Lack of effect of genetic polymorphisms of SLC01B1 on the lipid-lowering response to pitavastatin in Chinese patients

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Aim: To investigate the SLC01B1 388A>G and 521T>C polymorphisms in hyperlipidemia patients and evaluate the effect of the two polymorphisms on the lipid-lowering efficacy of pitavastatin.

Methods: The functional polymorphisms of SLC01B1 (388A>G and 521T>C) were genotyped in 140 Chinese patients with essential hyperlipidemia using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and one-step tetra-primers ARMS-PCR. Eighty-five patients were enrolled in the clinical trial and given 2 mg of pitavastatin daily for 8 weeks. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) serum levels were measured at baseline, after 4 weeks and after 8 weeks of treatment.

Results: The allele frequencies of SLC01B1 388A>G and 521T>C in essential hyperlipidemia patients were 71.1% and 11.1%, respectively. The 4- and 8-week treatment with pitavastatin significantly reduced TC, TG, and LDL levels, but there was no statistical difference among patients with wild type, SLC01B1 388A>G or SLC01B1 521T>C in the lipid-lowering efficacy of pitavastatin.

Conclusion: The present study found that the allele frequencies of SLC01B1 388A>G and 521T>C in Chinese patients with essential hyperlipidemia are comparable to those in healthy Chinese population. SLC01B1 388A>G and 521T>C do not affect the lipid-lowering efficacy of pitavastatin.

Keywords: essential hyperlipidemia; polymorphism; SLC01B1; pitavastatin

Introduction

The organic anion transporting polypeptide 1B1 (OATP1B1, also known as OATP-C, OATP2, and LST-1), exclusively expressed in the basolateral membrane of hepatocytes[1] is an important transporter of the OATP family, which contributes to the hepatic uptake of many endogenous and xenobiotic compounds including bile acids, sulfate and glucuronide conjugates, thyroid hormones, peptides and drugs such as pravastatin, pitavastatin, methotrexate and rifampin[2].

The highly polymorphic gene SLC01B1 encodes OATP1B1: to date, more than 20 single nucleotide polymorphisms (SNPs) have been identified, some of which are associated with altered function. SLC01B1 388A>G (Asn130Asp) and 521T>C (Val174Ala) are common variants and have altered transport activities in vitro. In addition, cumulative evidence indicates that SLC01B1 polymorphisms can significantly affect the pharmacokinetics of statins.

Pitavastatin (p-INN) is a novel synthetic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitor. It has a stronger hypolipidemic profile and a safer profile than other statins, and may be a better choice for treatment of hypercholesterolemic patients[3]. In vivo, pitavastatin is scarcely metabolized by cytochrome P450 2C9 and undergoes reversible lactonization[3, 4]. OATP1B1 is a transporter in the liver and plays an important role in the distribution of pitavastatin[5]. Some research[6-8] indicates that SLC01B1 polymorphisms...
have significant effects on the pharmacokinetics of pitavastatin. The dose-normalized area under the plasma concentration–time curve (AUC) and peak plasma concentration (C_{max}) values of pitavastatin in heterozygous subjects of SLCO1B1*15 (388A>G and 521T>C) or 521T>C were 1.4- and 1.8-fold higher, respectively, than in subjects without these SNPs\(^7\). SLCO1B1 polymorphisms had greater effects on the pharmacokinetic parameters of pitavastatin than those previously reported in pravastatin\(^6\). In addition, recent studies\(^9\) reported that SLCO1B1 polymorphisms were associated with the lipid-lowering response to HMG-CoA inhibitors and adverse effects of simvastatin and irinotecan. The present study was conducted to investigate the contribution of the SLCO1B1 polymorphisms to inter-individual response to pitavastatin in Chinese primary hyperlipidemia patients.

**Materials and methods**

**Subjects**

We genotyped 140 Chinese primary hyperlipidemia patients with total cholesterol (TC)≥5.72 mmol/L, triglyceride (TG)≥1.70 mmol/L, or high-density lipoprotein cholesterol (HDL-c)≤ 0.91 mmol/L after dietary intervention: 85 of those patients (ages 18–70) with varying genotypes were enrolled in this clinical trial. Exclusion criteria were as follows: hyperlipidemia caused by drugs or other causes, previous hypersensitivity to statins or other drugs, severe impairment of renal or hepatic function, history of thyroid hypofunction, history of mental illness, history of acute myocardial infarction, cerebrovascular accident, or myopathy, and treatment with other drugs (eg cyclosporin) that may increase the risk of toxic response to pitavastatin. Patients currently receiving treatment with lipid-regulating drugs were eligible to participate in the clinical trial after a 2-week washout period. This study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University. All subjects were informed of the detailed protocol of the clinical trial and gave their informed consent.

**Clinical trial protocol**

Subjects were treated with 2 mg of pitavastatin once daily after supper for 8 weeks. TC, TG, HDL, and low-density lipoprotein (LDL) plasma concentrations were determined at 0, 4, and 8 weeks of treatment. Vital signs, hepatic function, creatine kinase levels, fasting blood glucose levels and adverse events were monitored in safety evaluations.

