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Short communication

Organic anion transporting polypeptide-1B1 haplotypes in Chinese patients[1]

Lin-yong XU[2], Yi-jing HE[3], Wei ZHANG[3], Sheng Deng[4], Qing LI[3], Wei-xia ZHANG[3], Zhao-qian LIU[3], Dan WANG[3], Yuan-fei HUANG[3], Hong-hao ZHOU[3,5], Zhen-qiu SUN[2,5]

[2]Department of Epidemic and Health Statistics, School of Public Health, Central South University, Changsha 410078, China; [3]Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha 410078, China; [4]Affiliated Xiangya Hospital of Central South University, Changsha 410078, China

Key words

Abstract

Aim: To detect *388G>A* and *521T>C* variant alleles in the organic anion transporting polypeptide-1B1 (OATP1B1, encoding gene *SLCO1B1*) gene. **Methods:** One hundred and eleven healthy volunteers were screened for OATP1B1 alleles in our study. PCR–restriction fragment length polymorphism was used to identify the *388G>A* polymorphism and a 1-step tetra-primer method was developed for the determination of *521T>C* mutation. **Results:** The frequencies of the 388G>A and *521T>C* variant alleles in the Chinese population were 73.4% and 14.0%, respectively. The frequencies of the *SLCO1B1*1b and *15 haplotypes were 59.9% and 14.0%, respectively. **Conclusion:** The *SLCO1B1*1b and *SLCO1B1*15 variants are relatively common in the Chinese population. Their frequencies are similar to that in the Japanese, but significantly different from that in Caucasians and blacks.

Introduction

The members of the organic anion-transporting polypeptides (OATP) represent a family of important proteins involved in the membrane transport of endogenous and xenobiotic compounds. OATP are expressed in a wide variety of tissues, including the liver, kidney, brain, and small intestine[1–2]. Human OATP1B1 (also known as OATP-C, OATP2, *SLCO1B1* gene), a sodium-independent bile acid transporter, is specifically expressed on the basolateral membrane of hepatocytes and translocate a broad range of compounds, such as bile acids, bilirubin, sulfate and glucuronide conjugates, thyroid hormones, peptides, and drugs like 3-hydroxy-3-methylglutaryl-co-enzymeA (HMG CoA)-reductase inhibitors (pravastatin, rosuvastatin, pitavastatin) and methotrexate[3–5]. Recent studies have proven that OATP1B1 plays an important role in the hepatocellular uptake and consequently the elimination of numerous chemicals.

A number of single nucleotide polymorphisms (SNP)

have been identified in the encoding and regulating regions of the OATP1B1 gene among different populations. The frequency of the *SLCO1B1*5 (*521T>C*, Val174Ala) variant frequency is 14% in European Americans and the *SLCO1B1*9 (*1463G>C*, Gly488Ala) variant frequency is 9% in African Americans[6], but these 2 common polymorphisms are extremely low in Japanese populations, which exhibit significant ethnic difference. In Orientals, *SLCO1B1*1b (*388G*>A, Asn130Asp) and *SLCO1B1*15 (a haplotype of *SLCO1B1*1b and *SLCO1B1*5) are 2 common SNP with relatively high frequencies of 66% and 16%, respectively. Recent studies have elucidated that both *SLCO1B1*1a and *SLCO1B1*15 exhibit reduced transport function and play an important role in pravastatin, pitavastatin, rosuvastatin (a substrate for OATP1B1) systemic exposure and elimination[7–10]. *SLCO1B1*17 (*−11187G>A*, *388G*>A, and *521T>C*) was found to be associated with increased plasma concentrations of pravastatin in humans. However, there are no published studies concerning the functional signifi-

cance of the *−11187G>A* promoter SNP *in vitro* until now[9,10].

Therefore, this study was carried out to determine the *SLCO1B1*1b* and **15* polymorphisms in the Chinese population.

Materials and methods

Chemicals and reagents All primers for the PCR were synthesized by Bioasia Company (Shanghai, China). *Taq* DNA polymerase, dNTP mixture, PCR buffer, GeneRuler 500 bp DNA ladder, and the restriction endonuclease *Cla*I were all purchased from MBI Fermentas (Vilnius, Lithuania). All other chemicals were of highest grade and available from commercial sources.

