

# Reproductive Outcome of Women with 21-Hydroxylase-Deficient Nonclassic Adrenal Hyperplasia

C. Moran, R. Azziz, N. Weintrob, S. F. Witchel, V. Rohmer, D. Dewailly, J. A. M. Marcondes, M. Pugeat, P. W. Speiser, D. Pignatelli, B. B. Mendonca, T. A. S. Bachega, H. F. Escobar-Morreale, E. Carmina, F. Fruzzetti, and F. Kelestimur

*Mexican Institute of Social Security (C.M.), 06725 Mexico City, Mexico; University of Alabama at Birmingham (R.A.), Birmingham, Alabama 35294; Cedars-Sinai Medical Center (R.A.), Los Angeles, California 90048; Institute for Endocrinology and Diabetes (N.W.), Schneider Children's Medical Center, Petah Tiqva, 49202 Israel; Children's Hospital of Pittsburgh (S.F.W.), Pittsburgh, Pennsylvania 15213; Centre Hospitalier Universitaire D'Angers (V.R.), Angers, 49033 France; Centre Hospitalier Regional et Universitaire de Lille (D.D.), Lille, 59037 France; Hospital das Clinicas (J.A.M.M., B.B.M., T.A.S.B.), 05403 Sao Paulo, Brazil; Hospices Civils de Lyon (M.P.), Lyon, 69289 France; Schneider Children's Hospital (P.W.S.), New York, New York 11042; Faculty of Medicine of Porto (D.P.), 4200 Porto, Portugal; Hospital Ramon y Cajal (H.F.E.-M.), E-28034 Madrid, Spain; University of Palermo (E.C.), I-90139 Palermo, Italy; University of Pisa (F.F.), I-56100 Pisa, Italy; and Erciyes University Medical School (F.K.), 3805 Kayseri, Turkey*

**Context:** Because many women with 21-hydroxylase (21-OH)-deficient nonclassic adrenal hyperplasia (NCAH) carry at least one allele affected by a severe mutation of *CYP21*, they are at risk for giving birth to infants with classic adrenal hyperplasia (CAH).

**Objective:** Our objective was to determine the frequency of CAH and NCAH infants born to mothers with 21-OH-deficient NCAH.

**Design and Setting:** We conducted an international multicenter retrospective/prospective study.

**Patients and Methods:** The outcome of 203 pregnancies among 101 women with 21-OH-deficient NCAH was reviewed. The diagnosis of 21-OH-deficient NCAH was established by a basal or post-ACTH-stimulation 17-hydroxyprogesterone level of more than 10 ng/ml (30.3 nmol/liter). When possible, genotype analyses were performed to confirm CAH or NCAH in the offspring.

**Results:** Of the 203 pregnancies, 138 (68%) occurred before the mother's diagnosis of NCAH and 65 (32%) after the diagnosis. Spontaneous miscarriages occurred in 35 of 138 pregnancies (25.4%) before the maternal diagnosis of NCAH, and in only four of 65 pregnancies (6.2%) after the diagnosis ( $P < 0.002$ ). Four (2.5%; 95% confidence interval, 0.7–6.2%) of the 162 live births were diagnosed with CAH. To date, 24 (14.8%; 95% confidence interval, 9.0–20.6%) children, 13 girls and 11 boys, have been diagnosed with NCAH. The distribution of NCAH children and their mothers varied significantly by ethnicity ( $P < 0.0001$ , for both).

**Conclusions:** The risk of a mother with 21-OH-deficient NCAH for giving birth to a child affected with CAH is 2.5%; at least 14.8% of children born to these mothers have NCAH. (*J Clin Endocrinol Metab* 91: 3451–3456, 2006)

MUTATIONS OF BOTH alleles of *CYP21* result in defective activity of the enzyme P450c21 and symptomatic 21-hydroxylase (21-OH)-deficient congenital adrenal hyperplasia, one of the most common autosomal recessive disorders of man (1, 2). The spectrum ranges from classic adrenal hyperplasia (CAH), associated with excessive antenatal androgen secretion, to the milder forms of nonclassic adrenal hyperplasia (NCAH). Patients with CAH manifest clinical features apparent at birth or shortly thereafter, principally prenatal virilization of female infants. In contrast, patients with NCAH are typically asymptomatic at birth but develop symptoms as a result of hyperandrogenism in childhood, adolescence, or adulthood (1).

The endocrine diagnosis of 21-OH-deficient NCAH is based on markedly supranormal basal or ACTH-stimulated

17-hydroxyprogesterone (17-OHP) levels (3). The diagnosis should be confirmed by genotyping, with the V281L in exon 7 being the most common mutation (4–8). However, the complexity of the *CYP21* locus and the presence of rare mutations complicate the molecular genotype analysis of this disorder (9, 10). Affected patients with NCAH carry *CYP21* mutations on both alleles; some of these patients are homozygous, but most of them are compound heterozygotes (4, 6, 7). Different studies have reported variable percentages, from 27–76% of NCAH individuals carrying a severe loss-of-function mutation on one allele, which would result in CAH if present on both alleles (4–7). These individuals are therefore at risk for conceiving and giving birth to an infant with CAH if their partner also carries a severe loss-of-function *CYP21* mutation.