**Genotyping**

Venous blood (5 mL) was collected from patients in a sterile tube containing EDTA and stored at 4 °C. Genomic DNA was extracted by the QIAamp whole blood mini kit (Qiagen) following the manufacturer’s instructions. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed for 388G>A genotyping, and an amplification refractory mutation system (ARMS-PCR) was used for 521T>C genotyping as previously described\(^7\).

**Statistical analysis**

Genotype analysis

Genotype frequencies of SLCO1B1 388G>A and 521T>C were assessed for deviation from Hardy-Weinberg equilibrium using the χ²-test of goodness-of-fit. Differences between lipid parameters before and after pitavastatin treatment were tested using the paired t-test. Potential differences in drug response among the genotypes were tested using a one-way analysis of variance (ANOVA). The statistical significance level was defined as a P-value of less than 0.05 and statistical calculations were performed using the SPSS software (SPSS 11.0 for Windows; SPSS Inc, Chicago, IL).

**Results**

**Allelic frequencies of SLCO1B1 SNPs**

In the 140 Chinese hypercholesterolemic subjects, there were 57 (40.7%) heterozygotes and 71 (50.7%) homozygotes for the 388A>G mutation, and 27 (19.3%) heterozygotes and 2 (1.4%) homozygotes for the 521T>C mutation. The frequencies of the alleles and genotypes are listed in Table 1. The distribution of the genotypes of the 388G>A and 521T>C polymorphisms conformed to predictions of Hardy-Weinberg equilibrium (P>0.05).

| SNP     | Allele | Allele frequency (n) | Genotype  | Genotypic frequency (n) |
|---------|--------|----------------------|-----------|------------------------|
| 388G>A  | A      | 18.9% (81)           | AA        | 8.6% (12)              |
|         | G      | 71.1% (199)          | AG        | 40.7% (57)             |
|         |        |                      | GG        | 50.7% (71)             |
| 521T>C  | T      | 88.9% (249)          | TT        | 79.3% (111)            |
|         | C      | 11.1% (31)           | TC        | 19.3% (27)             |
|         |        |                      | CC        | 1.4% (2)               |

**Association of SLCO1B1 polymorphisms with pharmacodynamics of pitavastatin**

Subjects (41 males, 44 females) of the Han population were enrolled in and finished the clinical trial. The mean (±SD) age was 56±9 years and the mean (±SD) BMI was 24.85±2.73 kg/m². Our study showed that 4- and 8-week treatment with pitavastatin significantly reduced TC, TG, and LDL cholesterol plasma levels (P<0.05). However, the pitavastatin treatment did not affect the level of HDL cholesterol (P>0.05). Plasma lipid parameters of patients before treatment and after 4 and 8 weeks of pitavastatin treatment are listed in Table 2 and 3. There was no difference in age, sex, BMI, and lipid parameters.
before treatment among different genotypes, and after 4 and 8 weeks of treatment, serum lipid parameters did not show statistical difference among various genotype groups.

### Discussion

**SLCO1B1** is a newly identified transporter with functional genetic polymorphisms. Based on the functional variability of OATP1B1 and its broad range of substrates, SNPs in **SLCO1B1** may play an important role in pharmacology and toxicology. One such polymorphism, **521T>C**, has been consistently associated with altered transport activity both in vitro[14,15] and in vivo[7–9, 16–18] though results regarding the **388G>A** variant are contradictory[14, 15, 19, 20]. In the present study, the allele frequencies of **521C (11.1%)** and **388A (71.1%)** in Chinese primary hyperlipidemia patients were similar to those previously reported in healthy Chinese populations[13]. These results indicate that **SLCO1B1** 388G>A and **521T>C** variants are not associated with primary hyperlipidemia, although **SLCO1B1** has been reported to be involved in the transportation of cholesterol and statins.

Recent studies[7–9, 21–24] consistently showed **SLCO1B1** variants altered the pharmacokinetics of pitavastatin, rosuvastatin, simvastatin acid, atorvastatin and pravastatin, but our data revealed that there was no significant difference in therapeutic effect of pitavastatin among various genotypes. Interestingly, the alteration of pharmacokinetics did not extend to the response to pitavastatin. Similar results were also obtained for other statins in separate studies[24, 25]: the plasma concentration of pravastatin in subjects with haplotypes *15 or *17 (11187G>A, 388A>G, and 521T>C) was higher than in wild-

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**Table 2.** The lipid parameters on pre-therapy and post therapy in subjects with various genotypes for **SLCO1B1 521T>C** (*n*=85). Data are given as mean±SD.

