J Hum Genet (2006) 51:822–826
DOI 10.1007/s10038-006-0025-1

SHORT COMMUNICATION

Pharmacogenetic determinants of variability in lipid-lowering response to pravastatin therapy

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Received: 24 April 2006 / Accepted: 30 May 2006 / Published online: 18 August 2006

Abstract Pravastatin is mainly taken up from the circulation into the liver via organic anion-transporting polypeptide 1B1 (*SLCO1B1* gene product). We examined the contribution of genetic variants in the *SLCO1B1* gene and other candidate genes to the variability of pravastatin efficacy in 33 hypercholesterolemic patients. In the initial phase of pravastatin treatment (8 weeks), heterozygous carriers of the *SLCO1B1**15 allele had poor low-density lipoprotein cholesterol (LDL-C) reduction relative to non-carriers (percent reduction: –14.1 vs –28.9%); however, the genotype-dependent difference in the cholesterol-lowering effect disappeared after 1 year of treatment. Cholesterol 7α-hydroxylase (*CYP7A1*) and apolipoprotein E (*APOE*) are known to contribute to lipid metabolism. Homozygous carriers of the *CYP7A1* -204C allele or heterozygotes for both *CYP7A1* -204C and *APOE* ϵ4 alleles showed significantly poorer LDL-C reduction compared to that in other genotypic groups after 1 year of treatment (–24.3 vs –33.1%). These results suggest that the *SLCO1B1**15 allele is associated with a slow response to pravastatin therapy, and the combined genotyping of *CYP7A1* and *APOE* genes is a useful index of the lipid-lowering effect of pravastatin.

Keywords *SLCO1B1* · *CYP7A1* · *APOE* · Pravastatin · Cholesterol

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Introduction

Coronary heart disease is the leading cause of death worldwide. Several risk factors for cardiovascular disease are well known, especially increased low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C). Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis. Lipid-lowering therapy by statins has the potential to improve outcomes in patients at risk for cardiovascular disease. Despite these large effects, interindividual variability in the response to statins has been observed in clinical situations (Pazzucconi et al. 1995). Previous studies have demonstrated that the mechanisms responsible for variability in the statin response are due, at least in part, to genetic factors. Most studies have focused on the association between variants (ϵ2, ϵ3 and ϵ4) in apolipoprotein E (*APOE*) gene, which is a primary ligand for the LDL receptor found on the liver, and the response to statins (Ojala et al. 1991; Ordovas et al. 1995). In addition, recent studies have demonstrated

that variants in cholesterol 7alpha-hydroxylase (*CYP7A1*) (Pullinger et al. 2002), *ABCG8* (Kajinami et al. 2004) and HMG-CoA reductase (*HMGCR*) (Chasman et al. 2004) are important determinants of the lipid response to statin therapy.

Pravastatin, a hydrophilic HMG-CoA reductase inhibitor, is taken up efficiently from the circulation into the liver by an active transport carrier system, but is not metabolized by CYP enzymes. Human organic anion-transporting polypeptide 1B1 (OATP1B1), transporter of pravastatin, is expressed on the basolateral membrane in the hepatocytes responsible for the hepatocellular uptake of pravastatin (Hsiang et al. 1999). The major site of cholesterol synthesis, the liver, is the main target organ of statins. Recently, Niemi et al. (2005) have shown that the *SLCO1B1**17 allele (containing -11187G>A, 388A>G and 521T>C) is associated with the decreased acute effect of pravastatin on cholesterol synthesis; however, the impact of *SLCO1B1* genotypes on the lipid-lowering response to pravastatin during long-term treatment has not been well investigated.

The aim of this study was to describe the influence of *SLCO1B1* genotypes on the lipid-lowering response to pravastatin in Japanese hypercholesterolemic patients. Furthermore, we evaluated the contribution of genetic variants in other candidate genes (*APOE*, *CYP7A1*, *ABCG8* and *HMGCR*) to the variability in pravastatin efficacy.

