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Ethnic Specific Distribution of Mutations in 716 Patients with Congenital Adrenal Hyperplasia Owing to 21-Hydroxylase Deficiency

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Abstract

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) occurs worldwide. The most common mutations in the *CYP21A2* gene in 716 unrelated patients were analyzed and the mutations were grouped by ethnicity, as defined through self-declaration corroborated by review of pedigrees extending to two or three generations. Prevalent allelic mutations and genotypes were found to vary significantly among ethnic groups, and the predominance of the prevalent mutations and genotypes in several of these populations was significant. There are ethnic-specific mutations in the *CYP21A2* gene. A large deletion is prevalent in the Anglo-Saxons; a V281L (1685 G to T) mutation is prevalent in Ashkenazi Jews; an R356W (2109 G to A) mutation is prevalent in the Croatians; an IVS2 AS -13 (A/C to G) mutation is prevalent in the Iranians and Yupik-speaking Eskimos of Western Alaska; and a Q318X (1994 C to T) mutation is prevalent in East Indians. Genotype/phenotype non-correlation was seen when at least one IVS2 AS -13 (A/C to G) mutation in the *CYP21A2* gene was present.

Keywords

CYP21A2; 21-hydroxylase deficiency; congenital adrenal hyperplasia; steroidogenesis

Introduction

Congenital adrenal hyperplasia (CAH) is a family of inherited disorders of adrenal steroidogenesis resulting from a deficiency in one of the five enzymatic steps necessary for normal cortisol synthesis. Deficiency of the 21-hydroxylase enzyme accounts for more than 90% of CAH cases. The gene for the enzyme, *CYP21A2*, has been discovered and sequenced [1-3]. 21-hydroxylase deficiency (21OHD) CAH occurs in two severe forms, classical salt-

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wasting and classical simple virilizing, and a third, mild, nonclassical form. 21OHD has been found worldwide.

In classical 21OHD, prenatal androgen excess causes external genital ambiguity in female fetuses. After birth, males and females with the classical form exhibit progressive postnatal virilization, which may include progressive penile or clitoral enlargement, precocious pubic hair, hirsutism, acne, advanced somatic and epiphyseal development, and central precocious puberty. Reduced fertility and menstrual abnormalities in untreated women and testicular adrenal rests in untreated men have been observed [4-7]. Aldosterone deficiency characterizes the salt-wasting variant of the classical disease, which constitutes three-fourths of classical cases [8,9].

Nonclassical 21OHD (NC21OHD) results from a mild deficiency of the 21-hydroxylase enzyme. Female NC21OHD patients do not demonstrate genital ambiguity at birth. Males and females may manifest variable signs of androgen excess at any phase of postnatal development. This may include short stature, premature development of pubic hair, insulin resistance, acne, and reduced fertility in untreated males and females. Polycystic ovaries, hirsutism, and male-pattern baldness are symptoms in untreated females [8,9].

The standard hormonal diagnostic test for 21OHD is the ACTH (Cortrosyn, 0.25 mg) stimulation test, measuring the serum concentration of 17-hydroxyprogesterone (17-OHP) after intravenous ACTH administration. A logarithmic nomogram provides hormonal standards for assignment of the 21OHD form by relating baseline to ACTH-stimulated serum concentrations of 17-OHP [10]. Although hormonal parameters are valuable in classifying the forms of 21OHD, molecular genetic techniques are best for making a secure diagnosis, as 90-95% of the allelic mutations are detected by these techniques [11-16].

Epidemiology

Analysis of CAH incidence data from almost 6.5 million newborns screened in the general population worldwide has demonstrated an overall incidence of 1:13,000 to 1:15,000 live births for the classic form of CAH [17,18]. The prevalence in specific populations based on newborn screening results are 1:10,000 - 1:23,000 in the United States and Europe [19], 1:21,000 in Japan [20], and 1:23,000 in New Zealand [21]. NC21OHD has a much higher frequency than the classical forms of CAH [22]. The estimated incidence of the classical form of CAH in the Chinese (Taiwanese) population is 1:28,000 [23]. A previous study of a small population of patients with a *CYP21A2* mutation indicated the likelihood of ethnic specific mutations [24]. The current study amplifies the data on the ethnic specific frequency of mutations in the *CYP21A2* gene.