The calculated probability of having a child affected with CAH among NCAH patients is 1:480 (*i.e.*  $1/60 \times 1/2 \times 1/4$ ), assuming a prevalence of heterozygosity for severe *CYP21* gene mutations in the general population of 1:60 (11) and that approximately 50% of mothers with 21-OH-deficient NCAH are compound heterozygotes carrying one mild and one severe mutation (4–7). This calculated probability is signif-

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Abbreviations: CAH, Classic adrenal hyperplasia; CI, confidence interval; 21-OH, 21-hydroxylase; 17-OHP, 17-hydroxyprogesterone; NCAH, nonclassic adrenal hyperplasia.

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icantly greater than the general population risk for CAH of 1:12,000–23,000 (11, 12). The probability that a woman with NCAH will give birth to a child with NCAH will be much higher, because the carrier rate for mild mutations in the general population is about 1:16 (2, 13). Hence, there is approximately a 1:32 (*i.e.*  $1/16 \times 1/1 \times 1/2$ ) chance that a parent with 21-OH-deficient NCAH will have an offspring with the same disorder, compared with a prevalence of NCAH in the general population of 1:400–2000 (2, 13).

The true frequency of CAH and NCAH offspring among patients with NCAH is likely to be higher than calculated because the male partner is unlikely to be chosen at random from the general population; rather, in some communities, there is a tendency to marry within the same ethnic and racial subpopulation (14, 15). We undertook the following international multicenter study to determine the actual frequency of CAH and NCAH in infants born to mothers with a diagnosis of 21-OH-deficient NCAH.

### Patients and Methods

Patients were studied according to the ethical guidelines for human subjects research of each participating center. From a total of 331 women with 21-OH-deficient NCAH obtained at 14 tertiary referral centers from nine countries, those identified as having been pregnant were reviewed. Data were collected in a standardized fashion, either prospectively as the diagnosis was established in new patients or by retrospective review of the clinical file in those subjects in whom the diagnosis had been made previously. The time span of the retrospective study was variable in each center, from 1973–1995. The prospective study covered the period of 1996–2005.

Patients were classified as White non-Jewish, White Jewish, Hispanic, and other race/ethnic group. All patients with NCAH were diagnosed by either a basal or ACTH-stimulated 17-OHP level greater than 10 ng/ml (30.3 nmol/liter) and lower than 200 ng/ml (605.2 nmol/liter) (16–18). Some variation in the ACTH stimulation protocol was present among centers, such as in ACTH dose administered (0.25 mg or 1.0 mg) or the time of the post-stimulation sampling (30 or 60 min); however, these differences in stimulation are not generally associated with significant differences in the ACTH-stimulated 17-OHP values, at least among healthy subjects (19). The ACTH stimulations were performed in the morning (0800–1030 h) and in the follicular phase (d 3–8) of the menstrual cycle or after a progestogen-induced bleeding. For the purpose of standardizing the 17-OHP results from each center, 10 control serum samples with varying levels of 17-OHP were sent in masked fashion to the participant centers. The results had high correlation coefficients ranging from 0.87–0.99 (20).

Symptoms including premature pubarche, precocious puberty, primary amenorrhea, oligomenorrhea, and infertility were obtained by history. The examining physician documented the presence of hirsutism, acne, and clitoromegaly. The protocol for evaluation of all these signs and symptoms were reported previously (20). In brief, patients with menstrual cycles greater than 35 d were deemed to have oligomenorrhea and patients who never presented menstrual cycles as primary amenorrhea. Precocious puberty and premature pubarche were considered when present in girls younger than 8 yr and boys younger than 9 yr (21, 22). The degree of hirsutism was assessed using a modified Ferriman-Gallwey scoring method, and hirsutism was considered to be present when a score of at least 8 was evident (23, 24). Acne and alopecia were recorded, but their severity was not scored. Prader score was used to classify abnormalities of the external female genitalia, from mild clitoromegaly (stage 1) to complete fusion of labioscrotal folds with a phallic urethra (stage 5) (25). We considered that females with NCAH may have signs of postnatal androgen excess, including mild clitoromegaly, although they generally are born with nonambiguous external genitalia, as reported (11, 20).

### Statistical analysis

The clinical and hormonal values are expressed as median and range. The reproductive outcome frequencies are expressed as percentages. The comparison of reproductive outcome before and after the diagnosis of NCAH, as well as the proportion of children with CAH or NCAH and their mothers among the different ethnic groups, was performed by  $\chi^2$  analysis. A *P* value of <0.05 was considered as statistically significant. The 95% confidence interval (CI) was calculated for the prevalence of CAH and NCAH children born from NCAH patients and for the risk of these women having at least one CAH/NCAH-affected offspring.

