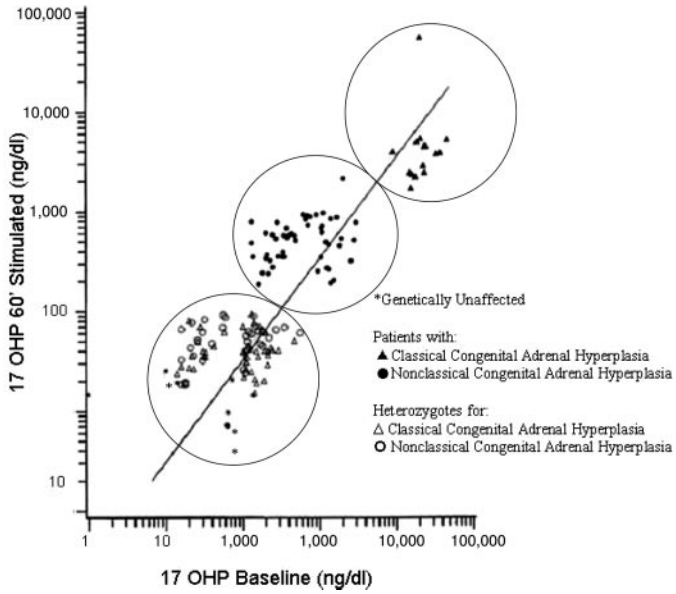

FIG. 5. Nomogram relating baseline to ACTH-stimulated serum concentrations of 17-OHP. The scales are logarithmic. A regression line for all data points is shown.

TABLE 2. Hormonal measurements of NC21OHD patients in neonates (unpublished data)

	Normal values (ng/dl)	0-d-old female	0-d-old male	Female treated until term
17-OHP	Males and females: <420	458	2094	212
Δ^4-Androstenedione	Males and females: <290	368	125	20
Testosterone	Males: <187		56	
	Females: <24	60		Blank
Mutations in 21-OH gene		V281L/V281L	V281L/R356W	Unknown

sound, may be difficult to differentiate from cystic ovarian tissue (50). A recent report from Montréal, Canada, by Dr. Geoffrey Hendy indicated that 10% of PCOS patients were proven to have NC21OHD (Hendy, G., personal communication).

It has been recognized that infertility of undetermined cause in women may be reversed with glucocorticoid therapy. In one report, five females with irregular menses and high 17-keto(oxo)steroids resumed regular menses and demonstrated adequate suppression of 17-ketosteroids and pregnanetriol within 2 months of beginning glucocorticoid therapy (51). This suggests the presence of an adrenal 21-hydroxylating defect, although no molecular genetic diagnosis was carried out (51). In another report of 18 infertile women with acne and/or facial hirsutism and hormonal criteria consistent with 21OHD (without molecular genetic confirmation of the disease), seven conceived shortly after initiating prednisone treatment alone; an additional four women conceived within 2 months of the addition of clomiphene to the therapeutic regimen (52). Hormonal profiles after initiation of therapy were not reported in this study.

Females with NC21OHD may present with hirsutism. In one study of 20 females with NC21OHD, 39% had isolated hirsutism (53). These studies did not include genetic analysis because they were published before the technique was available for diagnosis. Additionally, male-pattern baldness, classified by Ludwig scores (54), can be the sole presenting symptom of NC21OHD (Fig. 4).

In our large cohort of patients with NC21OHD, there were 28 women with the diagnosis of NC21OHD confirmed by genetic and hormonal analysis whose hirsutism was evaluated by Ferriman-Gallwey scores (55). Of the 28 NC21OHD women, 20 (71%) had scores above 8, and 15 (54%) had scores above 15. Ferriman-Gallwey scores of 8 or higher indicated significant hirsutism.

Hormonal Features

The most definitive hormonal diagnostic test for 21OHD is an ACTH (Cortrosyn 0.25 mg) stimulation test, which measures the serum concentrations of 17-hydroxyprogesterone (17-OHP) at 0 and 60 min after ACTH administration. Test values may be plotted in a nomogram (Fig. 5), which reflects the different degrees of hormonal compromise in patients with classical and nonclassical 21OHD, and heterozygous carriers of a *CYP21A2* mutation (57). As indicated by the nomogram, patients with NC21OHD typically aggregate midway down the regression line with 60-min stimulated 17-OHP values between 1,000 and 10,000 ng/dl.