Ethnicity

Ethnic groups were defined by self-declaration as corroborated by analysis of the patient's pedigree taken to include two to three generations in the maternal and paternal lineage.

Molecular Genetics

CAH due to 21OHD is a monogenic autosomal recessive disorder. The gene for adrenal 21-hydroxylase, *CYP21A2*, is located 30 kb from its pseudogene, *CYP21A1P*, on chromosome 6p21.3, adjacent to the HLA Class III region. The high degree of sequence similarity (96-98%) between *CYP21A2* and *CYP21A1P* permits two types of recombination events: 1) unequal crossing-over during meiosis, which results in large deletions/duplications of *CYP21A2* [2, 25,26], and 2) gene conversion events that transfer deleterious mutations present in the pseudogene to *CYP21A2* [27,28]. *In vitro* expression analysis demonstrates that the large

deletion of the *CYP21A2* gene results in no 21-hydroxylase enzymatic activity, while the IVS2 AS -13 (A/C to G) and I172N (1001 T to A) point mutations result in 1-5% of normal enzyme activity [29-31]. In contrast, the P30L (89 C to T), V281L (1685 G to T) and P453S (2580 C to T) mutations demonstrate 20-60% enzyme activity in *in vitro* expression analysis [24,28,32]. Table 1 indicates the grouping of mutations in the *CYP21A2* gene according to severity. In this study, we screened 716 unrelated patients and grouped mutations in the *CYP21A2* gene by ethnicity.

Methods and Materials

Subjects

This study was done under an IRB approved protocol and with informed consent. A total of 716 probands were studied. A total of 1388 unrelated alleles were studied from 655 patients.

Iranian patients—61 nonconsanguineous Iranian patients from the area near Tehran were studied. There were 42 patients from consanguineous families only resulting in 42 unrelated alleles. The other 19 patients were from non-consanguineous families resulting in 38 unrelated alleles, giving a total of 80 unrelated Iranian alleles studied.

Croatian patients—50 unrelated patients from Croatia were studied, some of whom were previously studied [33,34].

Italian patients—53 unrelated patients from Italy were studied.

Yupik Eskimos—7 Yupik Eskimos were studied, 4 of whom were previously reported [35].

Thai patients—72 unrelated patients from Thailand were studied.

The rest of the unrelated patients, some whom have been previously reported [15,16,36], were from North America (Anglo-Saxon, Ashkenazi, Asian, East Indian, French, Hispanic, Italo-American, Native American, and African American ethnicities). The ethnicity of these patients was not pure in all cases. Ethnicity was classified through self-declaration as corroborated by detailed pedigrees including multiple generations (at least two or three) in the paternal and maternal lineage.

Genetic analysis

The 8 most common mutations in the *CYP21A2* gene [large deletion; P30L (89 C to T); IVS2 AS -13 (A/C to G); 8 bp deletion (Δ 707-714); I172N (1001 T to A); V281L (1685 G to T); Q318X (1994 C to T); R356W (2109 G to A)] were studied by Southern blot analysis and allele-specific polymerase chain reaction (PCR) of the *CYP21A2* gene as described [37]. The large deletions consisted of chimeras of CYP21PA1/CYP21A2 deleting either exons 1, 2 and 3 or exons 1-7. The other seven mutations studied here are all found in the pseudogene and presumably resulted from some type of gene conversion event [3]. For the Iranian samples, there was not enough DNA for Southern blot analysis. Therefore, in cases where the allele-specific PCR indicated homozygosity and when the parental samples were not available for analysis, these samples may be hemizygous due to a large deletion of one allele.

Phenotypic analysis

The clinical phenotype and hormonal profile were evaluated in each case by a physician to categorize the patient with CAH with the salt-wasting, simple virilizing, or nonclassical forms

of 21OHD. The criteria for salt-wasting was either a salt-wasting crisis in the newborn period or elevated plasma renin activity and hyponatremia.

Statistical analysis

Chi square analysis was performed on alleles within an ethnic group as compared to the other groups combined. Pearson exact analysis was performed on ethnic groups with a low number of alleles and compared to the other groups combined.