## Results

### General findings of reproductive outcome

In total, we recruited 211 pregnancies in 107 women with 21-OH-deficient NCAH. Three pregnancies were electively terminated by three different women and were excluded from additional analysis. Another five pregnancies in three women did not have complete reproductive outcome data and also were excluded. Of the 101 women included in this study, 68 (67.3%) were White non-Jewish, 13 (12.9%) were White Jewish, 15 (14.8%) were Hispanic, and 5 (5.0%) were of other races or they did not have race/ethnicity recorded. Their mean age was  $29.7 \pm 9.7$  yr. The median basal 17-OHP level for all patients was 6.2 ng/ml (range, 0.3–156.0 ng/ml), and the median ACTH-stimulated 17-OHP level was 44.7 ng/ml (range, 12.6–185.2 ng/ml) (Fig. 1). Of the 91 women who were tested by ACTH stimulation, 84 (92.3%) had a poststimulation 17-OHP value of more than 20 ng/ml, and seven (7.7%) had values between 10 and 20 ng/ml. In 10 women, an ACTH test was not performed because the basal 17-OHP level was more than 10 ng/ml.

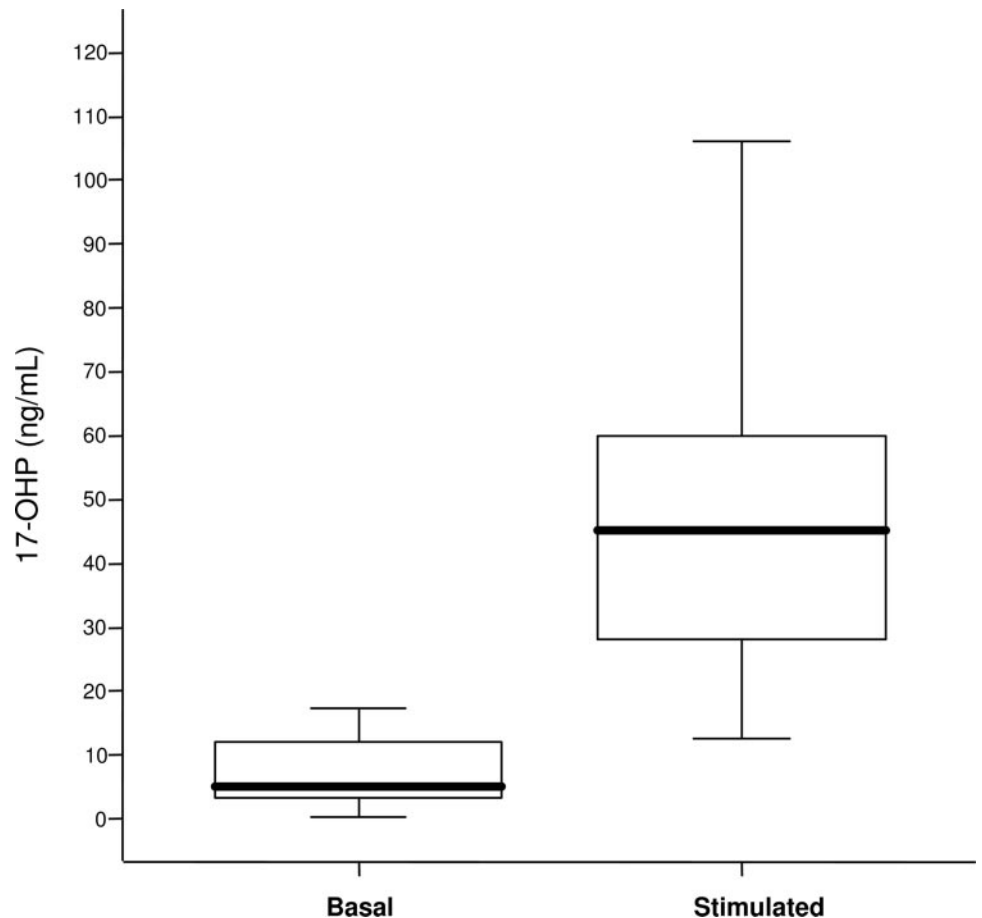
Of the 101 NCAH women included, 47 (46.5%) presented with oligomenorrhea and four (4.0%) patients with primary amenorrhea. Hirsutism was present in 51 (50.5%), and the median modified Ferriman-Gallwey score was 12.0 (range, 0–27). In addition, 28 (27.7%) patients presented with acne, 11 (10.9%) with alopecia, and 10 (9.9%) with mild clitoromegaly (Prader score stage 1). Premature pubarche was present in four (4.0%) patients. Infertility was a complaint in 22 (21.8%).

Of the 203 pregnancies included, singleton term live births were observed in 151 pregnancies (74.4%) and live-born twins in four (2.0%). Six (3.0%) of the pregnancies were singleton delivered preterm, with three infants surviving the neonatal period. Two (1.0%) infants were singleton stillborn at term. Finally, 39 (19.2%) pregnancies ended in a spontaneous miscarriage and one (0.5%) in an ectopic pregnancy.

