SNP Communication

**Novel Nonsynonymous Single Nucleotide Polymorphisms in the CYP2D6 Gene**

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Full text of this paper is available at http://www.jssx.org

**Summary:** Cytochrome P450 (CYP) 2D6 is an important drug-metabolizing enzyme, and its gene is known to be highly polymorphic. Here, we report five novel nonsynonymous single nucleotide polymorphisms (SNPs), and 65 other sequence variations detected from the gene coding for cytochrome P450 (CYP) 2D6 in 254 Japanese subjects. Two of the novel nonsynonymous SNPs were associated with the CYP2D6 gene as follows: 73 C>T (Arg25Trp, exon 1), 972 C>T (Ala30Val, exon 2), 1611 T>A (Phe120Le, exon 3), 1720 A>C (Glu196Ala, exon 3), 3172 A>C (Gln343Ala, exon 7). The SNPs, 73C>T, 972C>T, 1611T>A, 1720A>C and 3172A>C were linked with the key words: CYP2D6; SNP; nonsynonymous SNP

**Introduction**

Cytochrome P450 (CYP) 2D6 encoded by the CYP2D6 gene is clinically important since it metabolizes a wide variety of clinical drugs including antiarrhythmic and psychiatric drugs, as well as endogenous compounds. The CYP2D6 gene is highly polymorphic, and nearly 50 different alleles or haplotypes have been described (References 2 and 3 for review and http://www.imm.ki.se/CYPalleles/cyp2d6.htm). These include a deletion of the entire gene, amino acid substitutions, splicing defects, and insertions and deletions of nucleotide(s) resulting in frameshifts in the open reading frame.

During the course of our discovering new CYP2D6 variants by direct sequencing of leukocyte DNA of Japanese subjects who were administered antiarrhythmic drugs, we detected 70 sequence variations including 5 novel nonsynonymous single nucleotide polymorphisms (SNPs).

**Materials and Methods**

Human genomic DNA samples: Total genomic DNA was extracted from blood leukocytes from 254 Japanese individuals, who were administered antiarrhythmic drugs and/or β-blockers, and was used for DNA sequence analysis. The ethics committees of both the National Cardiovascular Center and the National Institute of Health Sciences approved this study. Written informed consent was obtained from all subjects participating in this study.

Polymerase chain reaction (PCR) conditions for sequencing: 5'-Flanking and exonic regions of the CYP2D6 gene were separately analyzed by a PCR-based

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SNP9 (131)
Table 1. Primers used for the amplification and sequencing of 5′-flanking region and all exons of CYP2D6 gene

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CYP2D6 exons: 1st PCR

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CYP2D6 exons: 2nd PCR and sequencing primers

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method. Primer sets used for the amplification and sequencing of the 5′-flanking region and all exons are described in Table 1. Two-step PCR reactions were used for the amplification of the 5′-flanking region of the CYP2D6.

The first-step PCR amplification of the 5′-flanking region was conducted in a reaction mixture (100 μL) containing 1× Ex Taq buffer, 3.0 mM MgCl₂, 5.0 mM dNTPs, 1 unit of Ex Taq polymerase (TaKaRa Shuzo, Kyoto, Japan), and 0.5 μM of each primer. The PCR amplification conditions were as follows: 94°C for 5 min, followed by 30 cycles consisting 94°C for 30 s, 64°C for 1 min, 72°C for 3 min, then 72°C for 5 min, followed by 4°C. For the analysis of each CYP2D6 exon and surrounding introns, a DNA fragment containing all CYP2D6 exons was amplified using the Dlo, Dlo,

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* A of the translation initiation codon ATG is numbered +1.
* This SNP position is at nucleotide 2431 in a reference sequence, AY45316.
* Assumes frequency could not be evaluated due to the surrounding repeat sequences.
DPK+, and DPKlow primers as described by the method of Hersberger et al., which identifies the CYP2D6*5 allele. The PCR product containing non-*5 5-kb fragment, was purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and subjected to a second round PCR for the analysis of all the CYP2D6 exons.

