Brief/Technical Note

Direct and Rapid Genotyping of *SLCO1B1* 388A>G and 521T>C in Human Blood Specimens Using the SmartAmp-2 Method

Kenta Yoshida,¹ Junichi Takano,¹ Yuri Ishizu,² Alexander Lezhava,² Ichiro Ieiri,³ Kazuya Maeda,¹ Yoshihide Hayashizaki,² and Yuichi Sugiyama^{4,5}

Received 21 December 2012; accepted 25 February 2013; published online 8 March 2013

Abstract. Organic anion-transporting polypeptide (OATP) 1B1, encoded by the solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene, mediates the active uptake of various organic anions into hepatocytes and determines their hepatic clearances as the first step in the detoxification pathway. Previous reports indicated that alterations in its function by drug–drug interactions or genetic polymorphisms affect the pharmacokinetics of the substrate drugs. In the present study, we developed a method to genotype *SLCO1B1* 388A>G (rs2306283) and 521T>C (rs4149056), which significantly affect the clinical pharmacokinetics and subsequent side effects such as myopathy caused by statins, OATP1B1 substrates in humans. We used a small aliquot of blood and the isothermal Smart Amplification Process version 2 (SmartAmp-2), which could complete the genotyping of 388A>G and 521T>C within 60 min. The genotypes of 101 genomic DNA samples and blood samples assessed by SmartAmp-2 matched perfectly to those determined previously by the conventional PCR-SSCP method. The SmartAmp-2 method enables the rapid identification of the 388A>G and 521T>C genotypes, saving time and effort in the genomic DNA preparation in clinical practice. This method will be useful for evaluating and predicting altered pharmacological and toxicological effects of substrate drugs caused by *SLCO1B1* polymorphisms.

KEY WORDS: membrane transporters; pharmacogenetics; single nucleotide polymorphisms.

INTRODUCTION

Organic anion-transporting polypeptide (OATP) 1B1, encoded by the solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene, is expressed predominantly on the blood side of the human liver and regulates the

hepatic uptake of various organic anions, including clinically important drugs such as HMG-CoA reductase inhibitors (statins) and angiotensin II receptor antagonists (sartans) (1,2). Recent evidence demonstrates the importance of OATP1B1 as a determinant of the pharmacokinetics of anionic drugs. For example, coadministration of a clinical dose of potent OATP1B1 inhibitors such as cyclosporin A and rifampicin significantly increases the plasma area under the concentration–time curves (AUCs) of various substrate drugs (3). In addition, several reports have demonstrated the impact of genetic polymorphisms in the *SLCO1B1* gene on the pharmacokinetics and subsequent pharmacological/toxicological effects of substrate drugs. In particular, two single nucleotide polymorphisms (SNPs) in *SLCO1B1*, 388A>G and 521T>C, are found frequently in several ethnic groups, and their effects on clinical pharmacokinetics have been investigated extensively.

Previous clinical studies indicated that the plasma AUCs of several OATP1B1 substrate drugs are higher in subjects with *SLCO1B1* 521T>C because this SNP decreases the transport activity of OATP1B1 and reduces the hepatic clearance of drugs (1,2). *SLCO1B1* 521T>C also decreases the hepatic clearance of the antidiabetes drug repaglinide and subsequently reduces the plasma glucose level (4). A genome-wide association study

Kenta Yoshida and Junichi Takano authors contributed equally to this work.

¹Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo Japan.

²Technology Development Unit, Omics Science Center, RIKEN, Yokohama Japan.

³Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka Japan.

⁴Sugiyama Laboratory, RIKEN Innovation Center, RIKEN Research Cluster for Innovation, Yokohama Japan.