Results

Important variations in allelic mutations frequencies were found in ethnic specific groups (Table 2 a-b). Similarly, phenotypes varied among ethnic groups (Table 3 a-d).

The allelic mutations found in the *CYP21A2* gene reveal that the V281L (1685 G to T) mutation is the most frequent mutation in Ashkenazi Jews (63%, $p=5 \times 10^{-54}$). A large deletion was observed in 40% of Native Americans and 28% ($p=2 \times 10^{-10}$) of Anglo-Saxons. The only mutation detected in the Yupik speaking Eskimos of Western Alaska was the IVS2 AS -13 (A/C to G) mutation ($p=0.003$). In the Iranian population, the IVS2 AS -13 (A/C to G) mutation represented 41% ($p=0.0003$) of the alleles. In the East Indians, the Q318X (1994 C to T) mutation constituted 16% ($p=0.003$) of the alleles. In the Croatians, the R356W (2109 G to A) mutation represented 14% ($p=0.00003$) of the alleles. Though the frequency of the V281L (1685 G to T) mutation was relatively high in European populations, this mutation was not detected in Yupik-speaking Eskimos of Western Alaska, Native Americans, East Indian or Asian populations.

Genotype analysis reveals that the homozygous V281L (1685 G to T) mutation is prevalent in Ashkenazi Jews (42%), while the homozygous IVS2 AS -13 (A/C to G) mutation is common in Iranians (41%) and Yupik-speaking Eskimos of Western Alaska (100%) (Tables 3a and 3b). The Iranian genotypes demonstrate a high percentage of the homozygous IVS2 AS -13 (A/C to G) mutation. Of the 25 patients with the homozygous IVS2 AS -13 (A/C to G) mutation, 20 were from consanguineous families. This resulted in 30 distinct alleles instead of 50. The remaining 5 patients with the homozygous IVS2 AS -13 (A/C to G) mutation may be hemizygous due to a large deletion. However, the large deletion could not be detected because there was not enough DNA available for Southern blot analysis.

In patients with the classical form of CAH, the IVS2 AS -13 (A/C to G) mutation and the large deletion were the most frequent mutations observed (Table 3 a-d). Among compound heterozygote patients, the Ashkenazi Jewish patients have high percentages of the following mutations: large deletion/ V281L (1685 G to T) (14%) or IVS2 AS -13 (A/C to G)/ V281L (1685 G to T) (16%) (3c).

Discussion

In the human *CYP21A2* gene, approximately 103 mutations have been described which cause congenital adrenal hyperplasia [38]. The pseudogene, *CYP21A1P*, duplicates the active gene, *CYP21A2*. The mutations in the active gene may result from unequal crossing over, resulting in deletion of the gene. The common point mutations in the active gene are present in the pseudogene. Thus, point mutations in the active gene may result from gene conversion. Steroid 21-hydroxylase deficiency is observed worldwide and the ethnic specificity of the mutations in the *CYP21A2* gene is of interest.

In the *CYP21A2* gene, the frequent V281L (1685 G to T) mutation observed in Ashkenazi Jews, the IVS2 AS -13 (A/C to G) mutations in Yupik speaking Eskimos of Western Alaska, and the large gene deletion in Native Americans demonstrate that there is ethnic specificity of the mutations in *CYP21A2* gene. This may have resulted from an ancient founder effect, a hot spot in the gene, unequal crossing over during meiosis or gene conversion of point mutations in the pseudogene. The mutation in V281L (1685 G to T) in Ashkenazi Jews also occurs in other populations, which makes a founder effect less likely. However, since the Ashkenazi Jews are an endogamous population, a combination of both a hot spot and the founder effect is possible. The periodic pogroms experienced by the Ashkenazi Jewish population in Europe may also have contributed to a genetic bottleneck effect. This would have resulted in a severe reduction in the population, which would have reduced the diversity of the original gene pool. The mutation could then have occurred in this small population; and then through genetic drift, the reduced population would have slowly increased. Thus, the mutation would have become prevalent in the expanded population.

