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Revisiting the prevalence of nonclassic congenital adrenal hyperplasia in US Ashkenazi Jews and Caucasians

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Purpose: Nonclassic 21-hydroxylase deficiency, a mild form of congenital adrenal hyperplasia (CAH), is estimated to be the most common autosomal recessive condition, with an especially high prevalence in Ashkenazi Jews (3.7% affected, 30.9% carriers), based on a 1985 HLA-B linkage study of affected families. Affected individuals, especially women, may suffer from hyperandrogenism and infertility. State-of-the-art genetic studies have not been done to confirm these remarkable rates.

Methods: *CYP21A2* genotyping was performed in 200 unrelated healthy Ashkenazi Jewish subjects and 200 random US Caucasians who did not self-identify as a specific ethnicity using multiplex minisequencing, real-time polymerase chain reaction and junction site analysis.

Results: Nonclassic CAH carriership was found similarly in 15% (95% confidence interval (CI): 10.4–20.7) of Ashkenazi Jews and 9.5% (95% CI: 5.8–14.4) of Caucasians ($P = 0.13$). The proportion of Ashkenazi Jewish nonclassic CAH carriers (0.15 versus 0.309, $P < 0.0001$) and disease affected (0.005 versus 0.037, $P = 0.009$) was not as high as previously reported. The estimated prevalence of nonclassic CAH in Caucasians was 1 in 200 (0.5%, 95% CI: 0.01–2.8).

Conclusion: Nonclassic CAH is a common condition, regardless of ethnicity, and should be considered with preconception and infertility counseling.

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Key Words: Ashkenazi Jews; congenital adrenal hyperplasia; infertility; nonclassic; prevalence

INTRODUCTION

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive disorders due to enzymatic defects in steroidogenesis. More than 95% of all cases of CAH are caused by 21-hydroxylase deficiency (*CYP21A2;* OMIM 201910), which manifests as cortisol deficiency, with or without aldosterone deficiency, and androgen excess. The clinical spectrum ranges from mild to life-threatening, determined by the functional impairment of the corresponding genetic mutation. Because CAH follows an autosomal recessive pattern of inheritance, the phenotype is typically correlated with the less affected allele. Neonatal screening for the classic or severe life-threatening form of 21-hydroxylase deficiency is performed in over 40 countries. Thus, the epidemiology of the classic form is well established (1:10,000 to 1:20,000 live births) due to the neonatal screening of millions of newborns worldwide.[1,2] However, neonatal screening was not designed to detect the nonclassic or mild form of CAH, which was previously estimated to be common (0.1% of Caucasians), especially in Ashkenazi Jews (3.7% affected, 30.9% carrier rate),[3] a population considered a genetic isolate at high risk for autosomal recessive diseases.[4] Given the dramatic advances in genetics in the past three decades, it is quite remarkable that the commonly cited epidemiologic rates for nonclassic CAH are based on a 1985 human leukocyte antigen linkage study of 210 affected families (167 families with the classic form and 43 families with the nonclassic form).[3]

The 21-hydroxylase enzyme is encoded by the *CYP21A2* gene on chromosome 6 within the human leukocyte antigen region. Hundreds of mutations have been described, with the 12 most common mutations accounting for approximately 95% of all mutations, including three mutations (p.V281L, p.P453S, p.P30L) associated with the nonclassic phenotype.[5] The mild phenotype of nonclassic CAH is conferred by mutations that retain 20 to 50% of enzyme activity.[6] A point mutation in exon 7 (p.V281L) accounts for the majority of nonclassic alleles worldwide and is the mutation previously associated with the Ashkenazi Jewish population.[7]

Nonclassic CAH may manifest with signs and symptoms of androgen excess or may be asymptomatic.[1,2] The most common presentation during childhood is precocious pubarche, while adult women may present with hirsutism, menstrual cycle disorders, or infertility.[8–10] In this study, we report, through state-of-the-art *CYP21A2* analysis, that nonclassic CAH is less common in US Ashkenazi Jews than

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previously suggested and that nonclassic CAH may be more commonly found than previously thought in the US general Caucasian population.

MATERIALS AND METHODS

We performed genotyping in 200 unrelated healthy subjects of Ashkenazi Jewish descent, defined as having four Ashkenazi Jewish grandparents, and 200 healthy Caucasians who did not self-identify as a specific ethnicity. DNA was collected from The Foundation for Jewish Genetic Medicine (Ashkenazi Jews), and the Coriell Institute for Medical Research Biorepository (Caucasians). DNA was analyzed for the 12 most common *CYP21A2* mutations (p.P30L, IVS2-13A/C > G, exon 3 and 8-bp deletion (p.G110Efs), p.I172N, exon 6 cluster (p.I236N, p.V237E, p.M239K), p.V281L, p.Leu307fs, p.Q318X, p.R356W, and p.P453S) and deletions using multiplex minisequencing, real-time polymerase chain reaction, and junction site analysis.[5] The following mutations were regarded as nonclassic as previously described:[5,9,10] p.V281L, p.P453S, and p.P30L. The 30-kb deletions were confirmed by a clinically validated Sanger sequencing methodology at a commercial CLIA/College of American Pathologists certified DNA diagnostics laboratory (PreventionGenetics, Marshfield, WI). p.Q318X in the setting of gene duplication was not considered pathogenic.