In rare instances, nonclassical patients have had hormonal measurements in the neonatal period and early infancy (Table 2). Although we have very few hormone analyses in the neonate, it appears that hormone values are variable. In the two infants without prenatal treatment we observed, 17-OHP is elevated in both the male and female, whereas testosterone and Δ^4-androstenedione were elevated only in the female. However, additional studies are required. In the fetus treated until term, the values were low, although the newborn had not shown signs of adrenal insufficiency.

Psychobiology of NC21OHD

NC21OHD appears to affect selected psychological domains, presumably through the effects of elevated androgen levels in the brain and/or by way of psychological reactions to clinical androgen-dependent symptoms. In a recent study, Meyer-Bahlburg *et al.* (58) showed slightly but significantly increased masculinization/defeminization on several measures of gender-related behavior in NC21OHD women when compared with normal control women, although markedly less so than in women with classical CAH. Similarly, a recent abstract from the same study indicated that the NC21OHD women had significantly increased bisexual or homosexual orientation (59). However, the sexual functioning of N21OHD women did not differ from that of normal control women, unlike women with classical CAH who as a group showed impaired sexual functioning (60). Retrospective clinical-qualitative interviews with these women revealed a history of discomfort and social stress related to their pretreat-

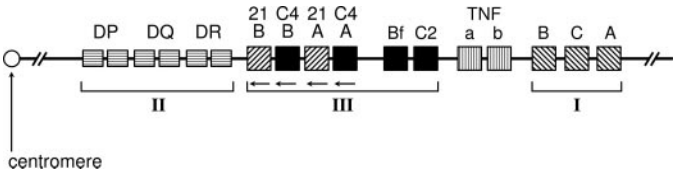

FIG. 6. HLA region of chromosome 6p. 21B is the active gene, whereas 21A is the pseudogene. I, II, III, Major Histocompatibility Complex I, II, and III; DP, DP-antigen gene; DR, DR-antigen gene; DQ, DQ-antigen gene; C4, C4 complement genes; Bf, complement factor B gene; C2, C2 complement genes; B, B-antigen gene; C, C-antigen gene; A, A-antigen gene.

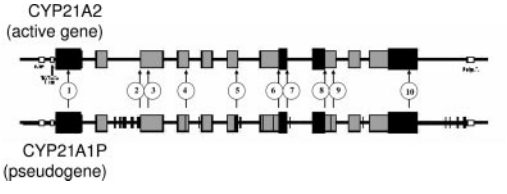

FIG. 7. The two homologs: CYP21A2 (the active gene) and CYP21A1P (the pseudogene). Noncorrespondent bases number less than 90 over a distance of 5.1 kb of DNA. Numbered are the pseudogene base changes that are frequently identified on mutant CYP21A2 genes responsible for 21OHD. Mutations associated with NC21OHD are indicated with *black squares* (exons 1, 7, 8, and 10).

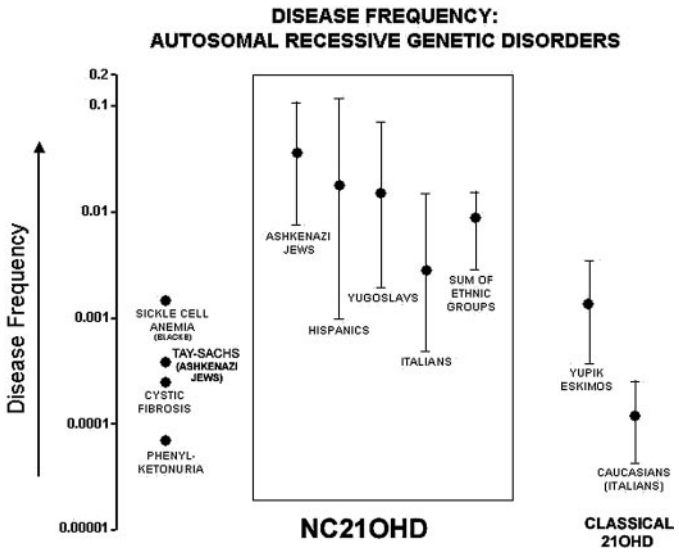

FIG. 8. Disease frequencies in different ethnic groups.

ment experiences with androgen-dependent signs such as acne, hirsutism, and conception difficulties. Unlike classical patients, NC21OHD patients are not treated from birth and often lack diagnosis and hormonal control for many years.