The V281L (1685 G to T) mutation results in a mild deficiency and is usually observed in the mild, nonclassical phenotype. In certain populations (Asian and Native American), this V281L (1685 G to T) mutation is not observed. This may be because NC21OHD does not occur in that population, or because NC21OHD has not been clinically diagnosed in the population. We have demonstrated that the severe mutations of IVS2 AS -13 (A/C to G) and deletion in the *CYP21A2* gene cause the severe form of 21OHD.

In general, our data is consistent with previously published reports of 21OHD mutation frequencies in specific ethnic populations. However, we have found some differences. The mutations in patients from the Emilia-Romagna province in northern Italy reported herein had a large deletion on only 3% of their 106 alleles. A previous report from Emilia-Romagna by Balsamo et al found 13% of 114 alleles with the large deletion mutation [39]. This is surprising as these patients originated from the same region in Italy. In the Italo-American population from a Southern Italian heritage studied in this paper, 15% of 100 alleles carried the large deletion, which resembles Balsamo et al's report from the northern province of Emilia-Romagna. Despite the similarity in mutation frequency, these two populations are very different in origin. In Iranian patients, we report that 33% of 80 alleles carried the IVS2 AS -13 (A/C to G) mutation, while a previous report from Vakili et al found only 15% out of 60 alleles with this mutation [40]. Vakili et al also found 10% of 60 alleles carrying the 8 bp deletion ($\Delta 707-714$) in exon 3, but this mutation was not detected in our patients. This variance in mutation frequency may be due to regional differences within Iran – our patients come from the area near Tehran, while the patients in Vakili et al's report reside in the Khorasan provinces of northeastern Iran – but has not been established. In our East Indian population, we have found a significant percentage of the Q318X (1994 C to T) mutation, which occurred in 6 of 38 (16%) alleles. This percentage is similar to previous data from Mathur et al, who reported 22% of 46 alleles with the Q318X mutation [41]. However, we found the large deletion in only 5% of 38 alleles in our East Indian patients, while Mathur et al found 16% of 46 alleles [41]. The mutation frequencies in our French patients match previous reports [42-45], with the exception of the IVS2 AS -13 (A/C to G) mutation. We found 41% of 32 alleles with the IVS2 AS -13 (A/C to G) mutation, whereas previous reports showed only 14% to 23%. The high frequency of the IVS2 AS -13 (A/C to G) mutation in our French population may be due to the lower number of alleles in this study; the previous studies included 3 to 8 times more alleles.

In 21OHD, there is largely a good correlation of genotype with phenotype, so that patients with homozygous mild and heterozygous mild/severe mutations express the nonclassical form of the disease. Patients with classical 21OHD have two severe mutations. However, there are rare examples of genotype/phenotype non-correlation, which will require further exploration of

factors that modify the expression of the *CYP21A2* gene. Genotype/phenotype noncorrelation occurs in patients with the IVS2 AS -13 (A/C to G) mutation [16,44,46,47], which may result from the leaky expression of this mutation. The leaky expression could be due to variants in RNA splicing factors. Buchner et al. have shown that a variant of the putative RNA splicing factor *SCNM1* resulted in a more severe phenotype in mice with a splice donor mutation in the *Scn8a* gene [48]. This *SCNM1* variant resulted in only 5% of the normal spliced transcript compared to 10% in the normal *SCNM1* variant.

The ethnic specificity of the *CYP21A2* mutations can guide physicians toward the diagnosis of 21OHD. NC21OHD is underdiagnosed by pediatricians, internists, endocrinologists, gynecologists, and reproductive urologists, as the signs of hyperandrogenism are often mild. Further, newborn hormonal screening for CAH does not detect NC21OHD because of modest or borderline elevation of the index hormone. The high frequency of specific mutations within ethnic groups may stimulate diagnosis of 21OHD by clinicians practicing in communities in which there is a predominant ethnic group described herein. Finally, the genotype/phenotype correlation data presented are valuable in prenatal diagnosis and treatment where diagnosis is usually based on genotype.

