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A frameshift variant of CYP2C8 was identified in a patient who suffered from rhabdomyolysis after administration of cerivastatin

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Abstract A hypercholesterolemic patient medicated with cerivastatin for 22 days resulted in acute rhabdomyolysis. CYP2C8 and CYP3A4 are the major enzymes responsible for the metabolism of cerivastatin, and a transporter, OATP2, contributes to uptake of cerivastatin to the liver. In this study, the patient's DNA was sequenced in order to identify a variant that would lead to the adverse effect of cerivastatin. Three nucleotide variants, 475delA, G874C, and T1551C, were found in the exons of CYP2C8. The patient was homozygous for 475delA variant that leads to frameshift and premature termination. Accordingly, the patient is most likely lacking the enzyme activity. The patient's children were both heterozygous for the mutation. The patient had three nucleotide variants in exon 4 (A388G) and exon 5 (C571T and C597T) of OATP2 that were all heterozygous. No nucleotide variation in the exons of CYP3A4 was identified. To our knowledge, this is the first report showing that the adverse effect of cerivastatin might be caused by the genetic variant of CYP2C8.

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Introduction

All hydroxymethylglutaryl-CoA reductase inhibitors (statins) potentially cause rhabdomyolysis as a serious adverse effect (Rossenson 2004). Cerivastatin was developed as a highly potent, lipophilic, and pure enantiomeric reductase inhibitor and has been evaluated in clinical efficacy and safety trials since 1993 (Stein 1998). Its dual mechanism of elimination mediated by CYP2C8 and CYP3A4 was originally thought to impart a safety benefit relative to other statins that were exclusively metabolized via the CYP3A4 pathway. However, despite the dual metabolizing pathways, cerivastatin caused rhabdomyolysis more frequently than other statins, especially when cerivastatin was given together with other drugs such as gemfibrozil (Farmer 2001; Staffa et al. 2002). In vitro experiment with human liver microsome demonstrated that gemfibrozil inhibited CYP2C8 activity leading to a decreased level of M-23 in the cerivastatin-metabolizing pathway, which at least partially explains the interaction between gemfibrozil and cerivastatin (Wang et al. 2002). Because of the adverse effects in the muscles causing rhabdomyolysis, cerivastatin was withdrawn from the market in August 2001. In the metabolic pathway of cerivastatin, CYP2C8 and CYP3A4 are equally important in the formation of M-1 and the subsequent formation of M-23, the major metabolite of cerivastatin, is mainly mediated by CYP2C8 (Mück 2000). It was also suggested that transporters OATP2 and MPR2 contributed to hepatic clearance of cerivastatin (Matushima et al. 2002). In the present study, we sequenced the above candidate genes in the DNA of a patient who suffered from rhabdomyolysis after taking cerivastatin.

Subjects and methods

Subject A 74-year-old hypercholesterolemic woman who showed rhabdomyolysis after taking cerivastatin

for 22 days, her son, and her daughter were recruited for the study. Serum creatinine phosphokinase was 19,190 IU/l, and myoglobin level was over 3,000 ng/ml. The participants gave informed consent, and the study was performed according to the Institutional Review Board of Toho University. The patient had taken pravastatin for the previous 2 years and simvastatin for 1.5 years before administration of cerivastatin, and no complaints with those drugs were reported. A consanguineous marriage was described for the patient's parents.

PCR condition and primer design Puregene DNA isolation kit was used to isolate genomic DNA from peripheral blood (Gentra systems, Inc., Minneapolis, MN, USA). Taq DNA polymerase (EX Taq HS) was from Takara (Kyoto, Japan). PCR amplification was under the following conditions: 94°C for 1 min, 40 cycles

of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min, then 72°C for 5 min. Primer sequences are shown in Table 1. Nucleotide variations were searched with direct sequencing using BigDye Terminator cycle sequencing on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Tokyo, Japan).

Results and discussion

The hypercholesterolemic patient showed severe rhabdomyolysis after taking cerivastatin. Cerivastatin is up-taken and metabolized in liver by OATP2, CYP2C8, and CYP3A4. To clarify the genetic susceptibility to the adverse effect of cerivastatin, these candidate genes were analyzed by direct sequencing in the patient and her children.