The second round PCR for 5'-flanking region and all exons was conducted by the same method as described previously. PCR amplification for the 5'-flanking subregions was done as follows: 94°C for 5 min, followed by 30 cycles consisting of 94°C for 30 sec, 55°C for 1 min and 72°C for 2 min, then, 72°C for 7 min, and kept at 4°C. Cycle sequencing after the incubation with exonuclease I (1 μL) and shrimp alkaline phosphatase (1 μL) was done according to manufacturer’s instruction. After cycling, the products were purified and separated on the ABI PRISM 3700 DNA autoscaler (Applied Biosystems, Foster City, CA, USA). Sequencing was done on both strands without any contradiction.

Results and Discussion

We amplified a 5'-flanking region and all exons of CYP2D6 gene by the two-step PCR and the resultant PCR products were subjected to sequencing as described under Materials and Methods. Sequencing the PCR products resulted in finding 70 sequence variations in the CYP2D6 gene (accession number, M33388) of the 254 Japanese individuals as described in Table 2. Besides the 70 variations, we detected the conversion to CYP2D7 in exon 9, which was a key variation of CYP2D6*56, in 37 samples (All were heterozygotes.). Proposal of Johansson et al., that a tandem arrangement of CYP2D6*108R and CYP2D6*10 (C) was associated with CYP2D6*56 suggested that CYP2D6*56 haplotype contained more than 2 CYP2D6 gene copies. Since assignment of SNPs found in the CYP2D6*56 possessing patients to either of the two copies is difficult, these 37 samples were excluded from the current calculation of SNP frequencies described in Table 2. Furthermore, 19 subjects were judged to possess CYP2D6*5 according to the method by Hersberger et al. We found, however, that 4 of the
19 subjects possessed heterozygous SNPs. This suggested that the 4 subjects possessed aberrant *5 (probably two CYP2D6 copies), though they were initially judged to have the CYP2D6*5 haplotype. Thus, we also excluded these 4 subjects from the calculation of SNP frequencies (Table 2). Recently, Ishiguro et al. have reported similar observation. (1)

Among the 5 nonsynonymous SNPs (Table 2), the 1611T>A (F120I, MPJ6_2D6074) was heterozygously detected in an individual who was a homozygous CYP2D6*10 subject. The 1720A>C (E156A, MPJ6_2D6075) was heterozygously detected in a homozygous *1 individual. The 972C>T, (A90V, MPJ6_2D6073) was found in a subject with *1/*10 genotype. Cloning and sequencing of DNA fragments obtained from the 972C>T subject revealed that 100C and 972T located on the same DNA strand, indicating the association of the MPJ6_2D6073 with the *1 haplotype. Allele-specific PCR analysis revealed that 3172A>C (E334A, MPJ6_2D6082), which was found in a subject with *1/*2 genotype, was associated with CYP2D6*2 haplotype. Similarly, by allele-specific PCR analysis, 75C>T (R25W, MPJ6_2D6065) was shown to be linked with C100T, a key SNP of *10 allele. For all the nonsynonymous SNPs, electropherograms are shown for each SNP and the SNP and surrounding nucleotide sequences are written in Fig. 1 (A–J).

Functional characterization of these SNPs is very important especially for those which resulted in the changes of their electronic charges (e.g. E156A and E334A), or for those which are associated with the *10 haplotype (e.g. R25W and F120I).

The known CYP2D6 alleles, so far observed specifically in Japanese and/or Chinese populations, *4 (1758G>A, G109R), *18 (9 bp insertion, insertion of VPT in exon 9), *21 (2573 ins C, frameshift), and *44 (IVS6 + 1 G>C, aberrant splicing) were also found, but their frequencies were very low (3/426, 1/426, 3/426, 1/426), respectively.

In conclusion, we detected 70 sequence variations in the CYP2D6 gene including 5 novel nonsynonymous SNPs. These amino acid alterations should further be
Fig. 1. Electropherogram of 5 nonsynonymous SNPs and their flanking sequences. The densitograms are shown in forward or reverse orientation as indicated. Arrows indicate the polymorphic and heterozygous positions and the heterozygous nucleotides are underlined. Bases colored with green, blue, black, and red represent adenine, cytosine, guanine, and thymine, respectively.

Note Added in Proof

The 5 CYP2D6 haplotypes that possess each of 73C>T (R23W), 972C>T(A90V), 1661T>A (F120I), 1720C>A (E156A), and 3172A>C (E334A) were assigned as *4E, *48, *49, *50, and *51, respectively, by the CYP Allele Nomenclature Committee (http://www.imm.ki.se/CYPalleles).

References