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Table 1Common mutations in *CYP21A2* gene causing 21-hydroxylase deficiency

Mutations	Location	% activity based on <i>in vitro</i> studies	Severity	References
Large deletion		0	severe	[26]
P30L	Exon 1	30-60	mild	[24]
IVS2 AS-13(A/C to G)	Intron 2	<5	severe	[47]
Δ707-714	Exon 3	0	severe	[49]
I172N	Exon 4	1	moderate	[30,31]
V281L	Exon 7	20-50	mild	[31,50]
Q318X	Exon 8	0	severe	[51]
R356W	Exon 8	0	severe	[52]

Table 2a
Frequency of Allelic Mutations in the CYP21A2 Gene in Ethnic Groups (Number of Alleles, (% of Alleles, p values)).

	Anglo-Saxon	Ashkenazi Jews	Croatian	French	Hispanic	Iranian	Italian
Del	52 (28%, 2×10 ⁻¹⁰)	27 (9%)	6 (6%)	5 (16%)	23 (14%)	*	3 (3%)
Int2	45 (24%)	43 (14%)	32 (32%)	13 (41%)	4 (2%)	33 (41%, 3×10⁻⁴)	23 (23%)
Ex3	5 (3%)	2 (1%)	3 (3%)	2 (6%)	7 (4%)	ND	1 (1%)
Ex4	11 (6%)	6 (2%)	8 (8%)	3 (9%)	14 (9%)	8 (10%)	14 (14%)
Ex7	7 (4%, 1×10⁻⁵)	196 (63%, 5×10⁻⁵⁴)	11 (11%)	2 (6%)	28 (17%)	1 (1%)	34 (34%)
Ex8 318	8 (4%)	6 (2%)	9 (9%)	1 (3%)	7 (4%)	8 (10%)	5 (5%)
Ex8 356	13 (7%)	5 (2%)	14 (14%, 3×10⁻⁵)	2 (6%)	8 (5%)	4 (5%)	6 (6%)
Ex1 Int2 Ex3	19 (10%)	11 (4%)	7 (7%)	1 (3%)	7 (4%)	9 (11%)	1 (1%)
Total Alleles	186	310	100	32	162	80	106

ND= none detected, Del=large deletion, Ex1=P30L (89 C to T), Int2= IVS2 AS -13 (A/C to G), Ex3=8 bp deletion (Δ707-714), Ex4=I172N (1001 T to A), Ex7= V281L (1685 G to T), Ex8 318=Q318X (1994 C to T), Ex8 356=R356W (2109 G to A).

* Deletions were not determined for Iranian samples. Therefore, homozygosity may be hemizyosity up to 15 out of the 19 families that are not consanguineous. There other 4 families were compound heterozygotes. Bolded number of patients and percents indicate statistical significance of < 0.002 as compared to the other ethnic groups utilizing the Chi Square test.

Table 2b
Frequency of Allelic Mutations in the CYP21A2 Gene in Ethnic Groups (Number of Alleles, (% of Alleles, p value)).

	Italo-American	Eskimo (Yupik)	Native American	Arabic	Asian	East Indian	Thai	African American
Del	15 (15%)	ND	4 (40%)	ND	6 (20%)	2 (5%)	11 (8%)	11 (18%)
Int2	21 (26%)	14 (100%)	4 (40%)	6 (38%)	7 (23%)	11 (29%)	47 (33%)	14 (23%)
Ex3	4 (4%)	ND	1 (10%)	ND	ND	ND	5 (4%)	1 (2%)
Ex4	13 (13%)	ND	ND	ND	4 (13%)	4 (11%)	22 (15%)	6 (10%)
Ex7	15 (15%)	ND	ND	ND	ND	ND	ND	3 (5%)
Ex8 318	6 (6%)	ND	ND	3 (19%)	ND	6 (16%) $\times 10^{-3}$	1 (1%)	4 (6%)
Ex8 356	ND	ND	ND	1 (6%)	2 (7%)	2 (5%)	6 (4%)	3 (5%)
Ex1 Int2	8 (8%)	ND	ND	3 (19%)	3 (10%)	6 (16%)	ND	4 (6%)
Ex3								
Total	100	14	10	16	30	38	142	62
Alleles								

ND= none detected, Del=large deletion, Ex1=P30L (89 C to T), Int2= IVS2 AS -13 (A/C to G), Ex3=8 bp deletion (Δ 707-714), Ex4=I172N (1001 T to A), Ex7= V281L (1685 G to T), Ex8 318=Q318X (1994 C to T), Ex8 356=R356W (2109 G to A). * Deletions were not determined for Iranian samples. Bolded number of patients and percents indicate statistical significance of < 0.002 as compared to the other ethnic groups utilizing the Chi Square test. Pearson exact test was performed for Eskimos ($p=0.003$), Native Americans ($p=0.02$), and Arabics ($p=0.02$). Statistical significance was considered with $p < 0.05$.