Our sample size was calculated based on carrier rates and the hypothesis that the nonclassic CAH carrier rate in our Ashkenazi Jewish group would be similar to those reported in various European population-based studies across different ethnicities (ranging from 4% to 7.5%).[11–14] Compared to the historical estimate of 30.9% carrier status among Ashkenazi Jews, our calculations yielded between 90 and 99% power to detect a statistically significant difference at an alpha level of 0.05. Calculation of expected prevalence was based on the Hardy–Weinberg equilibrium using the allele frequencies of our data. Proportions (with exact 95% confidence intervals (CI)) and comparisons between groups were analyzed using the binomial and Fisher's exact tests, respectively.

RESULTS

Of the 200 Ashkenazi Jews screened, 15% (95% CI: 10.4–20.7) were nonclassic CAH carriers, and 2.5% (95% CI: 0.8–5.7) were classic CAH carriers (**Table 1**). Of the Caucasians screened, 9.5% (95% CI: 5.8–14.4) were nonclassic CAH carriers, and 1.5% (95% CI: 0.3–4.3) were classic CAH carriers. One subject in each cohort (0.5%, 95% CI: 0.01–2.8) had a genotype consistent with being affected with nonclassic CAH. The proportion of Ashkenazi Jewish nonclassic CAH carriers (0.15 versus 0.309, $P < 0.0001$) and disease affected (0.005 versus 0.037, $P = 0.009$) was not as high as previously reported[3] and did not differ from our random sample of Caucasians (0.15 versus 0.095, $P = 0.13$). The estimated prevalence of nonclassic CAH in our general US Caucasian population was 1 in 200 (0.5%, 95% CI: 0.01–2.8).

DISCUSSION

Since 1985, the prevalence of nonclassic CAH has not been revisited in the United States using advanced genetic testing, despite the fact that prior rates are based on a cohort of patients,[3,15] not a random population-based study, and human leukocyte antigen linkage analysis does not optimally utilize genetic information. In 1990, a population-based study of 249 subjects (62 Ashkenazi Jews, 187 others) in New York City by the same group found similarly high estimated prevalence rates of nonclassic CAH of 1.14 and 3.23% among Caucasians and Ashkenazi Jews, respectively, but this was

Table 1 Population statistics for carriership of nonclassic and classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Ashkenazi Jewish and general US Caucasian populations.

	Carriers no.	Carriers % (95% CI)	Carrier frequency
Ashkenazi population ($N = 200$)			
Nonclassic CAH[a]	30	15.0 (10.4–20.7)	1 in 7
p.V281L	28	14.0 (9.5–19.6)	
p.P453S	2	1.0 (0.1–3.6)	
Classic CAH	5	2.5 (0.8–5.7)	1 in 40
IVS2-13A/C > G	3	1.5 (0.3–4.3)	
30-kb deletion	1	0.5 (0.01–2.8)	
p.I172N, exon 6 cluster[b], p.V281L, p.Leu307fs	1	0.5 (0.01–2.8)	
US Caucasian population ($N = 200$)			
Nonclassic CAH[a]	19	9.5 (5.8–14.4)	1 in 11
p.V281L	17	8.5 (5.0–13.3)	
p.P453S	2	1.0 (0.1–3.6)	
Classic CAH	3	1.5 (0.3–4.3)	1 in 67
IVS2-13A/C > G	2	1.0 (0.1–3.6)	
30-kb deletion	1	0.5 (0.01–2.8)	

CAH, congenital adrenal hyperplasia; CI, exact confidence interval for the binomial proportion.
Nomenclature at the protein level is based on conventional codon numbering.
[a]One possibly affected subject, resulting in a prevalence of nonclassic CAH of 1 in 200 (0.5%, 95% CI: 0.01–2.8). [b]Exon 6 cluster (p.I236N, p.V237E, p.M239K).

based on morning salivary 17-hydroxyprogesterone, not genetics.[15] Our study is the first to demonstrate, through state-of-the-art *CYP21A2* analysis, that nonclassic CAH is less common in Ashkenazi Jews than previously suggested. Our estimated carrier and disease rates for Ashkenazi Jews were significantly lower than previous reports.[3] Our study also suggests that nonclassic CAH is commonly found in the general US Caucasian population, with an estimated prevalence of 1 in 200 (0.5%, 95% CI: 0.01–2.8).