Molecular Genetics

Hormonally and clinically defined nonclassical and classical forms of CAH are associated with distinct genotypes (Table 1). These genotypes are characterized by varying levels of enzyme activity, as defined by *in vitro* expression studies (61–63).

The gene encoding 21-hydroxylase is a microsomal cytochrome P450 termed *CYP21A2* (64) (Fig. 6) (previously P450c21) mapped to the short arm of chromosome 6 within the HLA complex (65).

Understanding of the *CYP21A2* gene has been advanced, including its characterization in terms of location, duplication in tandem with the fourth component (C4) isotypes, structure, and the sequencing of the gene and its pseudogene and their arrangement on the chromosome (66–70). The *CYP21A2* gene and its homolog, the pseudogene *CYP21A1P*, alternate with two genes called C4B and C4A (70, 71) that encode the two isoforms of C4 of serum complement (Fig. 6) (72).

The *CYP21A2* and *CYP21A1P* genes, which each contain 10 exons, share 98% sequence homology in exons and approximately 96% sequence homology in introns (Fig. 7). In a large percentage of the patients with NC21OHD, the exon 7 (V281L) mutation has been associated with a duplication of the pseudogene and the C4B gene (73, 74). This is especially common in patients with the HLA haplotype B14DR1.

The deletional mutations of the 21OHD genes and characterized specific point mutations of the *CYP21A2* gene have been reported (75). The most common mutations appear to be the result of one of two types of meiotic recombination between *CYP21A2* and *CYP21A1P*: 1) misalignment and unequal crossing over, resulting in large-scale DNA deletions, and 2) apparent gene conversion events that result in the transfer of smaller-scale deleterious mutations present in the *CYP21A1P* pseudogene to the *CYP21A2* gene. To date, approximately 103 mutations in the *CYP21A2* gene have been reported (75) (see Table 1 for the most common mutations).

The genotype for the classical form of 21OHD is predicted to be a severe mutation on both alleles at the 21-hydroxylase locus, with markedly reduced 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 classical 21OHD, causes premature splicing of the intron and a shift in the translational reading frame (63, 76). Most patients who are homozygous for this mutation have low or absent serum aldosterone levels and the severe salt-wasting form of the disorder (77, 78). One mutation in exon 4 (I172N), specifically associated with simple-virilizing 21OHD (79), has been shown by *in vitro* expression studies to result in 1% of normal enzyme activity (79). Adrenal production of aldosterone is normally in the range of 1:100 to 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 21OHD.

In contrast to the classical form, patients with NC21OHD are predicted to have mild mutations on both alleles or one severe and one mild mutation of *CYP21A2* (compound heterozygote). Missense mutations in exon 7 (V281L) and exon 1 (P30L), which are predominantly associated with nonclassical disease, reduce enzymatic activity in cultured cells to 20–50% of normal (80). Nonclassical patients have normal aldosterone secretion and therefore do not experience salt wasting. A recent report found that missense mutations in exon 8 (R339H) and exon 10 (P453S) are associated with the nonclassical phenotype as well (84) (Table 1).

21OHD Genotype-Phenotype Correlations

In 98% of 21OHD patients, genotype corresponds with hormonal phenotype; however, rare cases of nonconcordance have important implications in prenatal diagnosis of 21OHD and genetic counseling (78). In our large cohort of

TABLE 3. Disease frequencies in various ethnic groups

Ethnic group	n	Disease frequency	Carrier frequency
Ashkenazi Jews	56	1:27	1:3
Hispanics	9	1:40	1:4
Slavics[a]	8	1:50	1:5
Italians	12	1:300	1:10
Heterogeneous population of New York City	249	1:100	1:6

[a] From Croatia.