Patients One hundred and eleven unrelated, healthy, Chinese Han male volunteers were recruited in this study. They were medical students at the Central South University, Xiangya Medical College (Hunan, China) and their mean age was 20±2 years. This research was approved by the Ethics Committee of the Central South University and written informed consent was obtained from each participant. The participants were assessed by medical history, physical examination, and clinical laboratory test of hematological, liver and kidney function, blood tests for human hepatitis B or C, and blood glucose. They were non-smokers who did not take any medication or alcohol in the 14 d before the study.

Blood acquisition and DNA isolation Five milliliters of venous blood was collected in a sterile tube containing EDTA and stored at -80 °C. Genomic DNA was isolated from leukocytes through a standard manual chloroform–phenol extraction procedure and stored at 4 °C until use.

PCR–restriction fragment length polymorphism assay for *388G>A* genotyping The 388G>A genotype was determined by means of PCR–restriction fragment length polymorphism (RFLP) analysis according to Torina *et al*[6] with some modifications. The PCR reaction was carried out in a total volume of 25 µL consisting of 2.5 µL 10×PCR buffer (with $MgCl_2$), 0.2 mmol/L of each dNTP, 60 pmol/L of each primer, 100 ng of genomic DNA as a template, and 2.5 U *Taq* polymerase. The sequences of the primers used for detection of *388G>A* were: forward primer, 5'-GCAAATAAA-GGGGAATATTTCTC-3' and reverse primer, 5'-AGAGATGT-AATTAAATGTATAC-3'. PCR amplification to detect *388G>A* was performed using the Gene Amplification PCR System 2400 (Perkin Elmer, Foster City, CA, USA) with an initial denaturation at 94 °C for 5 min, followed by 37 cycles of denaturation at 94 °C for 30 s, annealing at 46 °C for 30 s, and extension at 72 °C for 30 s. A final 5 min extension at 72 °C was adopted. After amplification, the PCR products (274 bp)

were digested with the *CLa*I restriction endonuclease at 30 °C for at least 6 h. Digested products were analyzed by electrophoresis on a 2.5% agarose gel in the presence of ethidium bromide.

Amplification refractory mutation system–PCR assay for *521T>C* genotyping The amplification refractory mutation system (ARMS) was introduced, avoiding the use of restriction enzymes[10]. The 4 primers used in ARMS–PCR were according to Torina *et al*[6] with slight modifications. PCR amplification was performed in a Perkin Elmer DNA Model PJ2000 thermal cycler (USA) in a total volume of 25 µL solution containing 2.5 µL 10×buffer (with $MgCl_2$), 0.4 mmol/L of each dNTP, 40 pmol/L of each primer, 2 U LA–Taq enzyme and approximately 200 ng genomic DNA as a template. The PCR conditions involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 30 s with a final extension at 72 °C for 7 min. Because the restriction endonuclease was unnecessary, the PCR products (totally 260 bp) were detected by means of 2% agarose gel electrophoresis and were detected by ethidium bromide staining. Two samples of *521T>C* of each genotype were directly sequenced to confirm our genotyping result. The primers and conditions are listed in detail in Table 1.

Data analysis Haplotypes were reconstructed on the basis of the phase-unknown genotype data using PHASE version 2 software (Stephens, *et al*, Seattle, Washington, USA), a computer-assisted statistical analysis based on Bayesian statistics. Haplotypes, as well as the genotype frequency deviation from the Hardy–Weinberg equilibrium, were evaluated by appropriate χ^2-test. The SPSS software package version 11.2 (Chicago, IL, USA) was used to perform the statistical analysis. A *P*-value of less than 0.05 was accepted as significant.

Results

The PCR product in different *388G>A* genotypes are shown in Figure 1. Patients with *388GG* produced 155 and 119 bp fragments, whereas those from *388AA* homozygotes generated an additional 274 bp fragment. Heterozygous *388G>A*-mutated genotypes produced 3 fragments of 155, 119, and 274 bp.

Four specific primers were used to amplify 3 fragments in different *521T>C* genotypes (Figure 2). The wild-type allele yielded 2 fragments of 260 and 179 bp in length, while the variant *521T>C* homozygotes resulted in 2 fragments of 260 and 123 bp in length. The heterozygotes resulted in 3 fragments of 260, 123, and 179 bp.

Table 1. Primers used in the study.

