Short Communication

Frequency of the Frame-Shifting CYP2D7 138delT Polymorphism in a Large, Ethnically Diverse Sample Population

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ABSTRACT:

Cytochrome P450 2D7 (CYP2D7) has long been considered a pseudogene. A recent report described an indel polymorphism (CYP2D7 138delT) that causes a frameshift generating an open reading frame and functional protein. This polymorphism was observed in 6 of 12 samples from an Indian population. Individuals with the 138delT polymorphism expressed CYP2D7 protein from a brain-specific, alternatively spliced transcript (J Biol Chem 279:27383–27389, 2004). The unexpectedly high frequency of the variant allele and resulting CYP2D7 expression could have important implications for brain-specific metabolism of CYP2D substrates including many psychoactive drugs. However, the 138delT variant has not been detected in other studies (Pharmacogenetics 11:45–55, 2001; Biochem Biophys Res Commun 336:1241–1250, 2005). Our goal was to determine the frequency of this variant in a larger, ethnically diverse population. CYP2D7 138delT genotypes for 163 Caucasians, 95 East Asians, 50 Indians, 68 Hispanic Latinos, and 68 African Americans were determined by Pyrosequencing. The 138delT allele was observed at a frequency of 1.0% in East Asians and 0.74% in Hispanic Latinos. The deletion was not observed in Indians or the other ethnic populations. In addition, in each of the three samples with 138delT, the putative brain-specific transcript contains a premature stop codon that would preclude protein expression. The low frequency of the CYP2D7 138delT polymorphism in our ethnically diverse sample, and particularly the absence from 50 Indian samples, is in contrast to the high frequency previously reported. Our results suggest that CYP2D7 138delT is unlikely to be highly relevant for population variation of pharmacokinetics or drug response.

The cytochrome P450 2D (CYP2D) locus on chromosome 22 comprises three highly homologous genes, CYP2D6, CYP2D7, and CYP2D8. CYP2D6 metabolizes a wide range of commonly prescribed drugs, and genetic polymorphisms in the corresponding CYP2D6 gene are recognized as important contributors to interindividual variability of pharmacokinetics and, in some cases, drug efficacy or safety (reviewed in Bernard et al., 2006). Relationships between genotype and metabolizer phenotype for CYP2D6 are well characterized, with individuals classified as poor, intermediate, extensive, or ultrarapid metabolizers based on genotype. The ultrarapid metabolizer phenotype is usually attributed to an amplification of the functional CYP2D6 gene (Løvlie et al., 2001). However, not all ultrarapid metabolizer phenotypes are explained by CYP2D6 gene duplications, suggesting the contribution of other, yet unknown factors (Løvlie et al., 2001). In addition to the functional CYP2D6, the CYP2D locus contains two nonfunctional pseudogenes, CYP2D7 and CYP2D8. CYP2D8 has multiple sequence differences relative to CYP2D6 that render it nonfunctional (reviewed in Zanger et al., 2004). In contrast, the open reading frame of CYP2D7 is disrupted only by a single base insertion within exon 1 (Kimura et al., 1989). A functional CYP2D7 gene that produces active enzyme might result from either deletion of the extra nucleotide or replacement of exon 1 by gene conversion and could contribute to the rapid-metabolizer phenotype in CYP2D6 amplification-negative individuals. For example, deletion of nucleotide 138T could restore CYP2D7 enzyme expression. Løvlie et al. (2001) examined this hypothesis by genotyping 17 CYP2D6 duplication-negative ultrarapid or extensive metabolizers at this site, but all individuals were homozygous for the wild-type nonfunctional CYP2D7 allele.

In a recent report, the 138delT polymorphism was observed in 6 of 12 samples in an Indian population (Pai et al., 2004). CYP2D7 protein translated from a brain-specific, alternatively spliced transcript was detected in samples containing the 138delT polymorphism. Protein expressed from the CYP2D7 variant transcript showed activity that differed from CYP2D6 in preferentially converting codeine to morphine rather than norcodeine. The reported high frequency of the CYP2D7 138delT variant, and the possibility that the resulting enzyme could be responsible for conversion of codeine to morphine in the human brain, suggested an important role for this variant in drug disposition. Furthermore, variability in expression of CYP2D6 in the brain may be a factor for variability in response to other psychoactive drugs that are CYP2D6 substrates.