TABLE 4. Ethnic-specific genotype frequencies (%) of NC21OHD probands

Ethnic group	n	Exon 7/ Exon 7	Exon 7/ Intron 2	Deletion/ Exon 7	Other
Ashkenazi Jews	140	50	18	18	14
African-Americans	4	25	0	0	75
Hispanics	33	24	27	21	28
Italian-Americans	48	17	19	15	49
Non-Jewish Caucasians	92	17	16	7	60
Total	317	31	18	15	36

TABLE 5. NC21OHD females with prenatal diagnosis and treatment

Patient	Dex	Birth year	Prader score	Dex treatment (wk)	Diagnosis procedure	Paternal mutation	Maternal mutation
1	No	2002	F		Amnio	Exon 7	Exon 7
2	No	2003	F		Amnio	Exon 7	Exon 7
3	No	2005	F		Amnio	Exon 7	Exon 7
4	No	2000	F		Amnio	Exon 7	Exon 7
5	Yes	1991	F	10–40	CVS	HLA	HLA
6	Yes	1997	F	4–41	Amnio	Exon 7	Deletion
7	Yes	1997	F	10–40	Amnio	Exon 1	Exon 7
8	Yes	2005	F	9–40	CVS	Exon 7	Exon 7

Dex, Prenatal dexamethasone treatment; Amnio, amniocentesis; CVS, chorionic villus sampling.

1502 patients with the genetically confirmed diagnosis of CAH, 440 patients have been assigned the clinical diagnosis of NC21OHD. In 438 of the 440 patients with NC21OHD, clinical phenotype correlated with nonclassical genotypes (homozygous mild/mild or heterozygous mild/severe), demonstrating a 99% concordance of hormonal phenotype with genotype. Of the 440 NC21OHD patients, 1% demonstrated genotype-phenotype nonconcordance. Nine individuals had a genotype indicative of classical 21OHD but did not have a classical phenotype: females did not have ambiguous genitalia and fell within the nonclassical range of stimulated 17-OHP values. However, the concordance of genotype with specific clinical signs of hyperandrogenism such as hirsutism, acne, or short stature has yet to be studied.

Frequency of NC21OHD

Through studies of kindreds including a proband with classical or NC21OHD (82) and molecular screening of the 21-hydroxylase alleles of the heterogeneous population of New York City (83), the frequency of NC21OHD has been estimated to be 1:100, making it the most common of all autosomal recessive diseases, even more common than sickle cell anemia, Tay-Sachs, cystic fibrosis, and phenylketonuria (a disease for which newborn screening is mandated in every state in the United States) (Fig. 8). Screening for classical CAH is conducted in 48 states in the United States. The nonclassical form of CAH is not detected by hormonal screening. This would require DNA screening, which is not yet available.

Because the gene for this disorder is autosomal, it occurs equally in men and women. It appears that males are significantly underdiagnosed. Of our 440 nonclassical patients, 344 are females and only 96 are males (unpublished data), indicating that males are infrequently diagnosed.

A higher frequency of NC21OHD was reported in certain ethnic groups (82). The results of this study were supported by a subsequent study of a random population of 100 people (83), which confirmed the highest ethnic-specific frequency to occur in the Ashkenazi Jews at 1:27 (82). Interestingly, whereas the prevalence of NC21OHD in Ashkenazi Jews is high, there is no increased prevalence of the severe classical form of 21OHD in this population (82). Other ethnic groups with high disease frequency included Hispanics (1:40), Slavs (1:50), and Italo-Americans (1:300) (Table 3) (82–86). These data were based on small groups that have recently been studied in larger groups (Wilson, R., S. Nimkarn, M. Dumic, J. Obeid, M. Azar, H. Najmabadi, F. Saffari, M. New, unpublished data).

The disparity in frequency of NC21OHD reported by different authors may be attributed to differences in ethnicity of study populations (6). In a study of 317 probands with NC21OHD, the most common genotype found was exon 7/exon 7 (V281L), which occurs most frequently in the Ashkenazi Jews. Table 4 presents data gathered from probands in common ethnic groups and their respective genotype frequencies (unpublished data).

Dr. Robert Wilson (Mount Sinai School of Medicine, New York, NY) has analyzed the molecular genotypes of more than 1300 patients with classical and NC21OHD over the past 10 yr and has identified more than 10 new mutations in the *CYP21A2* gene in exons 5, 6, 8, 9, and 10. The mutations are in exon 1 (H62L), exon 2 (R91X), exon 5 (H203Y), exon 8 (R316L, R341P, Q351K, and H365Y), and exon 10 (R426H; L442–1 nt; L433P; A457–1 nt; and a 9-bp deletion eliminating codons G424, A425, and R426) (unpublished data). In patients who demonstrate hormonal abnormalities indicating NC21OHD but whose genotype reveals only heterozygosity, we sequence the *CYP21A2* gene to establish a second mu-

