Analysis of CYP21 Coding Polymorphisms in Three Ethnic Populations: Further Evidence of Nonamplifying CYP21 Alleles Among Whites

I. CETIN OZTURK, MD,* WAN-LI WEI, MD,* LATHA PALANIAPPAN, MD,† MELVYN RUBENFIRE, MD,‡ ANTHONY A. KILLEEN, MD, PhD* Ann Arbor, Michigan

Background: Adrenal steroid 21-hydroxylase is essential for the synthesis of both mineralocorticoids and glucocorticoids. The gene for this enzyme, CYP21, contains several frequent coding polymorphisms. Because of its essential function in steroid synthesis, polymorphisms in this enzyme might influence a variety of disease processes. However, before disease-association studies are performed, it is important to understand the frequency of these polymorphisms among normal individuals.

Methods: Using polymerase chain reaction (PCR) with restriction enzyme digestion or size length polymorphism analysis, we measured the frequencies of the +Leu(10), Arg102Lys, and Ser268Thr polymorphisms in CYP21 in healthy whites, blacks, and Indian Americans. The subjects were all young female college students participating in a study of relative risks for cardiovascular disease in these populations.

Results: The frequency of each polymorphism among whites, blacks, and Indian Americans were as follows: +Leu(10), 0.55, 0.96, 0.75; Arg102, 0.63, 0.97, 0.82; and Ser268, 0.92, 0.68, 0.79, respectively. With the exception of the frequencies of the Ser268Thr polymorphism among blacks and Indian Americans, there were significantly different frequencies of each polymorphism among all groups (P < .05). Among whites, the distribution of genotypes for the +Leu(10) and Arg102Lys polymorphisms deviated significantly from expected Hardy-Weinberg values because of an excess of homozygotes.

Conclusions: Among the ethnic groups, there are statistically significant differences in the frequencies of these common coding polymorphisms in CYP21 that need to be considered in disease-association studies. Deviation from Hardy-Weinberg distributions might be explained by allelic dropout during PCR, a phenomenon previously reported at this locus.

Key words: CYP21, steroid 21-hydroxylase, allelic frequencies, polymorphisms, ethnic populations.

The genetic locus of the adrenal cytochrome P-450, steroid 21-hydroxylase, is in the class III region of the human major histocompatibility complex (MHC) [1]. This region normally contains the gene encoding the functional enzyme, CYP21, and a highly homologous pseudogene, CYP21P (Fig. 1) [2,3]. Mutations in CYP21 give rise to the usual form of congenital adrenal hyperplasia (CAH), an autosomal recessive disorder with an incidence of approximately one in 13,000 newborns for the classic form and up to 1% among some populations for the nonclassic form of the disease [4]. This disorder is characterized by impaired synthesis of glucocorticoids and mineralocorticoids and excessive production of adrenal androgens. The normal 21-
hydroxylase enzyme is also a frequent autoantigen in patients with Addison’s disease [5].

Several polymorphisms in the coding regions of CYP21 have been reported, including +Leu(10) in exon 1, Arg102Lys in exon 3, and Ser268Thr in exon 7 [6]. Steroid 21-hydroxylase activity is essential for the synthesis of both mineralocorticoids and glucocorticoids, and natural sequence variation might influence several disease processes as a result of alterations in the catalytic properties of the enzyme. A previous study analyzing the frequency of CYP21 coding polymorphisms among 12 Finnish patients with Addison’s disease found that the +Leu(10) and Arg102 polymorphisms were more frequent among patients than among 20 controls, although the differences were not statistically significant [7].

Before studying possible disease association with these polymorphisms, it is important to understand their frequencies among different populations, particularly if a disease of interest shows variation in prevalence among different ethnic groups. In addition to their possible association with disease, polymorphic genetic markers in the 21-hydroxylase genes are also of interest for determining the segregation of MHC haplotypes, especially in families with CAH. We recently reported frequencies of polymorphisms in introns 2 and 6 of CYP21 and intron 2 of CYP21P and showed the usefulness of these polymorphisms for both tracking the segregation of CYP21 alleles in families and showing deletions in CYP21 and CYP21P [8–10].

In this study, we examined the frequencies of the three coding polymorphisms in three ethnic groups: whites, blacks, and Indian Americans (i.e., descendants of immigrants from the Indian subcontinent).

Materials and Methods

Subjects from the three ethnic populations were recruited for participation in a comparative ethnic study of risk factors for cardiovascular disease among young women. Criteria for inclusion were age between 18 and 30 years, parents of the same ethnicity with no known family history of interracial marriage, and residents of the United States since the age of 10 years. Exclusion criteria were pregnancy, smoking habit, use of lipid-lowering medication, or use of oral contraceptives. None of the subjects was known to have cardiovascular disease. All subjects were women, and all were college students. The study was approved by the institutional review board. Data analyzing the cardiovascular risk factors in these subjects will be presented elsewhere. All DNA samples, which were available after the cardiovascular risks were analyzed, were used for the present study, which was also approved by the institutional review board.

DNA Extraction

DNA was extracted from peripheral-blood leukocytes by using a commercially available kit, Puregene (Gentra Systems, Minneapolis, MN), and redissolved in 10 mM Tris buffer, pH 8.0, and 1 mM EDTA.

Detection of Coding Polymorphisms

Portions of CYP21 were amplified by polymerase chain reaction (PCR) using the primers and reaction conditions shown in Tables 1 and 2 and Fig. 2. Selective amplification of CYP21 was by use of the CYP21-specific primers, b73R and P3 [11,12]. Both these primers recognize the 8 bp in CYP21 that are missing in the pseudogene, CYP21P (Fig. 2). All PCRs were performed using standard reagent concentrations: 20 mM Tris-Cl, pH 8.4; 50 mM KCl; 1.5 mM MgCl2; 200 μM each dNTP; 200 nM each primer; 2.5 U Taq polymerase; and 50 to 100 ng genomic DNA in a reaction volume of 50 or 100 μL.

For detection of the +Leu(10) polymorphism, a nested PCR reaction was used. First-round PCR reactions (30 cycles) used primers −43F and b73R. Second-round reactions (25 cycles) used 1 μL first-round products as template and primers 1F and 81R. The +Leu(10) polymorphism results in a sequence-length polymorphism in the PCR product (81 vs 84...