Table 3a
Phenotypes of Patients with Homozygous Mutations in the *CYP21A2* Gene in Various Ethnic Groups (Number of Patients, % of Genotypes).

	Anglo-Saxon	Ashkenazi Jews	Croatian	French	Hispanic	Iranian*	Italian	Italo-American
Del/Del	6 SW (6%)	ND	ND	1 SW (6%)	2 SW (2%)	24 SW, 1 SV (41%)	ND	2 SW (4%)
Int2/Int2	4 SW (4%)	5 SW (3%)	7 SW; 2 SV (18%)	2, SW (13%)	8 SW, 1 SV (11%)	1 SW (2%)	5 SW (9%)	6 SW (12%)
Ex7/Ex7	2 NC (2%)	65, NC (42%)	1 SW, 2 NC (6%)	ND	4 NC (5%)	1 SW (8%)	4 NC (7%)	3 NC (6%)
Ex3/Ex3	ND	ND	1 SW (2%)	ND	1 SW (1%)	ND	ND	ND
Ex4/Ex4	ND	1 SV (1%)	1 SV (2%)	ND	ND	1 SW, 4 SV (8%)	1 SV (2%)	ND
Ex8318/	ND	ND	1 SW (2%)	ND	ND	5 SW (8%)	ND	1 SW (2%)
Ex8318								
Ex8356/	2 SW (2%)	1 SW (1%)	4 SW (8%)	ND	ND	3 SW (5%)	ND	ND
Ex8356								
Ex1 Int2	2 SW (2%)	ND	ND	ND	1 SW (1%)	6 SW (10%)	ND	1 SW (2%)
Ex3/ Ex1								
Int2 Ex3								
Total	93	155	50	16	81	61	53	50
Patients								

SW= salt wasting, SV=simple virilizing, NC=nonclassical, ND= none detected, Del=large deletion, Ex1=P30L (89 C to T), Int2= IVS2 AS -13 (A/C to G), Ex3=8 bp deletion (Δ707-714), Ex4=1172N (1001 T to A), Ex7= V281L (1685 G to T), Ex8 318=Q318X (1994 C to T), Ex8 356=R356W (2109 G to A).

* Deletions were not determined for Iranian samples. Therefore, homozygosity may be hemizyosity up to 15 out of the 19 families that are not consanguineous. There other 4 families were compound heterozygotes. The rare genotypes are not indicated.

Table 3b

Phenotypes of Patients with Homozygous Mutations in the *CYP21A2* Gene in Various Ethnic Groups (Number of Patients, % of Genotypes).

	Eskimo (Yupik)	Native American	Arabic	Asian	East Indian	Thai	African American
Del/Del	ND	ND	ND	1 SW (7%)	ND	1 SW (1%)	2 SW (6%)
Int2/Int2	7 SW (100%)	1 SW (20%)	3 SW (38%)	1 SW (7%)	2 SW, 1 SV (16%)	4 SW, 1 SV (7%)	1 SW (3%)
Ex7/Ex7	ND	ND	ND	ND	ND	ND	1 NC (3%)
Ex3/Ex3	ND	ND	ND	ND	ND	ND	ND
Ex4/Ex4	ND	ND	ND	1 SV (7%)	2 SV (11%)	4 SV (6%)	ND
Ex8318/	ND	ND	1 SW (13%)	ND	1 SW (5%)	ND	ND
Ex8318	ND	ND	ND	ND	ND	ND	ND
Ex8356/	ND	ND	ND	ND	ND	ND	ND
Ex8356	ND	ND	1 SW (13%)	ND	2 SW (11%)	ND	ND
Ex1 Int2 Ex3/	ND	ND	ND	ND	ND	ND	ND
Ex1 Int2 Ex3	ND	ND	ND	ND	ND	ND	ND
Total	7	5	8	15	19	72	31
Patients							

SW= salt wasting, SV=simple virilizing, NC=nonclassical, ND= none detected, Del=large deletion, Ex1=P30L (89 C to T), Int2= IVS2 AS -13 (A/C to G), Ex3=8 bp deletion (Δ 707-714), Ex4=1172N (1001 T to A), Ex7= V281L (1685 G to T), Ex8 318=Q318X (1994 C to T), Ex8 356=R356W (2109 G to A). Rare genotypes are not indicated.