TABLE 6. NC21OHD males with prenatal diagnosis and treatment

Patient	Dex	Birth year	Dex treatment (wk)	Diagnosis procedure	Paternal mutation	Maternal mutation
1	No	1986		CVS	Exon 7	Exon 8356
2	No	1999		Amnio	Exon 1	Intron 2
3	No	2000		Amnio	Exon 7	Exon 7
4	No	2005		Amnio	Exon 7	Exon 8318
5	No	2003		Amnio	Exon 7	Exon 7
6	Yes	1994	8–11.7	CVS	Exon 7	Deletion
7	Yes	1995	3–7	CVS	Exon1, intron 2	Exon 7
8	Yes	1995	7–18	Amnio	Exon 7	Deletion
9	Yes	2000	1–40	CVS	Exon 7	Exon 8318
10	Yes	2001	4–13	CVS	Exon 7	Exon 8318

Dex, Prenatal dexamethasone treatment; Amnio, amniocentesis; CVS, chorionic villus sampling.

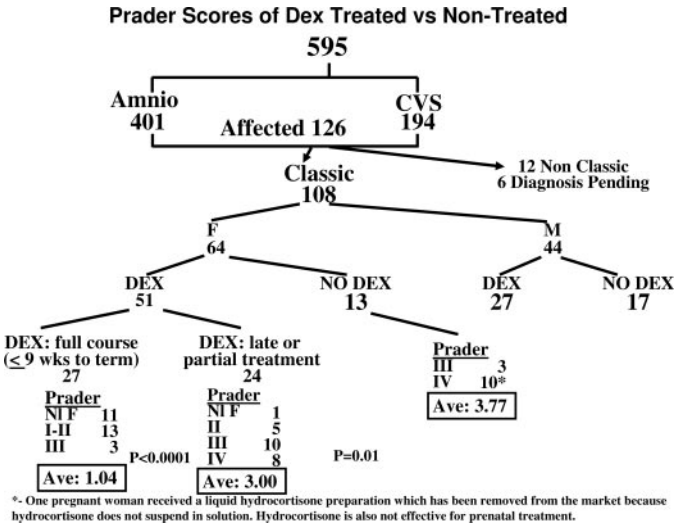

FIG. 9. Prenatal genetic diagnosis and treatment of pregnancies for classical 21OHD. Diagnosis was made by cells from amniocentesis and tissue from chorionic villus sampling. DEX, Dexamethasone; CVS, chorionic villus sampling; Nl F, normal female. [From New *et al*. (92) with permission.]

tation. If a new mutation is identified, *in vitro* expression studies are carried out. Wilson and colleagues (88–90) developed three new techniques for conducting molecular genetic studies. *In vitro* expression studies confirmed the reduced activity of all new mutations excluding the possibility of polymorphism.

Prenatal Diagnosis and Treatment of NC21OHD

We have made the prenatal diagnosis of NC21OHD in both males and females (Tables 5 and 6). The diagnosis was made by molecular genetic techniques, except for one female patient in which the diagnosis was made by HLA genotyping, as the patient was born in 1991 before routine molecular genetic testing was available. The genetic diagnosis was made on cells from amniocentesis and tissue from chorionic villus sampling (Fig. 9). In the newborn females, the genitalia were normal female whether or not dexamethasone treatment was administered to the mother according to the algorithm in Fig. 10. The treatment with dexamethasone was administered to the mother from the fourth to 10th week of gestation until term to four female fetuses. The male fetuses were partially treated from the first to 18th week of gestation. One male fetus was treated until term (Table 6) because the karyotype was initially reported erroneously as 46,XX and the genitalia on sonography showed a large phallic structure. The exon 7, 1, and 10 mutations have been associated with the nonclassical phenotype. The genotypes of the patients included an exon 7 (V281L) mutation in all female fetuses and in nine of the 10 male fetuses. The remaining male fetus had an exon 1 mutation. Two male fetuses were homozygous for the exon 7 mutation, whereas five female fetuses were ho-