Following on the above report, Gaedigk et al. (2005), using a CYP2D7-specific assay, did not detect the CYP2D7 138delT variant in their sample panel comprising Caucasian Americans (n = 109), African Americans (n = 112), Asian Americans (n = 43), Indians (n = 7), and unknowns (n = 14). However, their panel contained only seven individuals sharing geographic origin with the individuals studied by Pai et al. (2004). The high degree of homology among the three genes within the CYP2D locus makes genotyping a particular challenge. Whereas Pai et al. (2004) observed the CYP2D7 138delT variant at a frequency of 50%, it has not been observed by others (Løvlie et al., 2001; Gaedigk...
et al., 2005), and nonspecific assay design in the initial report has been postulated as an explanation for the disparate results (Gaedigk et al., 2005; Hoskins et al., 2005). In this study, we examined the occurrence of the CYP2D7 138delT variant in a larger, more diverse sample set using a CYP2D7-specific assay.

Materials and Methods

Genotyping. Anonymous samples used for genotyping were obtained from blood banks. The ethnically diverse sample set of 68 African Americans, 163 Caucasians, 95 East Asians, 68 Hispanic Latinos, and 50 Indians was genotyped for the CYP2D7 138delT variant. This study was carried out in accordance with the Declaration of Helsinki.

Amplicons used for genotyping were generated from 50 ng of genomic DNA in a 30-μl PCR containing 1 unit of Platinum Taq DNA Polymerase Hi Fidelity (Invitrogen, Carlsbad, CA), 2 mM MgSO₄, 0.2 mM deoxyribonucleoside-5’-triphosphates, and 0.3 μM concentrations of the forward and reverse primers (Table 1). When the amplicon was generated from cloned DNA, approximately 50 ng of purified plasmid was used as starting template. Genotype determinations were made using the PSQ 96MA instrument from Pyrosequencing (Biotage AB, Uppsala, Sweden) following the protocol suggested by the manufacturer. The reactions were assayed on the PSQ 96MA using the single-nucleotide polymorphism analysis software provided by the manufacturer. The Pyrosequencing primer (Table 1) was designed on the basis of the allele-specific PCR assay used to amplify each allele. CYP2D6 genotyping included *2 (2850C>G), *3 (1584C>T), *4 (1846G>A), *5 (gene deletion), *6 (1707delT), *9 (2613delAGA), *10 (100C>T), *17 (1023C>T), *41 (−1584C>G), and x2 (gene amplification) (Furman et al., 2004).

Cloning of CYP2D6 and CYP2D7 Fragments. CYP2D6 and CYP2D7 fragments were amplified with forward and reverse primers (Table 1) containing the attB1 and attB2 recombination sites, respectively, for cloning into the pDONR vector (Invitrogen) using the Gateway Technology (Invitrogen) as suggested by the manufacturer. Purified plasmid DNA was prepared using the PureLink HiPure Plasmid DNA Purification kit (Invitrogen).

Results and Discussion

PCR amplicons generated from genomic DNA with CYP2D7-specific primers were genotyped by Pyrosequencing. In this sample set, 3 of the 444 individuals were heterozygous for the CYP2D7 138delT polymorphism, resulting in an overall allele frequency of 0.34% (95% CI 0.071–0.60); specific frequencies for the ethnic groups were as follows: African American, 0 (95% CI 0–0.0298); Caucasian, 0 (95% CI 0–0.0114); East Asian, 0.011 (95% CI 0.0023–0.0392); Hispanic Latino, 0.007 (95% CI 0.0023–0.0448); and Indian, 0 (95% CI 0–0.0362). Direct sequencing was used to confirm the heterozygous genotypes observed in the Pyrosequencing assay (Fig. 1). The 138delT variant was observed neither in the 50 Indians in our study nor in the Gaedigk sample set containing 7 Indians (Gaedigk et al., 2005). In contrast, the study by Pai et al. (2004) observed it at a 50% frequency in their Indian sample set (n = 12); furthermore, the allele was always observed in the homozygous state. Gaedigk and colleagues (Gaedigk et al., 2005; Gaedigk and Leeder, 2006) have observed the intron 6 retention reported by Pai et al. (2004), but not the 138delT variant. The absence of heterozygotes and deviation of the Pai samples (Pai et al., 2004) from the Hardy-Weinberg equilibrium suggest that assay specificity may be in question.

