CYP3A5 GENETIC POLYMORPHISMS IN DIFFERENT ETHNIC POPULATIONS

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ABSTRACT:

Cyp3A5 activity varies within any given ethnic population, suggesting that genetic variation within the Cyp3A5 gene may be the most important contributor to interindividual and interracial differences in Cyp3A-dependent drug clearance and response. The full extent of Cyp3A5 polymorphism in a white and an indigenous African population was analyzed using DNA direct sequencing procedures. The presence of 10 and 12 single nucleotide polymorphisms was detected in the white and African samples, respectively. Thirteen novel mutations occurring at low frequencies were identified in these populations. Significant differences were observed in the distribution of Cyp3A5*3, Cyp3A5*6, and Cyp3A5*7 alleles among white and African populations. The frequency of Cyp3A5*3 allele in white Canadians (~93%) is higher than in Zimbabweans (77.6%) (p < 0.001). In contrast, Cyp3A5*6 and Cyp3A5*7 alleles are relatively frequent in African subjects (10–22%) but absent in white subjects (p < 0.001). These differences may reflect evolutionary pressures generated by environmental factors in geographically distinct regions. However, the genetic polymorphism of Cyp3A5 alone does not explain the interindividual differences in Cyp3A-mediated metabolism.

Materials and Methods

Our samples consisted of stored DNA extracts from 100 unrelated Zimbabweans of the Shona ethnic group recruited in the ZVITAMBO project and 77 unrelated whites from Quebec, Canada. The use of these samples for the present study was approved by ethics committees. DNA was extracted from whole peripheral blood using standard phenol-chloroform extraction procedures. The nucleotide sequence variants in the promoter region and the 13 exons of Cyp3A5 gene were determined by the direct sequencing method using 50 randomly selected samples of each population. The primers for PCR amplification of the promoter region and exon-specific fragments were designed from the wild-type Cyp3A5 sequence (GenBank accession number AC005020). The primers were designed to cover the entire promoter region and each exon, as well as sequences at the exon-intron boundaries that are important for messenger RNA (mRNA) splicing. Details regarding primer sequences and annealing temperatures for amplifying Cyp3A5 DNA fragments for sequencing are shown in Table 1. Amplification reactions contained 200 ng DNA template.
using chi square analysis. Genotypic frequencies were compared with Hardy-Weinberg expectations compared with published frequencies in other populations using the chi square test. Allelic frequencies in our study populations were according to the manufacturers' instructions (Table 2). The digestion products DNA products were then digested with the specific restriction enzymes ac- cording to the manufacturers’ instructions (Table 2). Genomic DNA was amplified with the specific conditions, resulting in modified mRNA that may generate A-RFLP analyses. The primer sequences, annealing temperatures, and restriction endonuclease enzymes for the A-RFLP analyses were determined by direct DNA sequencing. The primer sequences, annealing temperatures for amplifying the promoter and coding regions of Cyp3A5 for sequencing are indicated in Table 2. Genomic DNA was amplified with the specific temperatures, and restriction endonuclease enzymes for the A-RFLP analyses were determined by direct DNA sequencing. The reaction products were run in an automated DNA sequenc- ing ABI PRISM 3100 capillary sequencer (Applied Biosystems, Foster City, CA). All PCR products were sequenced in both directions. Specific polymorphisms with the potential to modify enzymatic activity and protein structure were typed by amplified restriction fragment length polymorphism (A-RFLP) in all the study samples. Cyp3A5*1B and Cyp3A5*1C alleles were determined by direct DNA sequencing. The primer sequences, annealing temperatures, and restriction endonuclease enzymes for the A-RFLP analyses are indicated in Table 2. Genomic DNA was amplified with the specific primers (Table 2) using the PCR conditions as described above. Amplified DNA products were then digested with the specific restriction enzymes according to the manufacturers’ instructions (Table 2). The digestion products were subjected to 2.5% agarose gel electrophoresis and detected by staining with ethidium bromide. Allelic frequencies in our study populations were compared with published frequencies in other populations using the chi square test. Genotypic frequencies were compared with Hardy-Weinberg expectations using chi square analysis.

**Results and Discussion**

The DNA sequence analysis of the promoter and the entire coding regions of the Cyp3A5 gene revealed the presence of 10 and 12 SNPs in the white and Zimbabwean samples, respectively (Table 3). In the current study, all sequences were compared with the wild-type Cyp3A5 sequence (GenBank accession number AC005020), and the positions reported are in reference to this sequence. Five novel variants, including two located in the promoter region, were identified in the white samples. The three novel nucleotide substitutions in coding regions are nonsynonymous mutations predicted to modify the amino acid composition of the protein: codons 309 (Thr→Asn), 323 (Thr→Asn), and 371 (Ile→Val). However, these mutations do not change the polarity of the protein: codons 309 and 323 (uncharged polar residue Thr to uncharged polar residue Asn) and codon 371 (nonpolar residue Ile to nonpolar residue Val). Eight new SNPs were detected in the African samples. Three are novel mutations in introns 3 (C→T), 8 (C→T) and 12 (T→C), and three others, at codons 218 (A→G), 295 (C→T), and 349 (C→T), are synonymous substitutions (silent mutations). The new nucleotide variants at codons 238 (T→C) and 460 (C→T) are nonconservative mutations resulting in a Val→Ala substitution and a stop codon leading to a nonfunctional truncated protein. The respective 238 substitution (nonpolar residue Val to nonpolar residue Ala) does not change the polarity and only slightly modifies the structure (addition of a methyl group) of the protein. This mutation would therefore be unlikely to cause a modification in the protein due to a change in polarity or sterical hindrance. All the novel nonsynonymous mutations found in the white and African samples may have the potential to modify the amino acid composition of the protein but do not affect the polarity of the protein and occur at a very low frequency in these populations. Therefore, it seems improbable that they would play an important role in the Cyp3A5-related metabolic activities in these populations.