### Pregnancy outcome according to time of diagnosis and glucocorticoid treatment

Of the 203 pregnancies, 138 (68%) occurred before the diagnosis of NCAH, and 65 (32%) occurred after the diagnosis was established. Spontaneous miscarriages occurred in 35 (25.4%) of 138 pregnancies occurring before the NCAH diagnosis and in only four (6.2%) of 65 pregnancies occurring after diagnosis was made ( $P < 0.002$ ). Thus, 95 (68.8%) of 138 pregnancies conceived before the diagnosis of NCAH was made resulted in a singleton term live-born infant, whereas 56 (86.2%) of the 65 pregnancies occurring after diagnosis experienced this outcome ( $P < 0.01$ ) (Table 1).

FIG. 1. Box and whiskers plot of basal and ACTH-stimulated values of 17-OHP in women with NCAH. The line within each box represents the median. Upper and lower boundaries of the box indicate 75th and 25th percentiles, respectively. The whiskers above and below show the upper and lower adjacent value, respectively. The diagnosis of NCAH was made when the basal or ACTH-stimulated 17-OHP values were above 10 ng/ml (30.3 nmol/liter) and under 200 ng/ml (605.2 nmol/liter).



Of the 138 pregnancies occurring before the diagnosis of NCAH was made, five (3.6%) were treated with glucocorticoids alone (administered empirically to improve ovulatory function), four (2.9%) with clomiphene alone, one (0.7%) with a combination of glucocorticoids and clomiphene, and another (0.7%) with glucocorticoids and menotropins. In 94 pregnancies (68.1%), there was no medical treatment, and in another 33 (23.9%), pregnancy data regarding preconception treatment were unavailable.

Of the 65 pregnancies occurring after the NCAH diagnosis, glucocorticoids alone were administered before conception in 35 (53.8%), clomiphene alone in five (7.7%), combined glucocorticoids and clomiphene in six (9.2%), combined glucocorticoids and menotropins in one (1.5%), and clomiphene and menotropins in the remaining (1.5%) pregnancy. No treatment

was used in 16 (24.6%) pregnancies, and no data on preconception treatment were available for one (1.5%) case.

Overall, preconception glucocorticoids either alone or in combination with other drugs were used before conception in 49 of the 203 pregnancies, seven before and 42 after the diagnosis of NCAH was made. The specific glucocorticoid used and the dosage varied widely; dexamethasone 0.25–0.75 mg was used in 26 cases, prednisone 5.0–12.5 mg in 13 cases, and hydrocortisone 6–20 mg in four cases. Six other pregnancies were treated with preconception glucocorticoids, although no specific data concerning the type or dose of glucocorticoids used were available for these cases. The 40 pregnancies treated with preconception glucocorticoids alone and the 110 pregnancies not receiving such treatment did not differ in the proportion of singleton term live births

TABLE 1. Comparison of pregnancy outcome before and after the diagnosis of 21-OH-deficient NCAH

Outcome	Before (n = 138)		After (n = 65)		Total (n = 203)	
	N	%	N	%	N	%
Singleton term	95	68.8 <sup>a</sup>	56	86.2 <sup>a</sup>	151	74.4
Twins	2	1.4	2	3.1	4	2.0
Preterm	4	2.9	2	3.1	6	3.0
Miscarriage	35	25.4 <sup>b</sup>	4	6.2 <sup>b</sup>	39	19.2
Ectopic	1	0.7	0	0.0	1	0.5
Stillborn	1	0.7	1	1.5	2	1.0

<sup>a</sup> Significant difference in percentages of singleton term deliveries ( $P < 0.01$ ).

<sup>b</sup> Significant difference in percentages of miscarriages ( $P < 0.002$ ).



(75.0 *vs.* 81.8%, respectively) or miscarriages (15.0 *vs.* 13.6%, respectively).

Sixteen of the 65 pregnancies occurring after the diagnosis of 21-OH-deficient NCAH was made were treated with glucocorticoids during gestation, including 0.25–0.5 mg/d dexamethasone in seven cases, 5.0–12.5 mg/d prednisone in six, and 10–20 mg/d hydrocortisone in the remaining three. There was no significant difference between the 16 patients treated with glucocorticoids during pregnancy and the 49 patients without such treatment in the number of singleton term live births (81.3 *vs.* 87.8%, respectively) or miscarriages (0 *vs.* 8.2%, respectively).