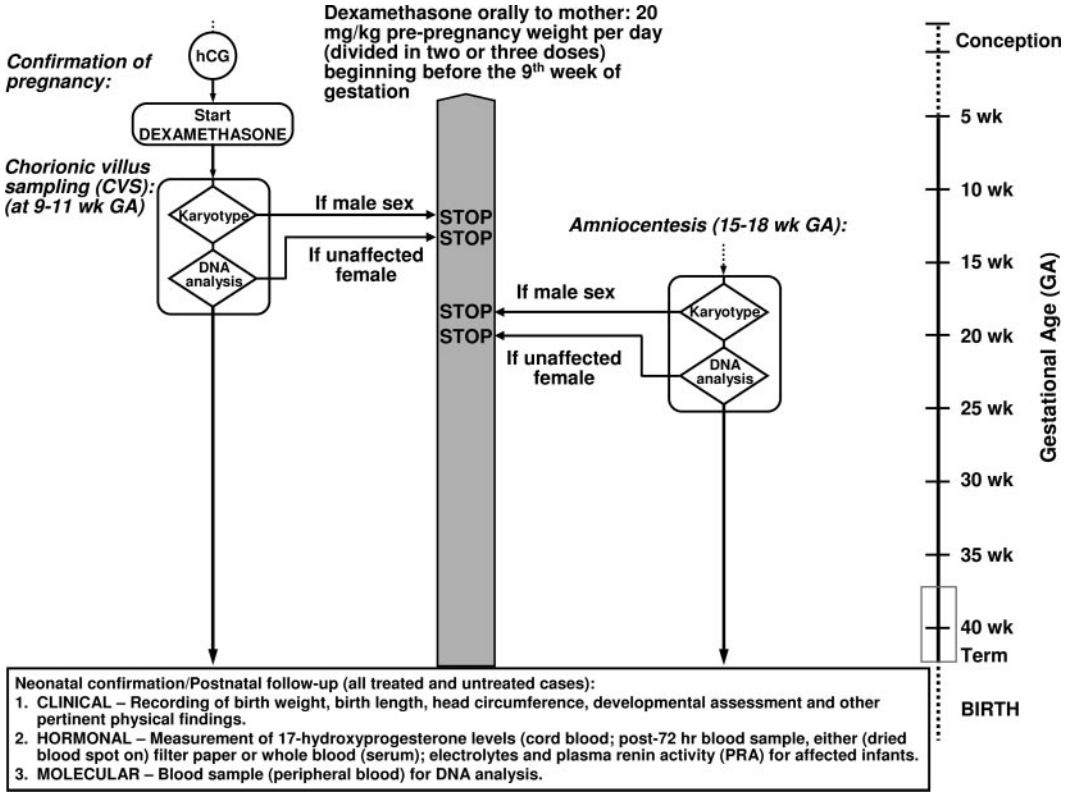

FIG. 10. Algorithm for prenatal diagnosis and treatment of 21-OHD. The mother comes for prenatal diagnosis, and dexamethasone treatment is started blind to the sex or affection status of the fetus. Dexamethasone treatment would be terminated if the fetus has a male sex, is an unaffected female fetus, or is a nonclassical patient based on genotype. A female fetus with classical 21OHD is treated until term. hCG, Human chorionic gonadotropin; GA, gestational age.

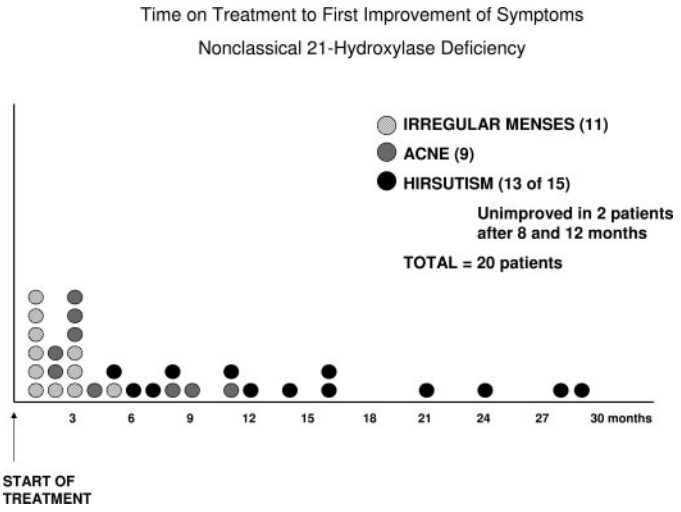

FIG. 11. Reversibility of symptoms in patients with NC21OHD (n = 20). Preliminary data indicate that irregular menses and acne can be reversed with glucocorticoids within 3 months, whereas hirsutism requires nearly 30 months. [Adapted from Ref. 91.]

mozygous for the exon 7 mutation. No fetuses carried the exon 10 mutation. The diagnosis was confirmed postnatally by hormonal tests.