| Genotypes | Lipid parameters (mmol/L) | Baseline | After 4-week treatment | Change (%) | After 8-week treatment | Change (%) |
|-----------|---------------------------|----------|------------------------|------------|------------------------|------------|
| TT (*n*=64) | TC                        | 6.4±1.2  | 4.9±1.0                | -19±16     | 4.8±0.9                | -22±18     |
|           | TG                        | 3±3      | 2.4±2.7                | -9±42      | 2.2±1.6                | -23±62     |
|           | HDL-C                     | 1.4±0.5  | 1.4±0.3                | -7±15      | 1.3±0.3                | -2±15      |
|           | LDL-C                     | 3.8±1.2  | 2.6±0.8                | -31±21     | 2.7±0.9                | -29±26     |
| CC+TC (*n*=21) | TC                        | 6.1±1.3  | 4.9±1.0                | -23±18     | 4.7±0.9                | -25±19     |
|           | TG                        | 3.0±2.2  | 2.7±2.0                | -29±71     | 2.2±1.6                | -36±62     |
|           | HDL-C                     | 1.3±0.4  | 1.2±0.3                | -5±26      | 1.2±0.4                | -6±31      |
|           | LDL-C                     | 4.2±1.1  | 2.7±1.0                | -30±26     | 2.6±0.8                | -27±29     |

TC: total cholesterol; TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol.

**Table 3.** The lipid parameters on pre-therapy and post-treatment in subjects with various genotypes for **SLCO1B1 388G>A** (*n*=85). Data are given as mean±SD.

| Genotypes | Lipid parameters (mmol/L) | Baseline | After 4-week treatment | Change (%) | After 8-week treatment | Change (%) |
|-----------|---------------------------|----------|------------------------|------------|------------------------|------------|
| AA (*n*=7) | TC                        | 6.3±0.8  | 5.0±0.6                | -20±17     | 5.0±0.9                | -21±24     |
|           | TG                        | 2.6±1.0  | 2.4±1.4                | -7±22      | 2.2±1.1                | -10±34     |
|           | HDL-C                     | 1.2±0.4  | 1.3±0.4                | 2±9        | 1.3±0.5                | 2±17       |
|           | LDL-C                     | 3.7±1.2  | 2.67±0.6               | -26±28     | 2.7±0.8                | -25±33     |
| AG (*n*=29) | TC                        | 6.3±1.3  | 4.9±1.3                | -22±18     | 4.8±1.0                | -24±20     |
|           | TG                        | 3.9±3.2  | 3.0±3.8                | -30±83     | 2.4±1.9                | -47±71     |
|           | HDL-C                     | 1.4±0.6  | 1.3±0.4                | -8±33      | 1.3±0.4                | -12±39     |
|           | LDL-C                     | 3.6±1.4  | 2.4±1.0                | -26±25     | 2.5±0.8                | -28±30     |
| GG (*n*=49) | TC                        | 6.4±1.3  | 4.9±0.8                | -23±18     | 4.8±0.9                | -24±18     |
|           | TG                        | 3.0±2.8  | 2.2±1.6                | -23±59     | 2.1±1.4                | -27±59     |
|           | HDL-C                     | 1.4±0.4  | 1.3±0.3                | -5±19      | 1.3±0.3                | -2±20      |
|           | LDL-C                     | 4±1±1.1  | 2.7±0.7                | -34±24     | 2.8±0.9                | -31±27     |

In this present study, the allele frequencies of **521C (11.1%)** and **388A (71.1%)** in Chinese primary hyperlipidemia patients were similar to those previously reported in healthy Chinese populations[13]. These results indicate that **SLCO1B1 388G>A** and **521T>C** variants are not associated with primary hyperlipidemia, although **SLCO1B1** has been reported to be involved in the transportation of cholesterol and statins.
type subjects when they were given 40 mg of pravastatin daily for 3 weeks, but there was no statistical difference in the lipid-lowering efficacy between genotypes.

In vivo, part of pitavastatin is reversibly converted to a lactone form, and the disposition of pitavastatin lactone is different from that of the parent drug. Chung et al. reported that SLC01B1 521T>C and 388G>A were not associated with a change in the pharmacokinetics of pitavastatin lactone. In addition, the alteration of pharmacokinetics due to polymorphisms in SLC01B1 may not be sufficient to affect the pharmacodynamics of pitavastatin. Takane et al. reported that the SLC01B1*15 allele was associated with a slow response to pravastatin therapy, and the combined genotype of CYP7A1 and APOE was a more useful index to predict the lipid-lowering effect of pravastatin. This implies that polymorphisms in other genes may play an important role in inter-individual variable response to statins.

In conclusion, the present study found that allele frequencies of SLC01B1 388G>A and 521T>C in patients with essential hyperlipidemia were comparable to those in healthy Chinese population and there was no significant difference in the lipid-lowering efficacy of pitavastatin among various genotype groups.

Author contribution
Guo-ping YANG, Hong YUAN, Zhi-jun HUANG and Hong-hao ZHOU designed the research; Guo-ping YANG and Bin TANG performed the research; Dong-sheng OU-YANG, Lian-hao ZHOU designed the research; Guo-ping YANG and Bin TANG wrote the paper.

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