#### Prevalence of CAH and NCAH among the children of women with NCAH

Of the 162 live-born infants observed, 83 (51.2%) were females and 79 (48.8%) were males. Four (2.5%; 95% CI, 0.7–6.2%) of the 162 offspring were diagnosed with CAH. However, the probability of a woman with NCAH in this study having at least one child with CAH is three of 101 (3.0%; 95% CI, 0.6–8.4%) NCAH women, because one mother had two children affected with CAH. Three male infants presented with salt-wasting CAH, and one female with simple virilizing CAH. One NCAH mother was a compound heterozygous (V281L/8BPdeletion) and her two male infants, with 17-OHP basal levels of 124 ng/ml and 128 ng/ml, had the ClusterE6/8BPdeletion genotype indicating that the ClusterE6 mutation was inherited from the father. The genotype of the other mother was V281L/R356W; her child had a 17-OHP basal level of 70 ng/ml and her genotype was R356W/I2 splice R356W. This infant (karyotype 46XX) demonstrated severe virilization of the external genitalia (Prader 5). The same NCAH mother subsequently had two children (one girl and one boy) affected with NCAH; the genotype for both children was V281L/I2 splice+R356W. In this instance, the affected paternal allele carried two mutations, the I2 splicing mutation and R356W. The genotypes of the remaining NCAH mother and male CAH child are unknown. Notably, the diagnosis of 21-OH-deficient NCAH in the three mothers was made *after* the birth of their affected infants. The genitalia were normal in all children, with the exception of the child with simple virilizing CAH.

**TABLE 2.** Clinical findings of children with 21-OH-deficient NCAH of NCAH mothers

Gender	Presentation	Age at diagnosis	17-OHP, basal/stimulated (ng/ml)	Genotyping
Female	Premature pubarche	8 yr 5 months	11.6/19.8	Homozygous V281L
Female	Premature pubarche	3 d	10.0	V281L/I2 splice+R356W
Female	Precocious puberty	4 yr 6 months	4.0/46.9	Homozygous V281L
Female	Precocious puberty	5 yr 1 month	8.3/49.6	Homozygous V281L
Female	Precocious puberty	2 yr 11 months	1.2/23.1	Heterozygous V281L/?
Female	Pubertal hyperandrogenism	15 yr 8 months	9.8/37.0	Homozygous V281L
Female	Pubertal hyperandrogenism	12 yr	19.7/41.5	Homozygous V281L
Female	Pubertal hyperandrogenism	7 yr 4 months	3.6/24.8	Homozygous V281L
Female	Pubertal hyperandrogenism	11 yr	2.2/15.4	Homozygous V281L
Female	Pubertal hyperandrogenism		8.7/80.0	Not known
Male	Premature pubarche	10 yr 2 months	4.0/25.8	Homozygous V281L
Male	Premature pubarche	1 yr 7 months	13.5/76.7	Homozygous V281L
Male	Premature pubarche	2 months	6.4/34.4	Q318X/V281L
Male	Premature pubarche	3 d	26.0	V281L/I2 splice+R356W
Male	Premature pubarche	8 yr	5.7/36.0	Heterozygous V281L/?
Male	Asymptomatic	14 yr		Homozygous V281L

The 17-OHP levels and genotyping of eight NCAH children (three females and five males) were not available.

To date, 24 (14.8%; 95% CI, 9.0–20.6%) of the 162 children, 13 females and 11 males, have been diagnosed with NCAH. In addition, this experience indicates that the probability that a woman with NCAH would have at least one child with NCAH is 12 of 101 NCAH women (11.9%; 95% CI, 5.0–18.7%). The available 17-OHP levels and genotypes of the NCAH children are denoted in Table 2. Of the 12 NCAH mothers who had these children, four were homozygous for V281L and two were compound heterozygotes carrying V281L/R356W and V281L/Q318X in one case each. The genotypes of the remaining six NCAH patients were not available.

Five additional female offspring developed signs of hyperandrogenism; none fulfilled the hormonal criteria for the diagnosis of NCAH. One of them had premature pubarche and was a carrier of the V281L mutation, and the other four females developed pubertal hyperandrogenism. Two were also found to be heterozygous for V281L and another heterozygous for I172N. Although the *CYP21* genotype is unknown for the remaining female, she is likely to be an obligate heterozygote because she could only inherit an affected allele from her mother.

Of the four children with CAH among the 162 live births, one of 94 (1.1%) was White non-Jewish, two of 31 (6.5%) were White Jewish, and one of 31 (3.2%) was Hispanic. Of the three NCAH mothers giving birth to a child affected with CAH, one of 68 (1.5%) was White non-Jewish, one of 13 (7.7%) was White Jewish, and one of 15 was Hispanic (6.7%). The calculated risk for a child of an NCAH mother of having NCAH and the risk for an NCAH mother of having a child affected with NCAH differed significantly by ethnicity ( $P < 0.0001$  for both distributions) (Table 3).