Position	PCR primers	Genotyping method	Fragments for genotyping (bp)
388G>A (Asn130Asp)	F: 5'-GCAAATAAAGGGGAATATTTCTC-3' R: 5'-AGAGATGTAATTAAATGTATAC-3'	RFLP (ClaI)	AA: 155+119 AT: 274+155+119 TT: 274
521T>C (Val174Ala)	F: 5'-AAGTAGTTAAATTTGTAATAGAAATGC-3' WT: 5'-GGGTCATACATGTGGATATAAGT-3' MT: 5'-AAGCATATTACCCATGAACG-3' R: 5'-GTAGACAAAGGGAAAGTGATCATA-3'	ARMS–PCR	TT: 260+179 TC: 260+123+179 CC: 260+123

F, forward primer; R, reverse primer. WT, forward primer for wild-type genotype, MT, reverse primer for mutant variants used in ARMS–PCR.
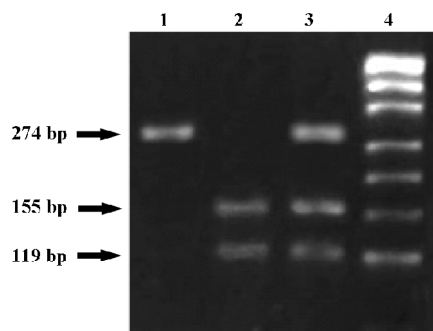

Figure 1. PCR–RFLP analysis result for the *388G>A* polymorphism. Lane 1, wide-type genotype with A/A at 388; lane 2, homozygotes with *388G>A* mutation; lane 3, heterozygotes with *388G>A* mutation, lane 4, DNA marker.

Of the 111 Chinese patients, 39 patients (35.1%) were heterozygotes and 62 (55.9%) were homozygotes for the 388G allele and 27 (24.3%) were heterozygotes and 2 (1.8%) were homozygotes for *521T>C* mutation. The frequencies of the alleles and genotypes were calculated and are listed in Table 2. The distribution of the 3 genotypes of the *388G>A* and *521T>C* polymorphisms conformed well to the predictions of Hardy–Weinberg equilibrium ($P>0.05$).

A haplotype comprising of the 2 common SNP was constructed for these 111 individuals (222 sequences). The results of the haplotype analysis using a pseudo-Bayesian algorithm revealed 3 types of haplotypes in our Chinese population. These haplotypes were A-T (*1a*), G-T (*1b*), and G-C (*15*). The haplotype frequencies were 26.1%, 59.9%, and 14%, respectively (Table 2). Of the 4 (2×2) possible combinations of the SNP, haplotype A-C (in order 388–521) was not present in our patients. In the population in our

Table 2. Allele and genotype frequencies of individual SNP of OATP-C in Chinese patients (*n*=111).

SNP	Allele	Allele frequency (*n*)	Genotype	Frequency (*n*)
388G>A	A	26.6% (59)	AA	9% (10)
	G	73.4% (163)	AG	35.1% (39)
			GG	55.9% (62)
521T>C	T	86.0% (191)	TT	73.8% (82)
	C	14.0% (31)	TC	24.3% (27)
			CC	1.8% (2)

present study, the diplotypes of the individuals were determined by PHASE 2.0 software consisting of 6 types in all: *1a/*1a*, *1a/*1b*, *1a/*15*, *1b/*1b*, *1b/*15*, and *15/*15*. The detailed haplotypes and their frequencies are shown in Table 3.

Table 3. Frequencies of haplotype and haplotype pairs based on 2 gene polymorphisms of OATP-C in Chinese patients.