Materials and methods

Study design

We studied 33 patients (14 males and 19 females; mean age 62.3 years; age range 34–83 years) with hypercholesterolemia treated in Tottori University Hospital. All subjects were initially prescribed pravastatin (mean dose range 9.4 mg/day) between January 1997 and October 2004. We used the electronic medical database available in the hospital to obtain precise information on patients' backgrounds, laboratory tests, prescribed drugs and adverse events. We collected these data retrospectively for each patient for at least 1 year from the day pravastatin was administered. Patients with serious or uncontrolled renal or liver disease, no drug compliance, other hypolipidemic treatment or uncontrolled diabetes were excluded. The average body mass index (BMI), total cholesterol (TC) and LDL-C values in this study patients were 23.9 kg/m^2 (range 17.3–30.9 kg/m^2), 259.6 mg/dl (range 225.8–315.0 mg/dl) and 167.4 mg/dl (range 112.0–240.7 mg/dl), respectively. This study was approved by the Tottori University Ethics Committee, and informed consent was obtained from all individuals.

Genotyping

All subjects were genotyped for variants in the candidate genes involved in the pharmacokinetics and pharmacodynamics of pravastatin. Details of the genotyping and haplotyping of *SLCO1B1**1b (388A>G), *5 (521T>C) and *15 (388A>G and 521T>C) were described previously (Nishizato et al. 2003). The promoter variant (-11187G>A) in the *SLCO1B1* gene was determined with PCR–SSCP analysis. The *SLCO1B1* -11187G>A variant was observed as heterozygosity (0.212) in this patient group suggesting it was tightly linked to the *SLCO1B1**15 allele. The genotypes in *CYP7A1* (-204A>C) (Hubacek et al. 2003), *APOE* (ϵ2, ϵ3 and ϵ4) (Hixon and Vernier 1990) and *ABCG8* (55G>C) (Kajinami et al. 2004) were examined by previously described methods using PCR restriction fragment length polymorphism analysis. Genetic variants (SNP12 and 29) in the *HMGCR* gene were found as functional variants for variable response to statin therapy in the previous study (Chasman et al. 2004) as determined with PCR–SSCP analysis.

Statistical analysis

Comparisons between two groups were performed using a Student *t*-test and between more than two groups using ANOVA (with Tukey–Kramer multiple comparison test). A 5% level of probability was considered to be significant.

Results and discussion

The mean percent reductions from the baseline in TC and LDL-C values at 8 weeks post-treatment with pravastain were significantly smaller in heterozygous carriers of the *SLCO1B1**15 allele than in homozygous carriers of the *1a and *1b alleles (Fig. 1a, $P<0.05$). Also, the mean percent reduction from the baseline in TC values at 8 weeks post-treatment was significantly smaller in *SLCO1B1**15 carriers than in non-carriers (–9.8 vs –20.9%; $P<0.05$; Fig. 1b). A similar trend was observed in the LDL-C level (–14.1 vs –28.9%, $P<0.05$; Fig. 1b) even though the pravastatin daily dose (mean±SD; non-carriers: 9.4±2.9 mg, carriers:

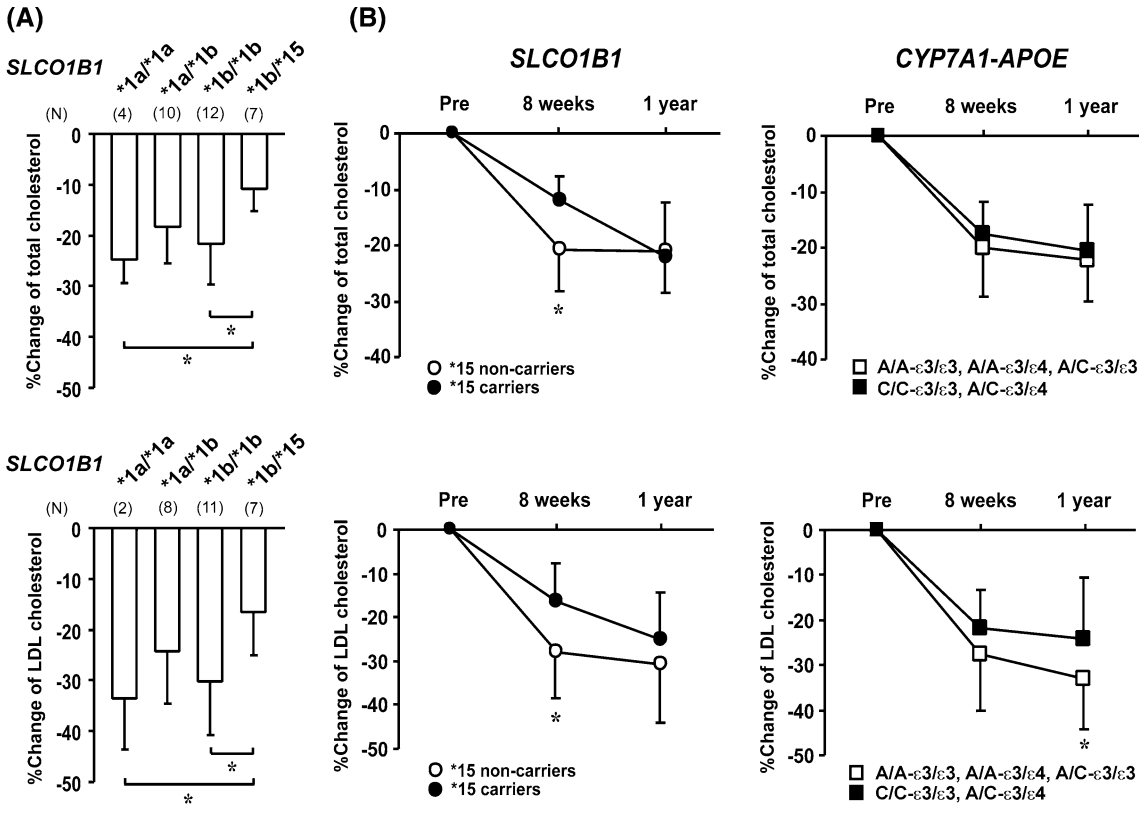

Fig. 1 **a** Influence of the *SLCO1B1* genotypes on percent reduction from baseline in TC and LDL-C values at 8 weeks after pravastatin treatment. *P<0.05 when compared between the two groups using Tukey–Kramer multiple comparison test. **b** Influence of the *SLCO1B1*, *CYP7A1* and *APOE* genotypes on time course of percent reduction from baseline in TC and LDL-C value after pravastatin treatment. *P<0.05 when compared between the two genotypes was analyzed with Student's t-test. Each value is the mean±SD

9.3±2.0 mg,) and BMI (non-carriers: 24.1±3.5 kg/m^2, carriers: 23.5±2.7 kg/m^2) were not significantly different between the two groups. In contrast, at 1 year post-treatment, there were no significant differences in the reduction of TC and LDL-C values between the two groups (Fig. 1b; Table 1).

In an in vitro experiment, Iwai et al. (2004) demonstrated that the transport activity of *SLCO1B1**15 allele is significantly decreased compared with that of the *SLCO1B1**1a or *1b allele using cDNA-transfected HEK293 cells. Previously, we found *SLCO1B1**15 allele was associated with higher plasma concentration of pravastatin, and the non-renal clearance of pravastatin in subjects with *SLCO1B1**1b/*15 and *15/*15 was reduced to 55 and 14% of *1b/*1b subjects, respectively (Nishizato et al. 2003). Thus, it is suggested that the *SLCO1B1**15 allele leads to an increase in plasma pravastatin concentrations but a reduction in the hepatocellular uptake of pravastatin, resulting in a decreased effect of pravastatin. However, interestingly, the genotype-dependent difference in this lowering effect disappeared after long-term treatment. Although its mechanism remains to be elucidated, one possible reason is that all of our patients with the *SLCO1B1**15 allele were heterozygotes for functionally active *1a or *1b alleles (Iwai et al. 2004). Thus, the lipid-lowering profiles in homozygotes for the *15 allele are of interest.

Multidrug resistance-associated protein 2 (MRP2/ABCC2) on the bile canalicular membrane is mainly involved in the biliary excretion of pravastatin (Matsushima et al. 2005). With regard to liver concentration of pravastatin, genetic polymorphisms of *MRP2* might affect response to pravastatin. However, *MRP2* variants have been observed at low frequency in Japanese (Itoda et al. 2002), and functional significance of these variants is not established. Therefore, association of *MRP2* genotypes should be analyzed by further studies.

