SNP Communication

A Novel Variant Allele of OATP-C (SLCO1B1) Found in a Japanese Patient with Pravastatin-induced Myopathy

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Summary: We have recently found that the frequency of OATP-C*15 is significantly higher in patients who experienced myopathy after receiving pravastatin or atorvastatin than in patients without myopathy. However, there were two patients who experienced pravastatin-induced myopathy despite the fact that they did not possess OATP-C*15 or other known mutations of OATP-C that have been reported to decrease the function of OATP-C. In this study, we sequenced all of the exons and exon-intron junctions of OATP-C of the two patients and found a novel mutation in exon 12 of OATP-C in one of the patients. In this mutation (1628T>G), there is a substitution of Leu to Trp at position 543 in transmembrane-spanning domain 10 of OATP-C. However, the frequency of this mutation in the Japanese population appears to be very low (<1%).

Key words: OATP-C (SLCO1B1); novel variant allele; nonsynonymous; statins

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, also known as statins, are the most effective drugs for treatment of elevated concentration of low-density lipoprotein cholesterol, and they have been shown to reduce cardiovascular events of coronary heart disease and cardiovascular-related morbidity and mortality rates. These drugs are tolerated well by most patients, but they can produce a variety of muscle-related complaints like myopathy and rhabdomyolysis, which have been the major clinical complication for statin treatment.2 We have recently studied genetic factors contributing to the risk of statin-induced myopathy and found that the frequency of OATP-C*15, a mutant allele of OATP-C (OATP1B1, gene SLC21A6/SLCO1B1), was significantly higher in patients with myopathy who were receiving pravastatin or atorvastatin than in patients without myopathy.3 We also found in another study that transporting activities for pravastatin and atorvastatin decreased significantly in HEK293 cells expressing OATP-C*15 compared to those in cells expressing OATP-C*1a, the reference allele of OATP-C.4 Based on these findings, we speculated that patients treated with pravastatin or atorvastatin did not possess OATP-C*15 or mutated alleles of OATP-C that have been reported to decrease the function of OATP-C. Therefore, we sequenced all of the exons and exon-intron junctions of OATP-C for the DNA samples of these patients, and we found a novel nonsynonymous mutation of OATP-C located in exon 12 of this gene.

Materials and Methods

Human genomic DNA samples: DNA samples obtained from the two patients who experienced myopathy after receiving pravastatin or atorvastatin did not possess OATP-C*15 were used in the present study. None of the known mutant alleles of OATP-C that have
been reported to decrease its activity29 were found in the DNA samples obtained from these patients. Written informed consent was obtained from the patients, and the study was approved by the Ethics Committee of the Graduate School of Pharmaceutical Sciences, Chiba University. We also studied fifty DNA samples obtained from healthy Japanese volunteers for the determination of allele frequency. Written informed consent was obtained from all of the volunteers, and the study was also approved by the Ethics Committee of the Graduate School of Pharmaceutical Sciences, Chiba University.

Polymerase chain reaction (PCR) conditions for sequencing: All exons and exon/intron boundaries of the OATP-C in the DNA samples obtained from the two patients were analyzed by PCR and direct sequencing. The primers used for amplification of the genomic DNA and direct sequencing are summarized in Table 1. DNA amplification was conducted in 2-mL reaction mixtures (50 μL) containing 1-2 μg/mL genomic DNA, 1.2 mM MgSO4, 5 mM dNTPs, 5 mM KOD buffer, 0.02 U/μL KOD-plus-polymerase and 0.2 μM of each primer. Thermocycling conditions consisted of initial denaturation for 3 minutes at 94°C followed by 35 cycles of denaturation at 96°C for 20 seconds, annealing at 57°C (reducing by 2°C every 3 cycles 2 times followed by 26 cycles at 51°C) for 30 seconds, and extension at 68°C for 25 seconds. Terminal elongation was performed at 68°C for 2 minutes. The PCR product was purified using Wizard® SV Gel and PCR Clean-Up System (Promega Corp., Madison, WI, USA) and directly sequenced on a CEQ88 2000 DNA Analysis System (Beckman Coulter, Inc., Fullerton, CA, USA) with a CEQ® 88 DTCS Quick start kit (Beckman Coulter, Inc.). The reference sequence of OATP-C was obtained from GenBank (NT_000122.9).