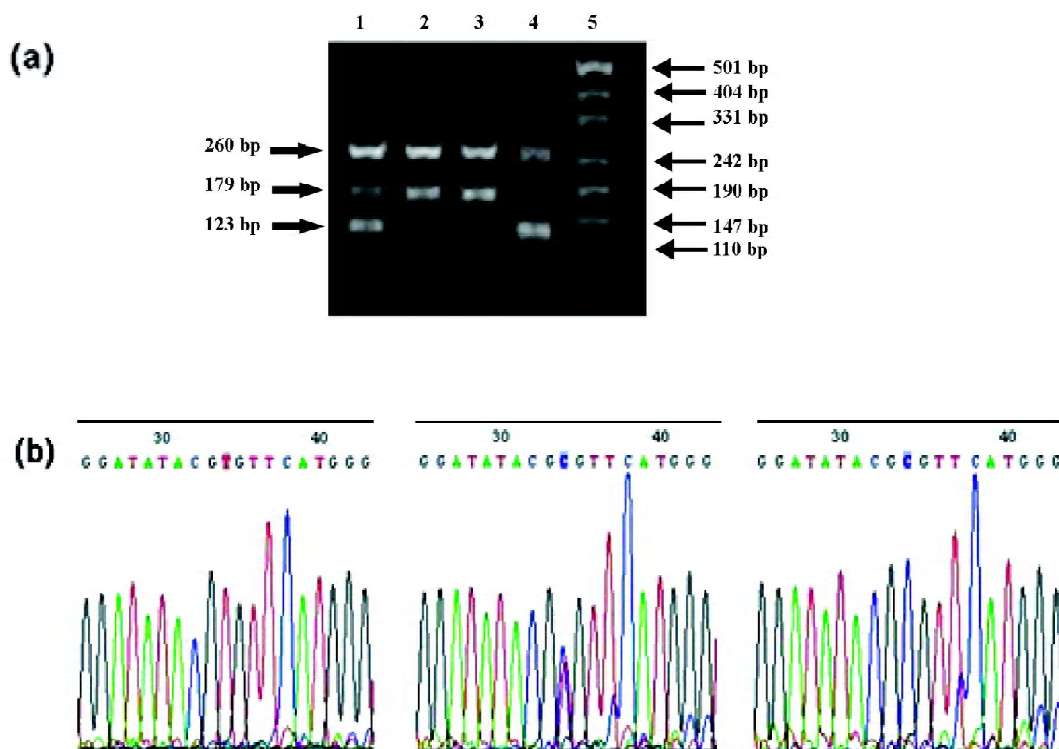|	Haplotype or Haplotype pairs	Frequency (*n*)
Haplotype (*n*=222)	AT (*1a)	26.1% (58)
	GT (*1b)	59.9% (133)
	GC (*15)	14% (31)
Haplotype pairs (*n*=111)	ATGT (*1a/*1b)	27.9% (31)
	GTGC (*1b/*15)	18.0% (20)
	GTGT (*1b/*1b)	36.9% (41)
	ATGC (*1a/*15)	6.3% (7)
	ATAT (*1a/*1a)	9.0% (10)
	GCGC (*15/*15)	1.8% (2)

Figure 2. Tetra-ARMS–PCR analysis result for the *521T>C* polymorphism. (A) lane 1, heterozygotes with *521T>C* mutation; lanes 2 and 3, wild-type genotype with T/T at 521; lane 4, homozygotes with *521T>C* mutation; lane 5, DNA marker. (B) Direct sequencing results for the *SLCO1B1 521T>C* allele.

Discussion

Drug metabolic enzymes and transporters have long been determined as major determinants of drug metabolism and disposition. However, accumulating evidence has proved that membrane transporters are also critical factors to the drug disposition process both *in vitro* and *in vivo*. Human OATP1B1 belongs to the OATP family of drug uptake transporters. It is specifically localized at the basolateral membrane of hepatocytes and is responsible for the hepatic uptake of a series of structurally-divergent compounds, including some endogenous chemicals and many clinically-used drugs.

More than 20 functionally-relevant SNP in the *SLCO1B1* gene have been identified in different populations[6,12–14]. The common SNP with impaired transport activity appeared to be *521T>C* (Val174Ala) in European Americans and Japanese and *G1463>C* (Gly488Ala) in African Americans. However, the clinical significance of these commonly seen mutations for large numbers of endogenous and xenobiotic substrates transported by OATP1B1 remains to be further studied.

We detected both the *SLCO1B1*1b* and *SLCO1B1*15* variants in this study to characterize OATP1B1 genetic polymorphisms in the Chinese population. The frequency of the *SLCO1B1*1b* haplotype was 59.9% in our study, which is similar to previous reports of Japanese (62.9%)[7] and African Americans (74%), but higher than that of European Americans (30%)[6]. The frequency of the *SLCO1B1*15* haplotype was 14% in Chinese, similar to that in Japanese (15.8%), but greater than that of Caucasians (2.4%) and African Americans (0%)[15].

The allele frequencies of drug transporter polymorphisms among different ethnic groups may contribute to drug therapeutic effects as well as toxicity. For example, pravastatin is one of the HMG-CoA reductase inhibitors (statins) widely used in the treatment of hypercholesterolemia[16], which experienced no obvious metabolism by cytochrome P450s, but could be efficiently taken up from circulation by the liver through OATP1B1[6]. It has been reported that increased systemic exposure and decreased non-renal elimination to pravastatin *in vivo* was significantly associated with the *521T>C* variant in both Europeans and Japanese. The *388G>A* site and novel mutation *−11187G>A* were also