We also examined the influence of the *CYP7A1* promoter (-204A/C) and *APOE* (ϵ2, ϵ3 and ϵ4) variants on the clinical outcome of pravastatin therapy. As shown in Fig. 1b and Table 1, the reduction from the baseline in LDL-C value at 1 year post-treatment was

Table 1 Association of *SLCO1B1*, *CYP7A1* and *APOE* genotypes with lipid changes

Gene	Genotype	Lipid concentrations (mg/dl)					
		N	Baseline	*N*	8 weeks	*N*	1 year
Total cholesterol							
*SLCO1B1**15	Non-carriers	26	260.9±24.4	26	205.8±22.2	20	201.9±18.5
	Carriers	7	254.8±10.6	7	227.9±19.6	6	204.0±16.5
	P value		NS		<0.05		NS
CYP7A1-APOE	A/A-ϵ3/ϵ3, A/A-ϵ3/ϵ4, A/C-ϵ3/ϵ3	19	261.9±23.9	19	210.3±27.9	14	198.9±12.7
	C/C-ϵ3/ϵ3, A/C-ϵ3/ϵ4	14	256.4±20.1	14	210.7±16.0	12	206.0±22.3
	P value		NS		NS		NS
LDL cholesterol							
*SLCO1B1**15	Non-carriers	22	170.7±27.4	22	124.0±20.7	17	115.1±23.9
	Carriers	7	157.0±29.3	7	132.0±32.7	6	110.5±10.9
	P value		NS		NS		NS
CYP7A1-APOE	A/A-ϵ3/ϵ3, A/A-ϵ3/ϵ4, A/C-ϵ3/ϵ3	19	168.6±34.4	19	124.0±29.9	12	106.3±20.6
	C/C-ϵ3/ϵ3, A/C-ϵ3/ϵ4	12	165.7±16.3	12	128.7±12.5	10	123.8±12.5
	P value		NS		NS		<0.05

Values are mean±SD

Statistical significance between the two genotypes was analyzed with Student's *t*-test

NS No significant difference

significantly decreased in carriers of A/A-ϵ3/ϵ3, A/A-ϵ3/ϵ4 or A/C-ϵ3/ϵ3 in *CYP7A1* and *APOE* genes compared with C/C-ϵ3/ϵ3 or A/C-ϵ3/ϵ4 carriers. There was no significant effect of genotypes (A/A-ϵ3/ϵ3, A/A-ϵ3/ϵ4 or A/C-ϵ3/ϵ3 vs C/C-ϵ3/ϵ3 or A/C-ϵ3/ϵ4) in the *CYP7A1* and *APOE* genes on pravastatin dose (10.0±2.9 vs 8.8±2.9 mg) and BMI (23.8±3.6 vs 24.5±3.0 kg/m^2). Only one patient was a heterozygous carrier of SNP12 in the *HMGCR* gene. However, no remarkable difference in the lipid-lowering effects was observed in this patient. Also, SNP29 in *HMGCR* and 55G>C in *ABCG8* were not detected.

In contrast to *SLCO1B1* gene, part of the interpatient variability in the efficacy of pravastatin after long-term treatment may be attributable to genetic variation, and combined genotyping of *CYP7A1* and *APOE* genes is useful for describing the lowering effects. Since the basal cholesterol synthesis rate is a key determinant for statin response, loss of CYP7A1 activity, which is involved in bile acid synthesis from cholesterol in the liver, may result in a poor response to statin treatment (Pullinger et al. 2002). A previous study has shown that the nucleotide sequence around position -204 negatively regulates CYP7A1 promoter activity (Cooper et al. 1997). Among the known variants, the *CYP7A1* -204A>C variant is expected to decrease promoter activity (Kajinami et al. 2005). Apolipoprotein E is known as one of the major determinants in lipoprotein metabolism. Previous studies (Ojala et al. 1991; Ordovas et al. 1995) demonstrated that the ϵ4 allele in primary hypercholesterolemia is associated with lower response to statin, when compared to ϵ2 and ϵ3 alleles, because the binding activity of ϵ4 allele to receptor is relatively higher than that of other alleles. These results suggest that decreased cholesterol 7alpha-hydroxylase activity and increased binding affinity of apolipoprotein E to LDL receptor enhance the intracellular cholesterol content in hepatocytes, resulting in lower HMG-CoA reductase activity, which may also lead to tolerance to statin treatment (Kajinami et al. 2005).

In conclusion, our results suggest that the *SLCO1B1**15 allele is associated with a slow response to pravastatin. Instead of *SLCO1B1**15, combined genotyping of *CYP7A1* -204A>C and *APOE* ϵ4 variants may be useful for describing the long-term clinical outcomes of pravastatin. Further study is necessary to confirm the influence of genetic variants in these candidate genes on the lipid-lowering efficacy of pravastatin as well as other statins in a large sample size.

Acknowledgements This study is supported by Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, Tokyo, Japan.

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