SNP Communication

Two Novel Haplotypes of CYP2D6 Gene in a Japanese Population

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Summary: Two novel haplotypes of the human CYP2D6 gene, newly designated as CYP2D6*44 allele, had both a novel single nucleotide polymorphism (SNP) of 2950G>C in intron 6 donor splice junction and a known SNP (82C>T). The newly detected mutation was as follows: SNP, 030418Tsukoku001; GENE NAME, CYP2D6; ACCESSION NUMBER, M33388; LENGTH, 25 bases; 5'CGATGTGACCG/CTGAGCCATCTG-3'. In addition, we found another haplotype, newly designated as CYP2D6*21B allele, containing -1584C>G, -1235A>G, -740C>T, -678G>A, and a gene conversion with CYP2D7 gene in intron 1 associated with CYP2D6*21. Both CYP2D6*44 and CYP2D6*21B alleles would cause a splicing error or a frameshift with impaired drug metabolizing function mediated by CYP2D6.

Key words: P450 2D6; genetic polymorphism; splice variant

Introduction

Human cytochrome P450 2D6 (CYP2D) is one of the most important drug metabolizing enzymes causing wide interindividual and ethnic differences of clinically used medicines.1,2 The CYP2D6 gene locus is extremely polymorphic, with over 70 known allelic variants (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). However, poor metabolizers associated with CYP2D6 function in the Japanese could not be accounted for by the known mutant alleles of CYP2D6 as yet.1,2 In the course of discovering new CYP2D6 variants, we identified a new haplotype containing the 2950G>C nucleotide transition in the invariant GT at the 5' donor splice site of intron 6 of the CYP2D6 gene. In addition, another haplotype with -1584C>G, -1235A>G, -740C>T, -678G>A, and a gene conversion with CYP2D7 gene in intron 1 associated with CYP2D6*21 was observed. These new alleles would cause splicing errors with impaired CYP2D6 function.

Materials and Methods

Detailed information on the subjects was described previously.3,4 Because of sample limitation, 75 subjects were used from the previous 98 samples5 along with new 11 subjects from JA Kutchan Kousei General Hospital. Total 86 DNA samples were sequenced. Informed consent was obtained from every volunteer. This study was approved by the ethics committee of Hokkaido University. The sequence of the complete human CYP2D6 gene described in the GenBank (accession number M33388) was used as a reference. The A of ATG start codon was defined as +1. The primers used for specific amplification and direct sequencing of all exons and exon-intron boundaries were described previously.2,5,6 To sequence the 5'-franking region of the CYP2D6 gene, four primers were designed; 2D6-1339R, 5'-ATT CTC GGC TCA CTA CAA CC-3', 2D6-1139R, 5'-ACT CAG TGG CCC AGG CCG GA-3', 2D6-760F, 5'-ATG TGT GTG TGA CTT GTG TG CTT TG-3', and 2D6-619R, 5'-ACC TGA TGG TGC ACA GAT CT-3'. Sequences were determined using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Results and Discussion

We found a novel polymorphism of the CYP2D6 gene as follows: SNP, 030418Tsukoku001; GENE

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As of April 29, 2003, the SNP did not appear either in the "Human Cytochrome P450 (CYP) Allele Nomenclature Committee database (http://www.imm.ki.se/CYPalleles/)" or the "JSNP's database (http://snp.lms.u-tokyo.ac.jp/)".
NAME, CYP2D6; ACCESSION NUMBER, M33388; LENGTH, 25 bases; 5'-CGGATGTCGACGG/CTGA-GCCCATCTG-3'. The SNP was 2950G>C in the first position of the 5' splice junction of intron 6 (Fig. 1). The subject also possessed another mutation at the position of 82 of the CYP2D6 gene (82C>T, known as CYP2D6*22) heterozygously. Haplotype analysis by subcloning indicated that 2950G>C and 82C>T existed in the same allele of the CYP2D6 gene. The other sequences of the amplified fragments were identical to the CYP2D6 sequence described in GenBank. Consequently, this novel haplotype containing a new SNP 2950G>C was formally designated as CYP2D6*44 allele by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee. One out of 86 individuals was heterozygous for the CYP2D6*44 allele, suggesting that the allele frequency was 0.6% in Japanese in the present study. The frequency of CYP2D6*44 might be lower because the CYP2D6*44 allele was not found out in a recent report\(^6\) with CYP2D6 genotyping of 98 Japanese subjects.

It is generally believed that consensus sequence in the intron 5' splice site donor sequence begins with a GT, whereas the 3' splice site acceptor sequence terminates with an AG. The nucleotide substitution G, at the position 2950 which corresponds to the first G of GT in intron splice donor site, to C could possibly affect splicing. The possible splicing defect of CYP2D6*44 allele was confirmed by a website software “Splice Site Prediction” (http://www.fruitfly.org/seq_tools/splice.html). This information suggested that the CYP2D6*44 allele could not yield the functional CYP2D6 protein. The capacity of dextromethorphan O-demethylation of this subject\(^9\) newly genotyped as CYP2D6*2/44 was lower (a metabolic ratio of 0.016) than those of subjects with CYP2D6*1/*2 (mean metabolic ratio of 0.0027, n = 6).

In addition, we found another haplotype CYP2D6*21B, containing −1584C>G, −1235A>G, −740C>T, −678G>A, and intron 1 conversion with CYP2D7 gene (214–245) together with known mutations associated with CYP2D6*21 (257InsC leading to stop codon, 2850C>T, and 4180G>C) by subcloning. Three out of 86 individuals were heterozygous for the CYP2D6*21B allele, suggesting that the allele frequency was 1.7% in Japanese. The metabolic ratios for dextromethorphan of the two subjects\(^9\) newly genotyped as CYP2D6*1/*21B and CYP2D6*10/*21B was 0.028 and 0.080, respectively. We could not re-evaluate the haplotypes of subjects firstly reported as CYP2D6*21\(^9\), simply because of sample limitations.


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