The distribution of Cyp3A5 alleles is shown in Table 4. The genotypic distribution of SNPs at each position is in Hardy-Weinberg equilibrium (p > 0.5) for both populations analyzed in the present study. There are some significant differences in Cyp3A5 allelic distribution between indigenous Africans and whites, particularly for Cyp3A5*3, Cyp3A5*6, and Cyp3A5*7 alleles. The frequency of Cyp3A5*3 allele in white Canadians (∼93%) is higher than in Zim- babweans (77.6%) (p < 0.001). In contrast, Cyp3A5*6 and Cyp3A5*7 alleles are relatively frequent in African subjects (10–22%) but absent in whites (p < 0.001). The Cyp3A5*3 and Cyp3A5*6 alleles both code for splicing defects (Hustert et al., 2001; Kuehl et al., 2001), resulting in modified mRNA that may generate

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer Name</th>
<th>Primer Sequence</th>
<th>Annealing Temperature</th>
<th>Fragment Size</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>Cyp3A5F</td>
<td>GCCATATACTCTCCACATGATCAGCAAA</td>
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<tr>
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<tr>
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<tr>
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<td>256 base pairs</td>
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<tr>
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<tr>
<td>6</td>
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<td>5Ex7f</td>
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<tr>
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<td>5Ex8f</td>
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<td>234 base pairs</td>
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<tr>
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<td>5Ex8r</td>
<td>GCCATATACTCTCCACATGATCAGCAAA</td>
<td>60°C</td>
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<tr>
<td>9</td>
<td>5Ex9f</td>
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<td>60°C</td>
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<tr>
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<tr>
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<td>5Ex13r</td>
<td>GCCATATACTCTCCACATGATCAGCAAA</td>
<td>60°C</td>
<td>234 base pairs</td>
</tr>
</tbody>
</table>

* Kuehl et al. (2001).
defective Cyp3A5 enzymes. The Cyp3A5*7 allele has a single base insertion at codon 346 that causes a frameshift resulting in a truncated, nonfunctional enzyme (Hustert et al., 2001). These findings raise the possibility that indigenous Africans may be greater metabolizers than whites, since the Cyp3A5*3 allele is observed less frequently in the former group. However, the presence of Cyp3A5*6 and Cyp3A5*7 in the indigenous African group may compensate for the relatively low frequency of the Cyp3A5*3 allele, resulting in metabolic capabilities similar to those of whites. Finally, SNP 31611, which is located in the upstream region of the Cyp3A5 gene, was detected in 10% of African samples and only in 8.6% of white samples (Lee et al., 2003).
Cyp3A5 activity can be achieved in any given ethnic population (Schuetz et al., 1994). This observation has led to the suggestion that the genetic variation in Cyp3A5 expression is a major determinant of Cyp3A-dependent drug metabolism in humans (Chou et al., 2001). Although some previous studies have attempted to correlate the metabolic capabilities of different patients with genotype, a clear relationship between the levels of Cyp3A5 expression and/or activity and genetic markers remains to be established (Wrighton et al., 1990; Jounaidi et al., 1996; Kuehl et al., 2001). It has also been reported that individuals homozygous for Cyp3A5*3 can express both wild-type and variant (SV1) mRNA (Westlind et al., 2001). Interestingly, the most frequent polymorphisms in all ethnic populations code for splicing defects (Cyp3A5*3 and Cyp3A5*6). This could suggest that these genetic factors predispose to downstream modulation of Cyp3A5 activity and expression and could therefore be the base of the interindividual variation that is observed for Cyp3A-mediated metabolism. Ultimately, it seems that the metabolic capacities of Cyp3A5 are intertwined into a complex procedure determined by the genetic makeup of an individual as well as external factors, such as xenobiotics, influencing gene expression (Gibson et al., 2002).

In summary, we have conducted a thorough analysis of the nucleotide sequence of the Cyp3A5 gene in samples collected from white Canadians and indigenous Zimbabweans. Several novel and nonconservative mutations, with respect to predicted amino acid composition and function of the protein, were identified, albeit at low frequencies. The data from this study and others (Wrighton et al., 1990; Chou et al., 2001; Hustert et al., 2001; Kuehl et al., 2001; Lee et al., 2003) demonstrate that the allelic distribution of the Cyp3A5 gene differs widely in populations from industrialized and developing countries, presumably due to geographically determined selection pressures. However, the genetic polymorphism of Cyp3A5 cannot explain the interindividual differences reported in Cyp3A-mediated metabolism. Additional studies will have to be undertaken examining a large number of parameters, including genotype, mRNA expression, and drug-drug interaction, as well as external influences, to more fully understand the factors that determine the metabolic capabilities of the Cyp3A5 family enzymes.

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References

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