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The Frequency of Candidate Alleles for CYP2D6 Genotyping in the Japanese Population with an Additional Respect to the \(-1584C\) to \(G\) Substitution

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Summary: The \(-1584C\)/G single nucleotide polymorphism (SNP) in the promoter region of CYP2D6 was suggested to have the potential to influence CYP2D6 activity. In this report, we demonstrated the frequencies of \(-1584C\) to \(G\) substitution-related alleles, such as CYP2D6*2, CYP2D6*21, CYP2D6*35 and CYP2D6*41, in the Japanese population. The frequencies of CYP2D6*2, *41 and *21 were 0.102, 0.026 and 0.005, respectively. We also showed a relationship between the SNP and other common alleles, CYP2D6*4, *5, *10, *14 and *18. Interestingly, the SNP was detected in all three subjects carrying CYP2D6*14. This finding suggests the \(-1584G\) is included in the CYP2D6*14 allele, which is a null-allele characteristic to the Japanese population. This report presents practical information on CYP2D6 alleles that should be considered in the pharmacokinetic study of CYP2D6 substrates in the Japanese population.

Key words: CYP2D6; frequency; CYP2D6*41; CYP2D6*35; CYP2D6*21; Japanese

Introduction

CYP2D6 metabolizes many clinically important drugs including antidepressants, neuroleptics, \(\beta\)-blockers and antiarrhythmics.\(^1\) There is a wide interethnic variation in the frequency of the CYP2D6 genotypes. In a previous study, we reported the frequencies of CYP2D6 genotypes in a Japanese population.\(^2\) However, some alleles have since been reported which were not included in our study.

In the present study, we focused on the \(-1584C/G\) substitution, because the subjects with \(-1584G\) were suggested to have higher CYP2D6 enzyme activity than those with \(-1584C\).\(^3\) The locations of the mutations in common CYP2D6 alleles are displayed in Fig. 1 according to the CYP nomenclature committee (http://www.imm.ki.se/CYPalleles/cyp2d6.htm), with some modifications. Since the single nucleotide polymorphism (SNP) in \(-1584\) seems to be mainly associated with CYP2D6*2, the committee has designated CYP2D6*2 with \(-1584G\) as CYP2D6*2A and CYP2D6*2 with \(-1584C\) as CYP2D6*41, according to Zanger et al.\(^3\)

Recent studies have suggested that individuals with CYP2D6*41 have lower CYP2D6 enzymatic activity \textit{in vivo} than those with CYP2D6*2A,\(^4\) possibly as a consequence of lower expression of CYP2D6 protein.\(^3\) The \(-1584G\) substitution is also found in the CYP2D6*35 allele, which has a \(31G\) to \(A\) substitution in addition to the SNPs of the CYP2D6*2 allele, but does not have the gene conversion mutation from CYP2D7 in intron 1 of CYP2D6. Although the allele has been identified in many duplication-negative “Ultra rapid” metabolizers,\(^5\) the activity of recombinant CYP2D6.35 is comparable to that of the wild-type.\(^6\) Furthermore, the \(-1584G\) is found in the CYP2D6*21 allele. However, these effective alleles have been classified as CYP2D6*2 according to previous detection criteria. Therefore, the consideration of these alleles may result in a better understanding of the phenotype-genotype correlation.

In view of the importance of the \(-1584C/G\) substitution-related alleles in the Japanese population, we examined the frequencies of CYP2D6*41 and *35, possibly included in CYP2D6*2, *21 and other alleles in the present study.

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When a cytosine is present at the first base. In contrast, in the present of a guanine no recognition site for SmaI is introduced. The PCR reaction was carried out in a 25-microl reaction volume containing 1.5 mM MgCl₂, 10 mM Tris/HCl (pH 8.3), 0.2 mM of each dNTP, 10 pmol of each primer (CYP2D6*41F; 5'-TTT TAC AAG ACC AGC CTG GAC AAC -3' and CYP2D6*41Mut.R), 30 ng genomic DNA and 1U AmpliTaqGold™DNA polymerase (Applied Biosystems). An initial denaturation step at 95°C for 10 min was followed by 35 cycles of 95°C for 30 sec, 60°C for 1 min and 72°C for 30 sec, and a final elongation step of 72°C for 5 min. PCR products were then digested with SmaI and separated on 4% agarose gel. Product from the −1584C allele was not cut and remained 53 bp in length, while the −1584G allele was cut into 32 and 21 bp.

The 31G/A substitution was detected to find the CYP2D6*35 allele according to Lovlie et al. In brief, a 341-bp fragment covering the SNP was amplified by a set of primers (CYP-511; 5'-AGG TTC ACT CAC AGC AGA GGG-3' and CYP-518; 5'-CCT GGT CGA AGC AGT ATG GTG-3'), then digested with NlaIII. The wildtype (31G) was cut into 305 and 36 bp fragments, while the mutant (31A) allele generated 193, 112 and 36 bp fragments. The PCR reaction product was purified and directly sequenced by DNA sequencer ABI 310 using dye-terminator chemistry to confirm the nucleotide substitution and search for other related substitutions. Then, to determine the haplotype, the fragment was cloned using the TOPO TA cloning kit (Invitrogen) according to the manufacturer’s instructions. Additionally, after genotyping, some clones with insertions were randomly selected and directly sequenced.

The presence of the CYP2D6*21 allele was determined by an allele specific PCR method using genotyping kit “SNP Typing Kit–Cytochrome P450 2D6*21 (2573C insert)” (TOYOBO, Japan) according to the manufacturer’s instructions.