Treatment and Reversal of Signs

Response to therapy of hyperandrogenic signs

The preliminary results in 20 females (91) suggest that screening would be beneficial and cost-effective in infertility and dermatology clinics. Irregular menstruation and acne are reversed within 3 months of treatment with dexamethasone (0.25 mg at the hour of sleep each day) (Fig. 11), whereas hirsutism may be reversed within 30 months (91). More studies on the response of NC21OHD to treatment must be performed. Correlation of genotype with remission and/or recrudescence of signs in response to treatment also warrants further investigation.

Treatment of short stature in children with NC21OHD

Children with NC21OHD do not achieve a final height that is owed them by their parents because they frequently suffer an advanced bone age and arrest their growth early. Some are very short (24, 28). However, there is now treatment with human GH and/or LHRH analog to increase the final height so that children will achieve the target height predicted from the heights of the parents (56).

Importance of Heightened Awareness of NC21OHD

Signs of hyperandrogenemia are of great concern to the American population, as evidenced by the expenditures on acne, hirsutism, frontal balding, and infertility. Table 7 indicates the money spent annually on current treatments for various signs of hyperandrogenemia. NC21OHD could be more effectively and economically treated with low-cost glucocorticoid treatment. Recently, infertility has been treated by assisted reproductive techniques including *in vitro* fertilization and intracytoplasmic sperm injection. Generally, one cycle of *in vitro* fertilization costs $30,000 and patients often undergo more than one cycle, with the second cycle followed by intracytoplasmic sperm injection at $2,500 per injection. In properly diagnosed nonclassical patients, fertility could be restored through inexpensive oral treatment with glucocorticoids, rather than difficult and expensive interventions. NC21OHD should be suspected and diagnosed in patients with hyperandrogenism, especially patients in ethnic groups at high risk for NC21OHD.

Although hyperandrogenic signs in NC21OHD are not fatal, they do cause anguish in many patients: severe acne in adolescents is often disabling, causing young patients to withdraw from social function. Loss of scalp hair in females and males is embarrassing, requiring treatment with 5α-reductase inhibitors and other hair-restoring treatments. Hirsutism, requiring shaving in women, is unacceptable to women who then undergo new treatments such as laser and depilatory treatment at great expense. Preliminary studies of the reversal of hyperandrogenic signs in patients with NC21OHD indicate that it took about 3 months of treatment to reverse acne and infertility (91). The treatment required for this reversal is generally 0.25 mg dexamethasone at the hour of sleep. The cost for a dexamethasone tablet is $0.50, and the 3-month treatment cost is estimated to be $45. Clearly, proper diagnosis and treatment of NC21OHD is cost-effective.

In summary, NC21OHD is a widely underdiagnosed disease causing a variety of hyperandrogenic symptoms, which are easily treated with glucocorticoid therapy. The availability of DNA analysis may encourage populations at risk for NC21OHD to obtain diagnosis and treatment. In ethnic groups such as Ashkenazi Jews, in whom the disease has a high prev-

TABLE 7. National health care burden for treatment of hyperandrogenic signs associated with NC21OHD

Annual expenditure on remedies	Cost	Source
Total skin care	$5 billion to $6 billion annually	Richard Granstein, M.D., Chairman of Dermatology, NYPH-WMC
Acne over-the-counter treatment	$324 million annually	www.drugtopics.com (D. P. Hamacher & Associates)
Acne prescription drugs	$350 per 2 wk of Benzaclin, topical retinoid, and antibiotics	Diane Berson, M.D., Dermatologist, Women's Center, NYPH-WMC
Hirsutism		
Laser treatment	$2000 per five sessions	Diane Berson, M.D., Dermatologist, Women's Center, NYPH-WMC
Electrolysis	$1200/yr (12 sessions/yr)	American Electrolysis Association

NYPH-WMC, New York Presbyterian Hospital-Weill Medical College.

alence, the diagnosis of NC21OHD should be considered. Genotyping is currently available and facilitates the diagnosis of NC21OHD. Because this disease is frequent and treatment is easy and cost-effective, it behooves clinicians to suspect the diagnosis in patients with hyperandrogenic symptoms.

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Address all correspondence and requests for reprints to: Dr. Maria I. New, Director, Adrenal Steroid Disorders Program, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, Box 1198, New York, New York 10029. E-mail: maria.new@mssm.edu.

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