## Discussion

These data suggest that the prevalence of 21-OH-deficient CAH among live-born children of NCAH women is 2.5%, higher than the 1:480 (0.2%) calculated prevalence. In addition, the risk of NCAH women for giving birth to at least one child affected with CAH is 3.0%. Alternatively, the prevalence of NCAH among children of NCAH mothers is approximately 15%, much higher than the predicted rate of 1:32

**TABLE 3.** Gender distribution of NCAH children and their mothers by ethnicity

NCAH patients	White non-Jewish	White Jewish	Hispanic	Other	Total
Children <sup>a</sup>	5/94	14/31	3/31	2/6	24/162
Mothers <sup>a</sup>	3/68	6/13	2/15	1/5	12/101

The data represent NCAH-affected patients/subjects in each ethnic group.

<sup>a</sup> Significant differences by ethnicity for NCAH children and mothers ( $P < 0.0001$  for both,  $\chi^2$  test).

(3.1%). Also, the risk of women with NCAH of giving birth to at least one child affected with NCAH is 11.9%. A likely explanation for the higher prevalence of affected offspring noted compared with calculated is the inherent tendency for individuals to intermarry within their ethnic subpopulation (14, 15), although this tendency lessens over generations among immigrants (14).

Although the numbers are too small to calculate the risk based on ethnicity, in this study, the probability of a child of an NCAH mother having NCAH and that of an NCAH mother having a child affected with NCAH differed significantly by ethnicity. The risk among women with NCAH of having a child with NCAH was significantly lower for White non-Jewish individuals than for other ethnic groups, perhaps because the background heterozygosity rate for *CYP21* mutations is lower in this population (2). Nonetheless, these data would suggest that biochemical screening of all children born to mothers with 21-OH-deficient NCAH is mandatory. Early diagnosis and treatment of children with NCAH may reduce the risk of developing clinically apparent hyperandrogenism and may improve final height (26, 27), although there are insufficient data to assess the efficacy and safety of presymptomatic treatment of NCAH infants and children (28). Hormonal screening with a basal 17-OHP level does not detect CAH in the first 24 h of life because 17-OHP is generally elevated in all infants; therefore, sampling should be performed between 48 and 72 h of age (28).

With the advent of newborn screening programs that detect 17-OHP in blood, fewer cases of CAH are missed. However, false-positive results may occur, especially in premature and low-birth-weight infants. Alternatively, false-negative results may be evident in some affected children (29), especially those with milder types of CAH, including NCAH. Newborn screening is efficient for diagnosing salt-wasting CAH but appears to be much less effective for identifying all patients with moderate forms of CAH, in which the false negative may be as high as 30% (30).

Of interest is the development of peri- or postpubertal hyperandrogenism not resulting from NCAH in five girls. All are likely to be carriers for maternally inherited mutations of *CYP21*. Taking into account the rare event of a false-negative ACTH-stimulated 17-OHP value and in the absence of gene sequencing data, it is unlikely that these patients have undiagnosed NCAH. Nonetheless, even with the use of low- and high-resolution genotyping, it is always difficult to identify all the *CYP21* gene mutations (31). Although we have previously reported that mothers who are obligate heterozygotes appear to be asymptomatic for the most part (32), it is possible that some *CYP21* mutation carriers develop hyperandrogenic symptoms. Prospective studies of larger populations of *CYP21* mutation carriers and examination of other factors associated with hyperandrogenism are needed.

In a previous study of 38 pregnancies in NCAH patients, Feldman *et al.* (33) reported on the outcome of 18 pregnancies in 10 women occurring before diagnosis and before the initiation of a specific treatment; 12 of these resulted in a term live birth and six (33%) ended in a first-trimester miscarriage. After hydrocortisone treatment, they observed that 19 pregnancies carried to term without any spontaneous abortion recorded. Similarly, in the present study, approximately 25% of pregnancies occurring before diagnosis ended in a miscarriage, significantly higher than for those pregnancies conceived after the maternal diagnosis of NCAH was established (~6%). Consequently, the fraction of pregnancies ending in a term delivery was lower before than after diagnosis (69 vs. 86%). Whether this improvement in pregnancy outcome is because of an effect of the glucocorticoids on the fetus or on the mother, or both, is not known. We should note that some glucocorticoids used, such as dexamethasone, cross the placenta (34), whereas others, such as hydrocortisone or prednisone, do not (35). Unfortunately, the numbers of patients treated with specific glucocorticoids were too few to compare.

It is also possible that the effect of glucocorticoids on pregnancy outcome may be mediated through an improvement in maternal hyperandrogenemia. Elevated serum androgen concentrations have been reported to be a risk factor for early pregnancy loss and recurrent miscarriages in women with and without polycystic ovary syndrome (36, 37), and an association between serum androgen levels and endometrial dysfunction has been noted (36). These findings, together with our data, suggest a possible detrimental effect of elevated serum androgen levels on the progress of early pregnancy. Prospective controlled trials will be needed to determine the true benefit and the mechanism of action of glucocorticoid therapy on pregnancy outcome in NCAH.

One limitation of the present study is that not all children with NCAH have yet been detected. Although in some centers, all the children born of mothers with NCAH were evaluated for NCAH, other centers evaluated only those children with clinical symptoms. Thus, some asymptomatic NCAH children could have been missed, although they may be diagnosed in the future.