Table 1 Primers used for sequencing CYP2C8, CYP3A4, and OATP2 exons

Exon(s)	Forward primer (5'–3') ^a	Reverse primer (5'–3') ^a	Product size (bp)
CTP2C8			
1	CAATTGGCTGGAGGAACATAA	GGCTTCAATGGAACCTTTTG	156
2	TAGGGCTCTGTTTTCCATCC	TTGCTGCTATTGCAATTC	212
3	TTTCTCCCTCACAACCTTGC	TGCTTCAAATCTCCCTCCAC	200
4	AAACCAAGTCTTCCCTACAACC	TTTTTGGACACATGGGGAAT	250
5	CGATGAATCACAAAATGGACA	ATTTCAATCAGGGCTTGGTG	209
6	GTGGAGGATACTGGCACCAT	TGTCCAGTATCTCTTTTGGCTAAC	264
7	GTTGGAACCAAAACCAGCACT	GGCCATGAATTGCTATGACA	268
8	CCAGAGGAGGTGCCATGTAA	GCGCTACGTGATGTCCACTA	256
9-1	TGGGAAGATTTGATGAGAGG	TGCATATTCTGTTGTGCAAGTC	340
9-2	TGAACCAGCAATTAATAACACTTTTT	TGAAGAATGCTAGCCCATCTG	320
CYP3A4			
1	AAGGGAAAGAGAGGCCTGAT	AAAGCTCCATGCACATAGCC	173
2	CCCCTGAGGAGAAGCATTTT	TCGTCTCTTGAGCATTCCA	210
3	TGTGCAGTGGGGTAAACTCA	CCCTTTTCAAATACCTCCTCTG	220
4	TTTCAACACATGAATGCTTACCC	TGTGTATTGTACAATAAGGGATTATG	182
5	ACACTGGGCATCTGGGATAA	CAGTGGACTACCCCTTGGAA	250
6	TTACCACAGCCCTCCTTTTG	GCCATGTCCTTCTGGGACTA	220
7	ATGTGGGTTTCCTGTTGCAT	AAAGCAGTTATTTTAAAGAGAGCAAGA	200
8	TGAGCAGTCTTCATGTAAAAAGC	GAAGGAATGGCTTCCAGTTG	304
9	AGATCAAGGACCAACGCTTG	GCATGCCCTCTAGAAAGTGCC	220
10	TTTTTCTTTTCAGAGCCTTCC	AGGGATTTGAGGGCTTCACT	340
11	GGCAGAATATGCTTGAACCAG	TCTCTGGAGCTCCTAATACTTCA	300
12	TTTGGCCCAGAGAACAAATTA	AATGACCAGCCCACAAAAGT	320
13	CCTCCTCACACTGATTGGTC	GTCCCTCAACACTGAAGGA	350
OATP2			
1-1	AGAGCTTCATTTTTGAGACTTGA	CTTGTTGCAGTTGCTGTAGGA	260
1-2	TGTICTTTTTAAACACTTGCTTTTC	TTCATGTAATCTGGTGTGTGATTC	400
2	TTTTTAACTTTGCAAGTTTGGTT	TGGCTGAGTAGTAGTACCTGGTAAA	266
3	TGGGTATCTTCTCAAAGGTAAC	TCAATGAGTGGTCTAATGTAGGTGA	250
4	TGTTGTTAATGGGCGAACTG	GGGGAAGATAATGGTGCAA	294
5	CCAAGAATGCATGGTCTTATTC	TGTCAAAGTTTGCAAAGTGAA	320
6	GGAGCTGGATTTTATATTTATCTGA	TTGTATGATCACTTTCCCTTTGTC	260
7	GCAAAAGAAAGCCAACTCCA	TCATACCATTATTTCCCTGAACC	320
8	GAGCAATAGTGACATCACAAGTTTTA	CAGGCTATTCTCACTCTTTGTGTT	370
9	TTTAGTTAAAATTGTTTTCTGCCTTA	TTGAATAAATTTGATTGACATACATTG	270
10	CATCACACCCATCACAATAACA	GGCAAAGATGGAGAGCGTAA	400
11	CAGCCTTGAGAGTTCATAGTAATTTT	AGTTTGAACAAGTGAGACTTCACTAAA	320
12	TGAAATTCCTCTTAAAGTCCA	TGAAATCATGATGCATTGAATAAA	250
13	TGACATGAGGAGAGTTTGGAA	TCAATGTGGAATATCATGCAGTT	330
14-1	TGGATCATATAGTCTAACCTTAACAC	GGATCTGGATACTGGAGAAAATG	430
14-2	TGGAATGTCTTTATTTCTTCCACA	GTTTCCAAACAGCATTGCAT	460