The specificity of the primers used for amplification of the CYP2D7 138delT region in our study was checked by a BLAST search. Our forward and reverse PCR primers (CYP2D7 138delT F3 and CYP2D7 138delT RB3) were complementary to CYP2D7P (X58467) and to the CYP2D7P1 region of NG_003180, but not to CYP2D8P2 (NG_000853) or CYP2D8P1 (NG_000854). A BLAST search with the primers from the study by Pai et al. (2004) showed perfect complementarity to all three CYP2D genes (Hoskins et al., 2005).

We experimentally confirmed the specificity of our assay by performing the CYP2D7 138delT Pyrosequencing assay on PCR products generated using cloned CYP2D6 and CYP2D7 138delT fragments as template. The Pyrosequencing reaction failed to generate any signal when performed on PCR product generated from cloned CYP2D6 using our CYP2D7-specific primers. Yet, when performed on PCR product generated from cloned CYP2D7 138delT, the same reaction showed the result expected for a sample containing only the variant allele (data not shown). When the primers used by Pai et al. (2004) were used to generate amplicon, the Pyrosequencing reaction generated a variant allele signal when either CYP2D6 or CYP2D7 138delT was used to generate amplicon (data not shown).

Gaedigk and colleagues (Gaedigk et al., 2005; Gaedigk and Leeder, 2006) reported that their CYP2D7 clone sequences as well as the genomic reference sequence for CYP2D7 (M33387) have a

<table>
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<th>Description</th>
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<th>Amplicon</th>
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</table>

**TABLE 1**

Primer sequences and descriptions for PCR and genotyping

One primer from each set used to generate an amplicon for genotyping was biotinylated at the 5′-end. Sequence added to primers for Gateway cloning subsequent to PCR is shown in bold.
“G” at position g.14408, but the CYP2D7 mRNA sequence deposited by Pai et al. (2004) (AY220845) has a “C” at this position. Position g.14408 is located within the 57-base pair intron retention found in the novel variant described by Pai et al. (2004), and the C at this position changes a TGA stop codon into a serine codon, allowing for a continued reading frame. We genotyped position g.14408 in our CYP2D7 138delT heterozygous samples and observed a G in all three samples. Thus, even though these individuals possess the potentially CYP2D7-activating 138delT allele, they would not be expected to produce functional protein resulting from a downstream stop codon.

Since CYP2D6 gene amplification could potentially confound CYP2D7 138delT genotyping, the three samples that were heterozygous for the CYP2D7 138delT allele were genotyped for CYP2D6 status. CYP2D6 amplification was not observed in any of the three samples; samples EA42 and EA46 were genotyped as *1/*1, and HL18 was *1/*4 (data not shown).

In conclusion, using a CYP2D7-specific assay in a larger and more diverse sample set, we show that the CYP2D7 138delT allele occurs at a low frequency. This finding contrasts the high frequency observed by Pai et al. (2004) as well as two reports that did not observe it at all (Løvlie et al., 2001; Gaedigk et al., 2005). However, since we observed the CYP2D7 138delT variant on the same allele as g.14408G, which results in a stop codon, no functional protein would be expected. Therefore, even though we report the detection of CYP2D7 138delT, we confirm prior reports (Løvlie et al., 2001; Gaedigk et al., 2005) that the CYP2D7 138delT variant does not contribute to the observed variability in CYP2D-catalyzed metabolism and drug disposition.

References

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