proven to be of pharmacokinetic significance[7–9]. In a recent study, patients with the *SLCO1B1*15* allele were proven to be associated with higher serum bilirubin levels[17–19]. Thus, the determination of *OATP1B1* gene polymorphisms in specific ethnic groups is very important for contributing to individualized drug therapeutics and the pathogenesis of hereditary diseases. However, the effect of the *521T>C* genotype on the pharmacokinetics of rosuvastatin was not observed significantly in the Chinese, Malay, and Asian–Indian populations[20]. Our study indicated that the *SLCO1B1* polymorphisms could not fully explain the interindividual difference of rosuvastatin disposition. The functional polymorphisms of CYP2C9 and some certain drug transporters, for instance breast cancer resistance protein (BCRP), may also contribute to the disposition of rosuvastatin[21], whereas pravastatin is not obviously metabolized by CYP450.

In summary, the present study has shown that the *SLCO1B1*1b* and *SLCO1B1*15* variants represent common genetic polymorphisms in the Chinese population. There were remarkable ethnic differences in the frequencies of these 2 haplotypes. Our findings suggest that about 26.1% of the Chinese population carrying the *SLCO1B1*15* variant might exhibit impaired transport activity of OATP1B1.

References

1   Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. Biochim Biophys Acta 2003;1609(1):1–18. Review.

2   Kim RB. Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. Eur J Clin Invest 2003; 33 (Suppl 2): 1–5.

3   Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem 1999; 120: 17159–63.

4   Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic organic anion transporting polypeptide 2 (OATP2): identification of a liver-specific organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA-reductase inhibitor transporters. J Biol Chem 1999; 274: 37161–8.

5   Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am J Physiol Gastrointest Liver Physiol 2000; 278: G156–64.

6   Tirona RG, Leake BF, Merino G, Kim RB. Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European and African–Americans. J Biol Chem 2001; 276: 35 669–75.

7   Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, et al. Polymorphisms of OATP-C (SLC21A6) and OAT3

8   Mwinyi J, Johne A, Bauer S, Roots I, Gerloff T. Evidence for inverse effects of OATP-C (*SLC21A6*) *5 and *1b haplotypes on pravastatin kinetics. Clin Pharmacol Ther 2004; 75: 415–21.

9   Niemi M, Schaeffeler E, Langb T, Fromma MF, Neuvonen M, Kyrklund C, et al. High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). Pharmacogenetics 2004, 14: 429–40.

10 Kameyama Y, Yamashita K, Kobayash K, Hosokawa M, Chiba K. Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+C1007G, by using transient expression systems of HeLa and HEK293 cells. Pharmacogenet Genomics 2005; 15: 513–22.

11 Ye S, Dhillon S, Ke XY, Collins AR, Day INM. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res 2001; 29: E88–8.

12 Kim RB. 3-Hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins) and genetic variability (single nucleotide polymorphisms) in a hepatic drug uptake transporter: What's it all about? Clin Pharmacol Ther 2004; 75: 381–5.

13 Tirona RG, Kim RB. Pharmacogenomics of organic anion-transporting polypeptides (OATP). Adv Drug Deliv Rev 2002; 54: 1343–52.

14 Morimoto K, Oishi T, Ueda S, Ueda M, Hosokawa M, Chiba K. A novel variant allele of OATP-C (SLCO1B1) found in a Japanese patient with pravastatin-induced myopathy. Drug Metab Pharmacokinet 2004; 19: 453–5.

15 Pasanen MK, Backman JT, Neuvonen PJ, Niemi M. Frequencies of single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide 1B1 SLCO1B1 gene in a Finnish population. Eur J Clin Pharmacol 2006; 62: 409–15.

16 Hatanaka T. Clinical pharmacokinetics of pravastatin mechanisms of pharmacokinetic events. Clin Pharmacokinet 2000; 39: 397–412.

17 Cui YH, Konig J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6*. J Biol Chem 2001; 276: 9626–30.

18 Ieiri I, Suzuki H, Kimura M, Takane H, Nishizato Y, Irie S, et al. Influence of common variants in the pharmacokinetic genes (OATP-C, UGT1A1, and MRP2) on serum bilirubin levels in healthy subjects. Hepatol Res 2004; 30: 91–5.

19 Nozawa T, Nakajima M, Tamai I, Noda K, Nezu JC, Sai Y, et al. Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. J Pharmacol Exp Ther 2002; 302: 804–13.

20 Lee E, Ryan S, Birmingham B, Zalikowski J, March R, Ambrose H, et al. Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment. Clin Pharmacol Ther 2005; 78: 330–41.

21 Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, et al. Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. Clin Chim Acta 2006; 373: 99–103.