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Frequency of the frame-shifting *CYP2D7* 138delT polymorphism in a large, ethnically diverse sample population

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CYP2D7 138delT polymorphism in a diverse 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 (Pai et al., 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 (Løvlie et al., 2001; Gaedigk et al., 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.

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The cytochrome P450 2D (*CYP2D*) locus on chromosome 22 comprises 3 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 inter-individual 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 non-functional 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 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 non-functional *CYP2D7* allele (Løvlie et al., 2001).

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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 *CYP2D* 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., 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) (Gaedigk et al., 2005). However, their panel contained only 7 individuals sharing geographic origin with the individuals studied by Pai et al (Pai et al., 2004).

The high degree of homology between the 3 genes within the *CYP2D* locus makes genotyping a particular challenge. While Pai et al 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 non-specific assay design in the initial report has been postulated as an explanation for the disparate results (Gaedigk et al., 2005; Hoskins et

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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 (AA), 163 Caucasians (CA), 95 East Asians (EA), 68 Hispanic Latinos (HL) and 50 Indians (I) was genotyped for the *CYP2D7* 138delT variant.

Amplicons used for genotyping were generated from 50 ng genomic DNA in a 30 μL PCR containing 1unit of Platinum Taq DNA Polymerase Hi Fidelity (Invitrogen; Carlsbad, CA), 2 mM MgSO4, 0.2mM dNTPs and 0.3 μM 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 SNP 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>T), *3 (2549delA), *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

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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).

This study was carried out in accordance with the Declaration of Helsinki.

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% C.I. 0.07 -1.06]; specific frequencies for the ethnic groups were as follows: African American – 0 [95% C.I. 0 – 0.0298], Caucasian – 0 [95% C.I. 0 – 0.0114], East Asian – 0.011 [95% C.I. 0.0023 – 0.0392], Hispanic Latino – 0.007 [95% C.I. 0.0023 – 0.0448] and Indian – 0 [95% C.I. 0 – 0.0362]. Direct sequencing was used to confirm the heterozygous genotypes observed in the Pyrosequencing assay (Figure 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 Pai study observed it at a 50% frequency in their Indian sample set (n=12); furthermore, the allele was always observed in the homozygous state. Gaedigk et al have observed the intron 6 retention reported by Pai, but not the 138delT variant (Gaedigk et al., 2005; Gaedigk and Leeder, 2006). The absence of heterozygotes and deviation of the Pai samples from Hardy-Weinberg equilibrium suggest that assay specificity may be in question.

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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 Pai primers showed perfect complementarity to all 3 *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 Pai primers 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 et al reported that their *CYP2D7* clone sequences as well as the genomic reference sequence for *CYP2D7* (M33387) have a 'G' at position g.14408, but the *CYP2D7* mRNA sequence deposited by Pai et al (AY220845) has a 'C' at this position (Gaedigk et al., 2005; Gaedigk and Leeder, 2006). Position g.14408 is located within the 57-bp intron retention found in the novel variant described by Pai et al., and the 'C' at this position changes a T**G**A stop codon into a serine codon allowing for a continued reading frame. We genotyped position g.14408 in our *CYP2D7* 138delT

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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 due to a downstream stop codon.

Since *CYP2D6* gene amplification could potentially confound *CYP2D7* 138delT genotyping, the 3 samples that were heterozygous for the *CYP2D7* 138delT allele were genotyped for *CYP2D6* status. *CYP2D6* amplification was not observed in any of the 3 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 contrasts the high frequency observed by Pai et al (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.

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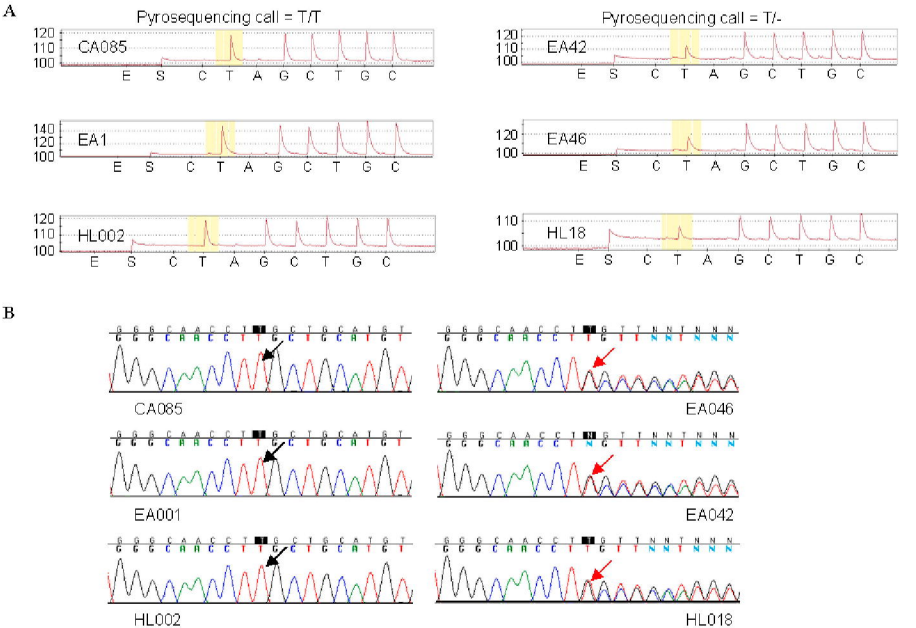
Figure 1. Genotyping of *CYP2D7* 138delT. A, Pyrograms for 3 samples homozygous at the 138T position are shown (left) compared to the 3 samples that were heterozygous (right). B, sequencing results for the samples shown in part A. Arrows indicate position 138.

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.

Description	Direction	Primer name	Sequence	Amplicon (bp)
CYP2D7 amplicon for 138delT genotyping	Forward Reverse	*CYP2D7* 138delT F3 *CYP2D7* 138delT RB3	ccagttggtagtgaggcagcc Bio-ggtggtggggcatcctcagg	254
CYP2D7 138delT pyrosequencing	Forward	Pyro 2D7 138delT-for	ggctgggcaacct	
Pai et al (Pai et al., 2004)	Forward Reverse		cttcctgctcctggtgga Bio-caccccttcatcctcga	506
CYP2D6 fragment for cloning	Forward Reverse	2D6 For attB1 2D6 Rev attB2	**ggggacaagtttgtacaaaaaagcaggctnn**agggaagggtcacgcgct **ggggaccactttgtacaagaaagctgggtn**ccggccctgacactccttct	3223
CYP2D7 fragment for cloning	Forward Reverse	2D7 For attB1 2D7 Rev attB2	**ggggacaagtttgtacaaaaaagcaggctnn**ccagttggtagtgaggcagcc **ggggaccactttgtacaagaaagctgggtn**aaggtgacaggctgagggcg	4798
CYP2D7 amplicon for g.14408 genotyping	Forward Reverse	2D7 14408 C>G FB 2D7 14408 C>G Rev	Bio-caacacaggacgaaggagagtgtcccctgggtgct ctaccaccggggctgatgct	303
CYP2D7 g.14408 pyrosequencing	Reverse	2D7 14408 C>G RevSeq	tgcgtccccacaat	

Figure 1


A
Pyrosequencing call = T/T
Pyrosequencing call = T/-
CA085
EA42
EA1
EA46
HL002
HL18
E S C T A G C T G C
B
CA085
EA046
EA001
EA042
HL002
HL018