Overall, our study suggests that the risk that a woman with NCAH will give birth to a child with CAH is approximately 2.5%, whereas the risk of having a child with NCAH is at least 15%. This study also emphasizes the importance of preconception screening and diagnosis of 21-OH-deficient NCAH, particularly in those hyperandrogenic or oligoovulatory women who desire pregnancy, and the possible benefit of preconception and/or antenatal glucocorticoid therapy on pregnancy outcome.

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Address all correspondence and requests for reprints to: Ricardo Azziz, M.D., M.P.H., M.B.A., Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, 8635 West Third Street, Suite 160 W, Los Angeles, California 90048. E-mail: azzizr@cshs.org; or Carlos Moran, M.D., M.Sc., Health Research Council, Mexican Institute of Social Security, 413 Interamerica Boulevard WH1, PMB 67–139, Laredo, Texas 78045. E-mail: cemoranv@hotmail.com.

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## References

- Speiser PW, White PC 2003 Congenital adrenal hyperplasia. *N Engl J Med* 349:776–788
- Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI 1985 High frequency of nonclassical steroid 21-hydroxylase deficiency. *Am J Hum Genet* 37:650–667
- Azziz R, Hincapie LA, Knochenhauer ES, Dewailly D, Fox L, Boots LR 1999 Screening for 21-hydroxylase deficient nonclassic adrenal hyperplasia among hyperandrogenic women: a prospective study. *Fertil Steril* 72:915–925
- Speiser PW, Knochenhauer ES, Dewailly D, Fruzzetti F, Marcondes JAM, Azziz R 2000 A multicenter study of women with nonclassical congenital adrenal hyperplasia: relationship between genotype and phenotype. *Mol Genet Metab* 71:527–534
- Weintrob N, Brautbar C, Pertzelan A, Josefsberg Z, Dickerman Z, Kauschansky A, Lilos P, Peled D, Phillip M, Israel S 2000 Genotype-phenotype association in nonclassical steroid 21-hydroxylase deficiency. *Eur J Endocrinol* 143:397–403
- Bachega TA, Billerbeck AE, Marcondes JA, Madureira G, Arnhold IJ, Mendonca BB 2000 Influence of different genotypes on 17-hydroxyprogesterone levels in patients with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)* 52:601–607
- Deneuve C, Tardy V, Dib A, Mornet E, Billaud L, Charron D, Morel Y, Kuttann F 2001 Phenotype-genotype correlation in 56 women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 86:207–213
- Stikkelbroeck NMML, Hoefsloot LH, de Wijs IJ, Otten BJ, Hermus ARMM, Siermans EA 2003 *CYP21* gene mutation analysis in 198 patients with 21-hydroxylase deficiency in The Netherlands: six novel mutations and a specific cluster of four mutations. *J Clin Endocrinol Metab* 88:3852–3859
- Witchel SF 1999 Molecular diagnosis in the congenital adrenal hyperplasia. *Endocrinologist* 9:197–207
- Speiser PW 2001 Molecular diagnosis of *CYP21* mutations in congenital adrenal hyperplasia. *Am J Pharmacogenomics* 1:101–110
- White PC, Speiser PW 2000 Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev* 21:245–291
- Pang S, Wallace MA, Hofman L, Thuline HC, Dorche C, Lyon ICT, Dobbins RH, Kling S, Fujieda K, Suwa S 1988 Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics* 81:866–874
- Sherman SL, Aston CE, Morton NE, Speiser PW, New MI 1988 A segregation and linkage study of classical and nonclassical 21-hydroxylase deficiency. *Am J Hum Genet* 42:830–838
- Kalmijn M 1993 Spouse selection among the children of European immigrants: a comparison of marriage cohorts in the 1960 census. *Int Migr Rev* 27:51–78
- Fiebert MS, Nugent D, Hershberger SL, Kasdan M 2004 Dating and commitment choices as a function of ethnicity among American college students in California. *Psychol Rep* 94:1293–1300
- New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack MS, Dupont B, Stoner E, Levy DJ, Pang S, Levine LS 1983 Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. *J Clin Endocrinol Metab* 57:320–326
- Dewailly D, Vantyghem-Haudiquet MC, Sainsard C, Buvat J, Cappoen JP, Ardaens K, Racadot A, Lefebvre J, Fossati P 1986 Clinical and biological phenotypes in late-onset 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 63:418–423
- Fiet J, Gueux B, Gournelen M, Kuttann F, Vexiau P, Couillin P, Pham-Huu-Trung MT, Villette JM, Raux-Demay MC, Galons H, Julien R 1988 Comparison of basal and adrenocorticotropin-stimulated plasma 21-deoxycortisol and 17-hydroxyprogesterone values as biological markers of late-onset adrenal hyperplasia. *J Clin Endocrinol Metab* 66:659–667