These results have important implications. As nonclassic CAH may result in infertility, which is easily treated with glucocorticoid therapy,[16] and nonclassic CAH women not receiving glucocorticoid therapy have higher miscarriage rates than those receiving treatment,[1,16] our new data will aid with preconception and infertility counseling. Importantly, current infertility guidelines[17] do not address nonclassic CAH. Our estimate of 1 in 200 disease rates for nonclassic CAH in the US Caucasian population argue for screening in the setting of female infertility, regardless of ethnicity, especially since hyperandrogenic symptoms may not be present.

The mild nonclassic form of CAH was first discovered during studies of family members of patients with the classic form of CAH. The lack of symptomatology led to the term cryptic CAH being used to describe an individual who is genetically found to have nonclassic CAH, but is apparently asymptomatic. Although individuals found to have nonclassic CAH by family genetic studies are mostly asymptomatic, female infertility and suboptimal cortisol response have been commonly described in such individuals.[18] In addition, there is some evidence that symptomatology worsens with age.[8] Affected males usually do not exhibit symptoms, but infertility and oligospermia have been described infrequently.[10] Affected women more commonly suffer from infertility than affected men due to hormonal imbalances that result in menstrual irregularities. In both males and females, infertility and gonadal dysfunction can be reversed with glucocorticoid treatment. The clinical presentation of women with nonclassic CAH may be identical to that of polycystic ovary syndrome, a common ovarian disorder associated with androgen excess in women and infertility. Since the treatment and management of these two conditions differ, it is essential to distinguish between these two disease entities. Our findings argue for ruling out the diagnosis of nonclassic CAH in the setting of female hyperandrogenism.

The p.V281L mutation is the most commonly identified *CYP21A2* variant associated with the nonclassic form of CAH in both studies of patients and population-based studies of unaffected individuals. Large cohort studies of patients with CAH report an allele frequency of the p.V281L and p.P30L mutations that ranges from 2.2 to 23.9% and 0.5% to 2.6%, respectively, and $< 1.0\%$ for p.P453S.[5,7] The allele frequency of *CYP21A2* mutations has been estimated in a few populations based on genetic studies of newborns undergoing neonatal screening. *CYP21A2* genotyping in a randomly chosen sample of 603 New Zealand neonates found a frequency of 4.8% (1 in 21) CAH carrier rate and a frequency of 2.0% (1 in 50) nonclassic CAH carrier rate, all p.V281L genotype.[11] Blood samples from 300 neonates in Spain were genotyped, and the p.V281L allele frequency was found to be 7.5%.[12] Similar population-based studies in Austria and Cyprus, Greece found carrier rates of nonclassic CAH to be 4.0% (3.5% p.V281L, 0.5% p.P30L) and 6.4% (4.3% p.V281L, 1.3% p.453S, 0.8% rare variant p.V304M) respectively.[13,14] Although it is possible that the high frequency of the p.V281L mutation amongst Cypriots is due to a founder effect, our study supports the concept that the p.V281L mutation may be a frequent mutation worldwide.

The high frequency of nonclassic CAH across ethnicities suggests that there may be an evolutionary advantage to the carrier state. Alterations in the hypothalamic–pituitary–adrenal axis have been described in carriers of *CYP21A2* mutations, with brisk cortisol response compared to controls.[19] In a study of patients with sex chromosome aberrations (Turner and Klinefelter syndromes), the *CYP21A2* p.V281L mutation was higher in the patients affected by chromosomal aberrations compared to the general population, suggesting a possible survival advantage of the fetus carrying the p.V281L mutation.[20] The *CYP21A2* gene is located in the human leukocyte antigen major histocompatibility complex, an area of the genome with a high frequency of crossover events and also an area of the genome that modulates autoimmune disease risk. Additional studies are needed to expand our understanding of the clinical implications of both the nonclassic CAH disease and carrier states.

Our study has limitations. First, the selection criteria for both cohorts were based on self-reported ethnicity data that may not be accurate. Second, the distribution of multiple mutations among alleles was unknown because parental DNA was not available; thus, disease status may have been overestimated. However, only two subjects, one from each cohort, were found to have multiple mutations. Each of these subjects was presumed to be affected. Third, rare mutations that may account for up to 5% of *CYP21A2* mutations were not identified.

Our results have informed our understanding of the prevalence of nonclassic CAH in the US Ashkenazi Jewish and Caucasian populations and suggest that nonclassic CAH is common across ethnicities. The commonly cited epidemiologic rates for nonclassic CAH underestimate the prevalence in the general US Caucasian population and overestimate the prevalence in Ashkenazi Jews. Genetic screening for this common and mostly undiagnosed condition would offer the opportunity for treatment. We encourage providers to have a low threshold for nonclassic CAH screening, especially in the setting of female infertility, regardless of ethnicity.

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DISCLOSURE

The authors declare no conflict of interest.

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