Table 3c

Phenotypes of Patients with Heterozygous Mutations in the *CYP21A2* Gene in Various Ethnic Groups (Number of Patients, % of Genotypes).

	Anglo-Saxon	Ashkenazi Jews	Croatian	French	Hispanic	Iranian*	Italian	Italo-American
Del/Ex1	1 NC (1%)	ND	ND	ND	1 SV, 1 NC (2%)	ND	ND	ND
Del/Ex1 Int2 Ex3	7 SW (8%)	5 SW (3%)	1 SW (2%)	ND	2 SW (2%)	ND	ND	1 SW (2%)
Del/Int2	17 SW (18%)	ND	1 SW (2%)	1 SW, 1 SV (13%)	1 SW, 2 SV (4%)	ND	ND	1 SW (2%)
Del/Ex3	2 SW (2%)	1 SW (1%)	ND	ND	ND	ND	ND	ND
Del/Ex4	1 SW, 1 SV (2%)	1 SV (1%)	ND	1 SV (6%)	5 SV (6%)	ND	ND	5 SV (8%)
Del/Ex7	ND	21 NC, 1 SW (14%)	ND	ND	4 NC (5%)	ND	3 NC (5%)	3 NC (6%)
Del/Ex8318	5 SW (5%)	ND	2 SW (4%)	ND	1 SW (1%)	ND	ND	ND
Del/Ex8356	2 SW, 1 SV (2%)	ND	1 SW (2%)	ND	1 SW (1%)	ND	ND	ND
Ex1 Int2	2 SW (2%)	1 SW (1%)	2 SW (4%)	ND	2 SW, 1 SV (4%)	2 SW (3%)	ND	1 SW (2%)
Ex3/Int2	1 SW, 1 SV (2%)	ND	1 SV (2%)	1 SV (6%)	ND	ND	ND	1 SV (2%)
Ex1 Int2	2 NC (2%)	1 SW, 3 NC (3%)	1 NC (2%)	ND	ND	ND	ND	1 NC (1%)
Ex3/Ex7	ND	ND	1 SW (2%)	ND	ND	ND	1 SW (2%)	ND
Ex3/Ex8356	1 SW, 1 SV, 3 NC (5%)	1 SV (1%)	1 SV, 1 NC (4%)	ND	1 NC (1%)	ND	ND	ND
Ex1/Ex3	ND	ND	1 SV (2%)	ND	ND	ND	ND	1 SV (2%)
Ex3/Int2	2 SW (2%)	ND	ND	1 SW (6%)	1 SV (1%)	1 SW (2%)	ND	1 NC (2%)
Ex4/Int2	3 SV (3%)	2 SV (1%)	1 SW, 1 SV (4%)	1 SV (6%)	1 SV (1%)	ND	1 SW, 2 SV (5%)	1 SW, 3 SV (8%)
Ex4/Ex7	ND	1 NC (1%)	2 NC (4%)	ND	5 NC (6%)	ND	5 NC (9%)	6 NC (6%)
Ex4/Ex8318	2 SV (2%)	ND	ND	ND	ND	ND	ND	1 SV (2%)
Ex4/Ex8356	1 SW (1%)	ND	1 SW (2%)	ND	ND	1 SW (2%)	1 SV (2%)	ND
Ex7/Int2	1 NC (1%)	1 SW, 1 SV, 23 NC (16%)	1 NC (2%)	2 NC (13%)	5 NC (6%)	ND	1 SW, 6 NC (13%)	2 NC (4%)
Ex7/Ex8318	ND	1 SV, 3 NC (3%)	1 NC (2%)	ND	1 NC (1%)	ND	5 NC (9%)	ND
Ex8318/Int2	1 SW (1%)	1 SW (1%)	2 SW 1 SV (6%)	1 SW (6%)	3 SW (4%)	2 SW (3%)	ND	ND
Ex8356/Int2	3 SW (3%)	2 SW (1%)	2 SW (4%)	1 SW (6%)	1 SW (1%)	ND	1 SW, 1 SV (4%)	ND
Total Patients	93	155	50	16	81	61	53	50