DNA samples from healthy volunteers were analyzed by PCR and direct sequencing using the primers used for sequencing exon 12 (Table 1). PCR and thermocycling conditions were the same as the described above.

### Results and Discussion

A novel nonsynonymous single nucleotide polymorphism (SNP) was found in one of the DNA samples from the two patients.

SNP: 04101050101001; GENENAME: SLCO1B1; ACCESSION NUMBER: NT_000102.9; LENGTH: 25 bases; 5'-GAATACAGTCGTT/GGAATTTATT-TC-3'. The SNP was 1628T>G in exon 12 of OATP-C (Fig. 1). In this SNP, Leu is substituted by Trp at position 543 in transmembrane-spanning domain 10 of OATP-C. Although the functional significance of this SNP is not known, it may cause functional impairment of OATP-C because it has been reported that non-synonymous SNPs within the putative transmembrane domains in OATP-C result in severely reduced function of OATP-C due to its decreased plasma membrane expression.29 This novel SNP was not found in any of the 50 DNA samples from healthy Japanese volunteers. The results suggest that the allele frequency of this novel variant allele of OATP-C is very low (<1%) in the Japanese population. However, OATP-C is responsible for the hepatocellular uptake of a broad range of endogenous and xenobiotic compounds, including bile acid, glucuronide and sulfate conjugates, methotrexate, pravastatin, rosuvastatin and cerivastatin.4,9 Therefore, further studies are required to clarify the exact frequency in the Japanese population and the functional characteristics of this novel variant allele of OATP-C.

In conclusion, we found a novel nonsynonymous mutation (1628T>G) located in exon 12 of OATP-C in a DNA sample from a patient who experienced myopathy after receiving pravastatin. In this mutation, there is a substitution of Leu to Trp at position 543 in transmembrane-spanning domain 10 of OATP-C. The

### Table 1. Primer sequences used for the analysis of OATP-C

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward Primer (5'→3')</th>
<th>Reverse Primer (5'→3')</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CATGACCTGACAGTGGGAAAG</td>
<td>GTGATCTATCCAAACAAAAAG</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>3</td>
<td>GAACTGACGTTTTACATGCT</td>
<td>CCTTGAGTATGAACCAACCC</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>4</td>
<td>CATCTTCCCTTTTCTTATCA</td>
<td>GTACACTTTATGGGATCTTCT</td>
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<tr>
<td>5</td>
<td>GTACCTGTTGAAATTTTGGGAA</td>
<td>CTTGGTTTTAATGGGCGAATT</td>
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</tr>
<tr>
<td>6-7</td>
<td>GGACTTTACACATATTGGTGA</td>
<td>GCTGATTTTATTTTTGATT</td>
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<tr>
<td>7</td>
<td>TCCCGCTGCCTCTTCTTGA</td>
<td></td>
<td>sequence</td>
</tr>
<tr>
<td>8</td>
<td>CCTAGACAGTATGTGGATTATGCA</td>
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<tr>
<td>9</td>
<td>TGAAATGACCCAGGATACAC</td>
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<td>CAACCTTCGGTGTCCCTTATTAG</td>
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<tr>
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</tr>
<tr>
<td>12</td>
<td>GTCCAAAAGATGTGATGTGCTG</td>
<td>CAGGCTGGAGGATCTGATA</td>
<td>sequence</td>
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</table>
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Wild type (sense)

![Wild type sequence](image1)

Variant (sense)

![Variant sequence](image2)

Fig. 1. Nucleotide sequences of OATP-C containing novel variant 041015OshT001 (1628T>G) in exon 12. Arrows indicate the positions of the nucleotide change.

frequency of this mutation in the Japanese population appears to be very low (<1%).

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References