- Azziz R, Bradley E, Huth J, Boots LR, Parker CR, Zacur HA 1990 Acute adrenocorticotropin-(1–24) (ACTH) adrenal stimulation in eumenorrheic women: reproducibility and effect of ACTH dose, subject weight, and sampling time. *J Clin Endocrinol Metab* 70:1273–1279
- Moran C, Azziz R, Carmina E, Dewailly D, Fruzzetti F, Ibañez L, Knochenhauer ES, Marcondes JAM, Mendonca BB, Pignatelli D, Pugeat M, Rohmer V, Speiser PW, Witchel SF 2000 21-Hydroxylase-deficient nonclassic adrenal hyperplasia is a progressive disorder: a multicenter study. *Am J Obstet Gynecol* 183:1468–1474
- Ritzen EM 2003 Early puberty: what is normal and when is treatment indicated? *Horm Res* 60(Suppl 3):31–34
- Charkaluk ML, Trivin C, Brauner R 2004 Premature pubarche as an indicator of how body weight influences the onset of adrenarche. *Eur J Pediatr* 163:89–93
- Ferriman D, Gallwey JD 1961 Clinical assessment of body hair growth in women. *J Clin Endocr* 21:1440–1447
- Hatch R, Rosenfield RL, Kim MH, Tredway D 1981 Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol* 140:815–830
- Prader A 1954 Der genitalbefund beim pseudohermaphroditismus femininus des kongenitalen androgenitalen syndroms. *Helvetica Paediatr Acta* 3:231–248
- New MI, Gertner JM, Speiser PW, Del Balzo P 1989 Growth and final height in classical and non classical 21-hydroxylase deficiency. *J Endocrinol Invest* 12(Suppl 3):91–95
- Weintrob N, Dickerman Z, Sprecher E, Galatzer A, Pertzelan A 1997 Non-classical 21-hydroxylase deficiency in infancy and childhood: the effect of time of initiation of therapy on puberty and final height. *Eur J Endocrinol* 136:188–195
- Clayton PE, Miller WL, Oberfield SE, Ritzen EM, Sippell WG, Speiser PW, LWPE/ESPE CAH Working Group 2002 Consensus statement on 21-hydroxylase deficiency from the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology. *J Clin Endocrinol Metab* 87:4048–4053
- Gudmundsson K, Majzoub JA, Bradwin G, Mandel S, Rifai N 1999 Virilising 21-hydroxylase deficiency: timing of newborn screening and confirmatory tests can be crucial. *J Pediatr Endocrinol Metab* 12:895–901
- Votava F, Torok D, Kovacs J, Moslinger D, Baumgartner-Parzer SM, Solyom J, Pribilinkova Z, Battelino T, Lebl J, Frisch H, Waldhauser F, Middle European Society for Paediatric Endocrinology–Congenital Adrenal Hyperplasia (MESPE-CAH) Study Group 2005 Estimation of the false-negative rate in newborn screening for congenital adrenal hyperplasia. *Eur J Endocrinol* 152:869–874
- Dolzan V, Solyom J, Fekete G, Kovacs J, Rakosnikova V, Votava F, Lebl J, Pribilinkova Z, Baumgartner-Parzer SM, Riedl S, Waldhauser F, Frisch H, Stopar-Obreza M, Krzisnik C, Battelino T 2005 Mutational spectrum of steroid 21-hydroxylase and genotype-phenotype association in Middle European patients with congenital adrenal hyperplasia. *Eur J Endocrinol* 153:99–106
- Knochenhauer ES, Cortet-Rudelli C, Cunningham RD, Conway-Myers BA, Dewailly D, Azziz R 1997 Carriers of 21-hydroxylase deficiency are not at increased risk for hyperandrogenism. *J Clin Endocrinol Metab* 82:479–485
- Feldman S, Billaud L, Thalabard JC, Raux-Demay MC, Mowszowicz I, Kuttann F, Mauvais-Jarvis P 1992 Fertility in women with late-onset adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 74:635–639
- Speiser PW, Laforgia N, Kato K, Pareira J, Khan R, Yang SY, Whorwood C, White PC, Elias S, Schriock E, Simpson JL, Taslimi M, Najjar J, May S, Mills G, Crawford C, New MI 1990 First trimester prenatal treatment and molecular genetic diagnosis of congenital adrenal hyperplasia (21-hydroxylase deficiency). *J Clin Endocrinol Metab* 70:838–848
- Akker ES van den, Stikkelbroek MM, Menheere PP, Roumen FJ, Otten BJ 2002 Successful treatment and pregnancy in a woman with the non-classic form of congenital adrenal hyperplasia. *Ned Tijdschr Geneesk* 146:268–270
- Okon MA, Laird SM, Tuckerman EM, Li TC 1998 Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril* 69:682–690
- Tulppala M, Stenman UH, Cacciatore B, Ylikorkala O 1993 Polycystic ovaries and levels of gonadotrophins and androgens in recurrent miscarriage: prospective study in 50 women. *Br J Obstet Gynaecol* 100:348–352