SW= salt wasting, SV=simple virilizing, NC=nonclassical, ND= none detected, Del=large deletion, Ex1=P30L (89 C to T), Int2= IVS2 AS -13 (A/C to G), Ex3=8 bp deletion (Δ 707-714), Ex4=I172N (1001 T to A), Ex7= V281L (1685 G to T), Ex8 318=Q318X (1994 C to T), Ex8 356=R356W (2109 G to A). Rare genotypes are not indicated.

* Deletions were not determined for Iranian samples.

Table 3d

Phenotypes of Patients with Heterozygous Mutations in the *CYP21A2* Gene in Various Ethnic Groups (Number of Patients, % of Genotypes).

	Eskimo (Yupik)	Native American	Arabic	Asian	East Indian	Thai	African American
Del/Ex1 Del/Ex1 Int2 Ex3	ND ND	1 SV (1%) ND	ND ND	ND ND	ND 1 SW (5%)	ND 1 SW, 1 SV (3%)	ND 1 SW (3%)
Del/Int2	ND	1 SW, 1 SV (40%)	ND	1 SW, 1 SV (13%)	ND	2 SW, 1 SV (4%)	4 SW (13%)
Del/Ex3	ND	1 SW (1%)	ND	ND	ND	1 SW (1%)	ND
Del/Ex4	ND	ND	ND	ND	ND	1 SV (1%)	1 SV (3%)
Del/Ex7	ND	ND	ND	ND	ND	ND	ND
Del/Ex8318	ND	ND	ND	ND	ND	ND	ND
Del/Ex8356	ND	ND	ND	1 SW (7%)	ND	ND	ND
Ex1 Int2 Ex3/ Int2	ND	ND	ND	1 SW (7%)	ND	7 SW, 1 SV (11%)	1 SW (3%)
Ex1 Int2 Ex3/ Ex4	ND	ND	ND	1 SW, 1 SV (13%)	ND	1 SV (1%)	ND
Ex1 Int2 Ex3/ Ex7	ND	ND	ND	ND	ND	ND	ND
Ex1 Int2 Ex3/ Ex8356	ND	ND	ND	ND	1 SW (5%)	2 SW (3%)	ND
Ex1/Int2	ND	ND	ND	ND	1 SW (5%)	ND	1 SW (3%)
Ex1/Ex3	ND	ND	ND	ND	ND	ND	ND
Ex3/Int2	ND	ND	ND	ND	ND	ND	ND
Ex4/Int2	ND	ND	ND	ND	ND	1 SW, 8 SV (13%)	1 SV (3%)
Ex4/Ex7	ND	ND	ND	ND	ND	ND	ND
Ex4/Ex8318	ND	ND	ND	ND	ND	ND	1 SW (3%)
Ex4/Ex8356	ND	ND	ND	ND	ND	2 SV (2%)	1 SW (3%)
Ex7/Int2	ND	ND	ND	ND	ND	ND	ND
Ex8318/Int2	ND	ND	ND	ND	2 SW, 1 SV (16%)	1 SW (1%)	1 SW, 1 SV (6%)
Ex8356/Int2	ND	ND	ND	ND	1 SW (5%)	1 SW (1%)	ND
Total Patients	7	5	8	15	19	72	31

SW = salt wasting, SV = simple virilizing, NC = nonclassical, ND = none detected, Del = large deletion, Ex1 = P30L (89 C to T), Int2 = IVS2 AS -13 (A/C to G), Ex3 = 8 bp deletion (Δ707-714), Ex4 = I172N (1001 T to A), Ex7 = V281L (1685 G to T), Ex8 318 = Q318X (1994 C to T), Ex8 356 = R356W (2109 G to A). Rare genotypes are not indicated.