⁵To whom correspondence should be addressed. (e-mail: ychi.sugiyama@riken.jp)

ABBREVIATIONS: AmC7-Q, 3'-Amino modifier C7; AUC, Area under the concentration–time curve; HMG-CoA, Hydroxymethylglutaryl-CoA; MT, Mutated type; OATP1B1, Organic anion-transporting polypeptide 1B1; RFLP, Restriction fragment length polymorphism; *SLCO1B1*, Solute carrier organic anion transporter family member 1B1; SmartAmp-2, Smart Amplification Process version 2; SNP, Single nucleotide polymorphism; SSCP, Single-strand conformation polymorphism; WT, Wild type.

Table I. Advantages of SmartAmp-2 Method Over Conventional Genotyping Methods

	SmartAmp-2 method	Conventional method
DNA purification	Not required	Required
Continuous temperature control	Not required Isothermal at 60°C	Required
Total reaction time	<1 h	~2 h

indicated that *SLCO1B1* 521T>C is highly linked with the elevated susceptibility to myopathy, a typical side effect of statins, in simvastatin-treated patients, possibly because of the increased plasma and muscle exposure to statins (5). By contrast, *SLCO1B1* 388A>G tends to decrease the plasma AUCs of ezetimibe, pravastatin, and repaglinide (2).

We have developed a rapid detection system of genetic polymorphisms, the Smart Amplification Process version 2 (SmartAmp-2®), for genotyping *SLCO1B1* 388A>G and 521T>C very quickly. SmartAmp-2 enables genotyping of genes under isothermal conditions using small aliquots of blood, which should save time and effort by avoiding genomic DNA preparation (6) (Table I). In the SmartAmp-2 reaction, the folding primer (FP) and turn-back primer (TP) participate in primer extension events, producing an intermediate single-stranded DNA product. These intermediate species then undergo a self-priming reaction to generate long DNA concatemeric products. The boost primer (BP), outer primer 1 (OP1), and outer primer 2 (OP2) support the functions of the FP and TP and therefore increase the reaction speed. The nonspecific amplification can be suppressed by a competitive probe (CP) and *Thermus aquaticus* MutS (*Taq* MutS) (7).

In the present study, we designed six and eight primers to detect *SLCO1B1* 388A>G and 521T>C, respectively, and we validated their specificity and reliability using 101 human blood and genomic DNA

specimens. The genotypes of these samples were determined previously using a PCR-single-strand conformation polymorphism (SSCP) method (8).

MATERIALS AND METHODS

Collection and Preparation of Human Blood Samples

Peripheral blood was collected from 101 healthy Japanese volunteers, and DNA was extracted by the standard protocol. The samples were numbered, unlinked, and tested anonymously. This study was approved by the Institutional Ethics Review Board, and a written informed consent was obtained from all subjects before the study. The DNA concentration was about 10 ng/μl. Two volumes of 75 mM NaOH were added to each blood specimen.

The SmartAmp-2 Assay Using Human Genomic DNA and Blood Samples

The primer sets for the SmartAmp-2 assay were designed for amplification and genotyping of *SLCO1B1* 388A>G and 521T>C as described in Table II and Fig. 1a. Samples were denatured at 98°C (388A>G) or 95°C (521T>C) for 5 min, and the SmartAmp-2 reactions proceeded at 60°C for 60 min. For 388A>G, the reaction mixture contained 2.67 μM of FP and TP, 1.33 μM of BP, 0.600 μM of OP1 and OP2, 163 ng/μl (genomic DNA) or 80.6 ng/μl (blood) of *Taq* MutS (DNAFORM K.K., Kanagawa, Japan), 0.4 M betaine, and 0.2 mg/ml of tannic acid. For 521T>C, the reaction mixture contained 2.40 μM of FP and TP, 1.20 μM of BP and CP, 0.300 μM of OP1 and OP2, 5% DMSO, and 20 ng/μl (genomic DNA) or 40 ng/μl (blood) of *Taq* MutS. The reaction mixtures also contained 1.4 mM dNTPs, 20 mM Tris-HCl (pH 8.0), 10 mM KCl, 10 mM (NH₄)₂SO₄, 8 mM MgSO₄, 0.1% Tween-20, 1/100,000 diluted original SYBR® Green I dye (Molecular Probes, USA), 0.24 units/μl of *Aac* DNA polymerase (DNAFORM K.K.), and prepared genomic DNA (2 μl