^aThese primers were designed with gene arrangement of complementary DNA expect CYP2C8 exon 3 and CYP3A4 exons 5, 7, and 9

Fig. 1 Schematic drawing of the CYP2C8 gene and variant sites. ↑: Exonic nucleotide substitutions reported by others. ↓: Exonic nucleotide substitutions indicated in this study. 475delA was shown in underlined bold and subsequent truncated peptide sequence was depicted underneath the nucleotide sequence

CYP2C8, comprising nine exons, was screened for molecular variant as shown in Fig. 1. The direct sequencing of the patient DNA demonstrated three

Fig. 2 The nucleotide sequencing of CYP2C8 in the patient, her children, and a control (a–c). A-1, A-2, A-3, and B denote the patient, her son, her daughter, and the control, respectively. Arrows indicate the nucleotide positions of the variant

nucleotide variations, 475delA, G874T, and T1551C (Figs. 1, 2). The patient was homozygous for the 475delA and T1551C mutations that were identified in the coding region. Deletion of adenine at nucleotide 475 resulted in premature termination at the amino acid residue 177 due to creating an early stop codon. Nakajima et al. (2003) reported that the 475delA mutation, denoting CYP2C8*5a, was detected in one subject after screening 200 subjects, showing that the allele frequency of the variant was estimated to be 0.0025. We screened 384 Japanese subjects for the variant; however, we failed to detect the 475delA mutation in our sample set.

Consanguinity, as evident from the clinical record, might explain the homozygous state of the patient. DNA samples obtained from the patient's son and daughter were also examined by direct sequencing; both were heterozygous for the 475delA mutation (Fig. 2a). C1551T and G874T variants were newly identified in this study. The patient was homozygous for C1551T variant, and her children were both heterozygous for the variant (Fig. 2c). The patient and her children were heterozygous for G874T in exon 6 (Fig. 2b), and the nucleotide change resulted in Ala²⁹² to Pro²⁹². The nonsynonymous G874T variant appears to be common in the general population because seven Japanese individuals examined were all heterozygous.

All 14 exons of OATP2 were screened, and three nucleotide variations, A388G in exon 4, and C571T and C597T in exon 5, were identified; however, all variants were silent and were also observed in control individuals. CYP3A4 consists of 13 exons (Lamba et al. 2002) and the patient did not show nucleotide variations.

CYP2C8 is a pivotal enzyme in the catalyzing pathway of cerivastatin that is also highlighted by the recent report showing that the drug gemfibrozil, when interacting with cerivastatin, inhibits CYP2C8 activity in human liver microsome (Wang et al. 2002). The patient most likely lacks the enzyme activity of CYP2C8 due to the homozygous mutation of 475delA that loses 64% of the protein structure. Indeed, our patient showed an elevated level of M-1 and a decreased level of M-23 that would be caused by the deficiency of CYP2C8 activity (Ozaki et al. manuscript in preparation). Therefore, the lack of enzyme activity of CYP2C8 might plausibly explain the etiology of cerivastatin-induced rhabdomyolysis. Because the allele frequency of the mutation is extremely low (<0.0000068 in our set), the homozygous individuals should be very rare and might experience little clinical impact despite the severity of the rhabdomyolysis. However, a possibility remains that the heterozygous subject is susceptible to the administration of cerivastatin, leading to rather mild myopathy. This possibility could not be answered easily because the drug was withdrawn from the market. As CYP2C8 plays important roles in metabolizing therapeutic drugs such as paclitaxel, an anticancer drug (Rahman et al. 1994); troglitazone, an antidiabetes drug (Yamazaki et al.

2000); and amiodarone, an antiarrhythmic drug (Ohyama et al. 2000); attention should be paid when heterozygous subjects take those drugs.

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