SNP Communications

Polymorphisms and Linkage Disequilibrium of the OATP8 (OATP1B3) Gene in Japanese Subjects

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Summary: OATP8, a member of the organic anion-transporting polypeptide family, is expressed on the sinusoidal membrane of hepatocytes, and transports endogenous organic anions, such as 17β-glucuronosyl estradiol, and xenobiotic substances, such as digoxin. The objective of this study is to search for polymorphisms of the OATP8 gene and to assess the allele frequency of the polymorphisms in the Japanese population.

Analysis of the OATP8 gene in 79 subjects revealed complete linkage of two deletion polymorphisms in the 5' regulatory region, deletion from position -28 to -11 and deletion from position -7 to -4, with an allele frequency of 0.196 for the deletion allele. The polymorphisms T334G (Ser112Ala) and G699A (Met233Ile) were also shown to be in complete linkage disequilibrium, with an allele frequency of 0.728 for the variant (112Ala/233Ile) allele. Interestingly, linkage disequilibrium was identified between the ins/del polymorphism and SNPs at 112 and 233. The predicted major haplotype was the insert-variant type with a haplotype frequency of 0.60.

Key words: organic anion transporting polypeptide (OATP)8; genetic polymorphisms; linkage disequilibrium; haplotype

Introduction

Uptake from blood into hepatocytes is the first step in the hepatic elimination of endogenous and xenobiotic substances, and is often mediated by specific transporter(s) expressed on the sinusoidal membrane of hepatocytes. Among them, organic anion-transporting polypeptide 8 (OATP1B3, SLCO1B3) is known to transport various organic anions, including endogenous and xenobiotic substances. Endogenous substrates for OATP8 include 17β-glucuronosyl estradiol, dehydroepiandrosterone-3-sulfate, bile acids such as cholytaurine and cholyglycine, and peptide hormones such as cholecystokinin-8. In addition, several xenobiotic substances, such as rifampicin, ouabain, digoxin and an endothelin receptor antagonist BQ-123, have been identified as substrates for OATP8.

On the other hand, it has been reported that genetic polymorphisms of OATP-C (OATP1B1, SLCO1B1), one of the transporters expressed on the sinusoidal membrane of hepatocytes, affect the blood concentration of pravastatin, a typical substrate of OATP-C. In addition, it has been reported that genetic polymorphism of OATP-B (OATP2B1, SLCO2B1), another member of the OATP family, affects the transport properties. Thus, genetic polymorphisms of members of the OATP family may affect the pharmacokinetics of substrates.

The objective of this study is to search for polymorphisms of the OATP8 gene and to assess the allele frequency of the polymorphisms in the Japanese population.
Table 1. Primer sequences of polymerase chain reaction and restriction enzymes used for the analysis of polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Exon</th>
<th>Effect</th>
<th>Primer Sequence</th>
<th>Restriction Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>deletionA</td>
<td>exon 1</td>
<td>—</td>
<td>F: ATTGAGCTTTGTGGCTTTTCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: CTTTTTGACAGTTAGTGGCC</td>
<td>—</td>
</tr>
<tr>
<td>deletionB</td>
<td>exon 1</td>
<td>—</td>
<td>F: ATTGAGCTTTGTGGCTTTTCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: CTTTTTGACAGTTAGTGGCC</td>
<td>Tru 1</td>
</tr>
<tr>
<td>T334G</td>
<td>exon 3</td>
<td>Ser 112 Ala</td>
<td>F: GAAGGTACAATGTCTTGGGC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: CTCTCAAAAGGTAACTGCCC</td>
<td>Alu I</td>
</tr>
<tr>
<td>G699A</td>
<td>exon 6</td>
<td>Met 233 Ile</td>
<td>F: ATGATTACATTCCCTGGATC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: ACTATCATGGTACCTTTGTC</td>
<td>Rsa I</td>
</tr>
</tbody>
</table>

Fig. 1. Identification of the OATP8 variants in exons 1, 3, and 6.

Materials and Methods

Subjects: We investigated the allele frequencies of the polymorphisms, haplotype frequency, and linkage disequilibrium of the OATP8 gene.

Search for genetic polymorphisms: We defined OATP8 mRNA in Gene Bank (accession No. NM 019844, OATP8 mRNA) to be wild type, and primers synthesized with reference to NT 024388 (OATP8 genome) were used for polymerase chain reaction (PCR). The search for genetic polymorphisms was performed by comparison of NM 019844 with AK055874, another OATP8 mRNA sequence. We found deletion polymorphism from position −28 to −11 (designated as deletion A) and from position −7 to −4 (deletion B) near the translation initiation site. In addition, we examined single nucleotide polymorphisms T334G (Ser112Ala) and G699A (Met233Ile), which have been found in Caucasians.11)

Polymerase chain reaction (PCR): Firstly, we extracted genome DNA from blood with a Genomix Kit (TALENT, Trieste, Italy) according to the manufacturer’s instructions. Primer pairs (Invitrogen, Carlsbad, CA) were designed to amplify polymorphic regions (Table 1). PCR was carried out in 50 μL volumes containing 0.1 μg of genomic DNA, 0.25 μM each of the
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Fig. 2. Genotyping of OATP8 in human genomic DNA by PCR-RFLP
(A) Exon 1 of the OATP8 gene was amplified by PCR. (B) PCR product of exon 1 digested with Tru I. (C) Exon 3 of the OATP8 gene was amplified by PCR and digested with AluI. (D) Exon 6 of the OATP8 gene was amplified by PCR and digested with RsaI.

primer set, Gene Amp® 10xPCR buffer II (Amersham Pharmacia Biotech, Uppsala, Sweden), 1.5 mM MgCl2, 200 μM each dNTP and 1.25 U of AmpliTaq Gold™ DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden), with a GeneAmp® PCR System 9700 (PE Applied Biosystems, Foster City, CA). PCR amplification was performed as follows: initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50–60°C for 30 sec, extension at 72°C for 2 min, and final extension at 72°C for 5 min.