Table II. The Primer Sets for Genotyping *SLCO1B1* 388A>G and 521T>C with SmartAmp-2 Assay

(A) 388A>G	TP	5'-TGGATAAGGTTGATGTTCTTACAGTTACAG-3'
	FP	5'-TTTATATATATATAAACAGGTGATGCTCTATTGAGTGA-3'
	BP (WT)	5'-GTTGAATTTTCTGATGAATT-3'
	BP (MT)	5'-TGTTGAATTTTCTGATGAATC-3'
	OP1	5'-TCTTACCTTTTCCCACTAT-3'
	OP2	5'-GCAAAATGTTTAATTCAGTGAT-3'
(B) 521T>C	TP	5'-ACATAGGTTGTTTAATCCACGAAGCATATTAC-3'
	FP	5'-ACCTTCTATACCTCAGAAGGTAATGAAACACTCTCTATC-3'
	BP (WT)	5'-ACATGTGGATATATGTG-3'
	BP (MT)	5'-TACATGTGGATATATGCG-3'
	CP (WT)	5'-GATATATGCGTTCATGG[AmC7-Q]-3'
	CP (MT)	5'-GATATATGTGTTTCATGG[AmC7-Q]-3'
	OP1	5'-GAATAATTAAGAGTTTACAAGTAG-3'
	OP2	5'-TGGGAGTCTCCCCTATTC-3'

AmC7-Q 3'-amino modifier C7, *BP* boost primer, *CP* competitive primer, *FP* folding primer, *OP* outer primer, *TP* turn-back primer, *WT* wild type, *MT* mutated type

a Primers for WT sequence of 388A>G and 521T>C

b Amplification profiles

Fig. 1. Genotyping of *SLCO1B1* 388A>G and 521T>C by SmartAmp-2. **a** The primers constructed for the genotyping of *SLCO1B1* 388A>G and 521T>C SNPs for the SmartAmp-2 assay. **b** Representative amplification–time profiles generated by the SmartAmp-2 assay in human blood specimens from **a**, **d** homozygotes of the WT, **b**, **e** heterozygotes, and **c**, **f** homozygotes of the mutated type of 388A>G **a–c** and 521T>C **d–f**. The primer sets for 388A>G **a–c** and 521T>C **d–f** were used in each experiment. Black circles and gray triangles indicate amplifications using the primer set for the WT and MT, respectively. AU arbitrary unit

in a 25- μ l reaction mixture) or blood (1 μ l for 388A>G or 2 μ l for 521T>C in 25 μ l) (6,9). The Mx3000P™ Real-Time PCR system (Agilent Technologies, USA) and LightCycler® 480 System II (Roche) were used for maintaining isothermal conditions and monitoring the changes in fluorescence intensity.

RESULTS AND DISCUSSION

We designed various sets of candidate primers for genotyping *SLCO1B1* 388A>G and 521T>C by placing the 3' end of the BP at the site of the SNPs. Two types of BPs for each wild type (WT) and mutated type (MT)

sequences were prepared for each primer set. For genotyping 521T>C, in addition to *Taq* *MutS*, we used a CP to suppress nonspecific amplifications (10). The CP was designed to bind to the mismatch sequence of the genomic templates; the CP with the MT sequence was included in the WT primer sets, and that with the WT sequence was included in the MT sets. Because the 3' end of the CP was modified with the AmC7-Q, mismatch amplifications could be suppressed in the presence of the CP.