Methods

All subjects in the present study were healthy males who had enrolled for several clinical trials. We regenotyped 206 subjects whose data had previously been reported. An additional 79 subjects were newly included, and consequently a total of 285 subjects were examined for the CYP2D6 genotype. This study was approved by the ethic committees of Osaka University. Written informed consent for genetic analysis was obtained from all subjects. Genomic DNA was isolated from peripheral lymphocytes from each subject.

In our previous study, each CYP2D6 genotype was defined from the results of PCR-RFLP methods for 100C/T, 1846G/A, 2850C/T and 4180G/C and from the results of XbaI and EcoRI-RFLP methods or the long-PCR method for the CYP2D6*5 allele.

A PCR-RFLP method for the −1584C to G substitution was developed for its direct detection. A mismatch PCR-RFLP assay was based on a recognition site for the restriction enzyme SmaI by utilizing an oligonucleotide mismatch primer (CYP2D6*41 Mut.R; 5'-TTT TAT TTG TAG TAG AGC CC -3'; the letter with the underline is the mismatch nucleotide). This antisense primer introduces a SmaI recognition site by extension when a cytosine is present at the first base. In contrast, in the present of a guanine no recognition site for SmaI is introduced. The PCR reaction was carried out in a 25-microl reaction volume containing 1.5 mM MgCl₂, 10 mM Tris/HCl (pH 8.3), 0.2 mM of each dNTP, 10 pmol of each primer (CYP2D6*41F; 5'-TTT TAC AAG ACC AGC CTG GAC AAC -3' and CYP2D6*41Mut.R), 30 ng genomic DNA and 1U AmpliTaqGold™DNA polymerase (Applied Biosystems). An initial denaturation step at 95°C for 10 min was followed by 35 cycles of 95°C for 30 sec, 60°C for 1 min and 72°C for 30 sec, and a final elongation step of 72°C for 5 min. PCR products were then digested with SmaI and separated on 4% agarose gel. Product from the −1584C allele was not cut and remained 53 bp in length, while the −1584G allele was cut into 32 and 21 bp.

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Results

The −1584G alleles were detected in the subjects previously determined as having the CYP2D6*2 genotype. The frequency of −1584G substitution in the Japanese population was estimated to be 0.114 and CYP2D6*41 allelic frequency 0.026 (Table 1).

Interestingly, there were four unusual subjects among all of the subjects with the −1584G allele. One subject was heterozygous for −1584G and had substitutions classified as CYP2D6*1/*10. Two subjects were also heterozygous for −1584G although both had CYP2D6*1/*14. The last was homozygous for −1584G in spite of CYP2D6*2/*14 carrier according to the previous classification.

The insertion of cytosine at 2573 was detected in three
subjects, which indicates that they had the CYP2D6*21 allele. Consistent with the allelic information on CYP2D6*21, the heterozygous for −1584G was detected from all three. Therefore, one subject was genotyped as CYP2D6*10/*21 and the other two as CYP2D6*1/*21. As a result, the frequency of CYP2D6*21 allele was estimated to be 0.005 in the present study.

One subject was found to be heterozygous for the 31G to A substitution with 31A, which may be a new type of allele related to CYP2D6 enzyme activity, these alleles should be considered as related-alleles with the 31A mutation as a minor novel allele. This was not regarded as CYP2D6*35. Although the 31G/A substitution is not likely to influence CYP2D6 enzyme activity and protein expression level in vitro, the CYP2D6*35 allele is found in many ultra rapid metabolizers. The present findings suggest that CYP2D6*35 allele frequency in the Japanese population is very low, compared with some other ethnic populations, which is consistent with the fact that the ultra rapid metabolizers are rare in Japanese and occur in less than about 10% of Caucasians.

The result from four unusual subjects suggests that the −1584G substitution might be associated with CYP2D6*14 and possibly CYP2D6*1 or *10 in addition to CYP2D6*2, *21 and *35. Zanger et al. also mentioned that one subject with CYP2D6*1/*1 possessing −1584G was detected in their study, supporting the present result. On the other hand, Gaedigk et al. suggested that CYP2D6 poor metabolizers (PMs) can be detected by the selection of −1584G carriers, as −1584G is exclusively linked to functional allelic variants. Their study seems to be of value for the simple clinical use of the SNP information. However, the connection between the *14 allele and the −1584G substitution was found in the present study in addition to *21. Since CYP2D6*14 is a null-allele characteristic to the Japanese population, these connections may limit the genotyping strategy to Japanese.

In conclusion, we examined the distribution of −1584C/G substitutions in the Japanese population mainly associated with CYP2D6*2, and found the frequency showed interethnic variation. Since the −1584C/G substitution-related alleles potentially affect CYP2D6 enzyme activity, these alleles should be consi-

Table 1. The allele frequency of CYP2D6 in Japanese compared with frequencies in other ethnic populations

<table>
<thead>
<tr>
<th>(Number of subjects)</th>
<th>Allele frequency (95% confidential interval; lower/upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−1584C</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td></td>
</tr>
<tr>
<td>Japanese (n = 285)</td>
<td>0.026</td>
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<tr>
<td></td>
<td>(0.016/0.043)</td>
</tr>
<tr>
<td>Caucasian (n = 203)</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>(0.002/0.015)</td>
</tr>
<tr>
<td>African-American (n = 193)</td>
<td>0.114</td>
</tr>
</tbody>
</table>

# Allelic frequency of CYP2D6*2×n and CYP2D6*1×n was reported to be 0.01 (n = 206).

The CYP2D6*41 and *35 alleles in Japanese
ndered in CYP2D6 genotyping. The present study found that the −1584C/G substitution has a potential link to other alleles, and further studies on the related alleles in various ethnic populations are necessary.

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