Analysis of genetic polymorphisms: Deletion A in exon 1 (−28 to −11 deletion): the PCR product of the insert-type allele yields a fragment of 309 bp, and the deletion-type allele yields a fragment of 287 bp. The electrophoresis was conducted with 2% agarose gel (Agarose21, Nippon Gene, Tokyo, Japan) for 2 hours at 75 V.

Deletion B in exon 1 (−7 to −4 deletion): the PCR-RFLP product of the insert-type allele yields a major fragment of 125 bp, and the deletion-type allele yields a major fragment of 131 bp. RFLP analysis was conducted with Tru I (Fermentas, Vilnius, Lithuania) for 2 hours at 37°C. The electrophoresis was conducted with 3% agarose gel for 4 hours at 50–60 V.

Polymorphism T334G in exon 3: the PCR-RFLP product of the wild-type allele yields a major fragment of 253 bp, and the variant-type allele yields a major fragment of 213 bp. RFLP analysis was conducted with AluI (Fermentas) for 2 hours at 37°C. The electrophoresis was conducted with 1.5% agarose gel for 1.5 hours at 75 V.

Polymorphism G699A in exon 6: the PCR-RFLP product of the wild-type allele yields a major fragment of 242 bp, and the variant-type allele yields a major fragment of 275 bp. RFLP analysis was conducted with RsaI (Toyobo, Osaka, Japan) for 2 hours at 37°C. The electrophoresis was conducted with 1.5% agarose for 1.5 hours at 75 V.

In addition, the sequence of the PCR product was determined with a DNA sequencer (model 373, Perkin Elmer Applied Biosystems, Foster City, CA) by using a
polymorphic, with strong linkage disequilibrium. Since
Fig. 2B, 2C, and 2D successfully determined by PCR-RFLP (RT) and the two SNPs (T334G, and G699A) were
for the deletion allele, and we designated this as Linkage
linkage disequilibrium, with an allele frequency of 0.196
polymorphisms in exon 1 were shown to be in complete
linkage disequilibrium was further identified between Linkage A and Linkage B. The predicted major haplotype was the insert/variant type with a haplotype frequency of about 0.60.

The present results show that the OATP8 gene is polymorphic, with strong linkage disequilibrium. Since

Table 2. Frequencies of OATP8 polymorphisms in the Japanese population (n = 79)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>w/w</td>
</tr>
<tr>
<td>DeletionA</td>
<td>51</td>
</tr>
<tr>
<td>DeletionB</td>
<td>51</td>
</tr>
<tr>
<td>T 334 G</td>
<td>7</td>
</tr>
<tr>
<td>G 699 A</td>
<td>7</td>
</tr>
</tbody>
</table>

BigDye Terminator Cycle Sequencing Kit (Perkin Elmer) as the positive control.

Analysis of linkage disequilibrium: Linkage disequilibrium was analyzed by application of the chi-square test, and p values of less than 0.05 were considered statistically significant.

Results and Discussion

The deletion polymorphism in exon 1 (deletion A; −28 to −11 deletion) was successfully determined by agarose gel electrophoresis (Fig. 2A). The other deletion polymorphism in exon 1 (deletion B; −7 to −4 deletion) and the two SNPs (T334G, and G699A) were successfully determined by PCR-RFLP (Table 1, Fig. 2B, 2C, and 2D). In addition, the two deletion polymorphisms in exon 1 were shown to be in complete linkage disequilibrium, with an allele frequency of 0.196 for the deletion allele, and we designated this as Linkage A. Tirona et al. have reported these two deletion polymorphisms as separate polymorphisms of “−28 to −11 deletion” and “−7 to −4 deletion”. They did not find complete linkage disequilibrium between these polymorphisms. The two SNPs (T334G and G699A) were also in complete linkage disequilibrium with an allele frequency of 0.728 for the variant (112Ala/233Ile) allele, and we designated this as Linkage B. The present study is the first to demonstrate these two complete linkage disequilibriums in the OATP8 gene. Letschert et al. have already reported the SNPs T334G and G699A in Caucasians. They reported the allele frequencies of 0.734 and 0.699 to be 0.71 and 0.78, respectively, in good agreement with the results of this study (0.728). However, complete linkage disequilibrium between these SNPs has not been demonstrated. These results suggest that allele frequency was not influenced by ethnicity, though the nature of linkage disequilibrium may be slightly dependent upon ethnicity.

Interestingly, statistically significant linkage disequilibrium was further identified between Linkage A and Linkage B. The predicted major haplotype was the insert/variant type with a haplotype frequency of about 0.60.

The present results show that the OATP8 gene is polymorphic, with strong linkage disequilibrium. Since both of the deletion polymorphisms were located immediately upstream of the translation initiation site, these polymorphisms may affect the stability of the mRNA. The SNPs, T334G and G699A, are located at the transmembrane domain and the extracellular loop, respectively, so that they might possibly affect the substrate specificity. Therefore, polymorphisms of the OATP8 gene may affect the pharmacokinetics of substrates, such as digoxin. In fact, SNPs T334G and G699A have been demonstrated to affect the affinity for bromosulfophthalein and cholytaurine, substrates of OATP8 (Table 2). It remains to be examined whether these polymorphisms affect the pharmacokinetics of OATP8 substrates such as digoxin in humans.

In conclusion, the present study is the first to establish the allele frequencies of polymorphisms, and their linkage disequilibrium, of the OATP8 gene in the Japanese population.

References

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