Screening of these primer combinations and reaction conditions yielded an ideal primer set, which completed the specific amplification of human blood

Table III. Comparison of the Results Between SmartAmp-2 and Conventional PCR-SSCP Method

(A) <i>SLCO1B1</i> 388A>G		SmartAmp-2 assay Genomic DNA sample			Blood sample		
		388AA	388AG	388GG	388AA	388AG	388GG
Conventional method	388AA	9			9		
	388AG		47			47	
	388GG			45			45
(B) <i>SLCO1B1</i> 521T>C		SmartAmp-2 assay Genomic DNA sample			Blood sample		
		521TT	521TC	521CC	521TT	521TC	521CC
Conventional method	521TT	75			75		
	521TC		22			22	
	521CC			4			4

The genotypes of *SLCO1B1* 388A>G and 521T>C were analyzed by the SmartAmp-2 method, and the results were compared with the previous results obtained using the conventional PCR-SSCP method (3). This table shows a comparison of the two methods applied to the identification of 388A>G (A) and 521T>C (B) SNPs in 101 genomic DNA and blood samples

specimens with corresponding genotypes within 60 min, as shown in Fig. 1b. Genotypes of *SLCO1B1* 388A>G and 521T>C were then tested for 101 genomic DNA and human blood samples, and the results were compared with the previous genotyping results obtained using PCR-SSCP (Table III) (8). The genotypes obtained by the conventional method and the SmartAmp-2 assay were identical, indicating that this new SmartAmp-2 assay is valid.

Conventionally, the genotyping of *SLCO1B1* 388A>G and 521T>C was performed using PCR-SSCP (8,11) or PCR-RFLP (12) method. In another paper, a real-time PCR-FRET method was used to shorten the reaction time (13). As summarized in Table I, the most striking difference between the SmartAmp-2 assay and the previous methods is that SmartAmp-2 is faster because it avoids the need for genomic DNA preparation and the genotyping can be completed within 60 min. Another advantage of the SmartAmp-2 assay is that the reaction proceeds in an isothermal condition and only simple equipment is needed to maintain the appropriate temperature. The method can be improved further with fluorescence-labeled oligonucleotides called “exciton primers,” where visible fluorescent signals are emitted using UV or regular halogen lamps (14). These inventions will allow medical personnel to determine these genotypes even at the bedside in the near future.

CONCLUSION

We have developed a novel SmartAmp-2 assay kit for the rapid and convenient genotyping of *SLCO1B1* 388A>G and 521T>C. The genotyping results obtained by this method matched perfectly to those determined by

the conventional PCR-SSCP method. This system will help the clinician include the patient's genetic information when deciding on the dosing regimen to maximize the pharmacological effects and to avoid the side effects of OATP1B1 substrate drugs.

ACKNOWLEDGMENTS

This work was supported partly by a Grant-in-Aid for Scientific Research (S) (24229002) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

REFERENCES

1. Maeda K, Sugiyama Y. Impact of genetic polymorphisms of transporters on the pharmacokinetic, pharmacodynamic and toxicological properties of anionic drugs. *Drug Metab Pharmacokinet.* 2008;23(4):223–35.
2. Niemi M, Pasanen MK, Neuvonen PJ. Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev.* 2011;63(1):157–81.
3. Yoshida K, Maeda K, Sugiyama Y. Transporter-mediated drug–drug interactions involving OATP substrates: predictions based on *in vitro* inhibition studies. *Clin Pharmacol Ther.* 2012;91(6):1053–64.
4. Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, *et al.* Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther.* 2005;77(6):468–78.
5. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, *et al.* *SLCO1B1* variants and statin-induced myopathy—a genomewide study. *N Engl J Med.* 2008;359(8):789–99.

6. Mitani Y, Lezhava A, Kawai Y, Kikuchi T, Oguchi-Katayama A, Kogo Y, *et al.* Rapid SNP diagnostics using asymmetric isothermal amplification and a new mismatch-suppression technology. *Nat Methods*. 2007;4(3):257–62.
7. Biswas I, Hsieh P. Identification and characterization of a thermostable MutS homolog from *Thermus aquaticus*. *J Biol Chem*. 1996;271(9):5040–8.
8. Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, *et al.* Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther*. 2003;73(6):554–65.
9. Kawai Y, Kikuchi T, Mitani Y, Kogo Y, Itoh M, Usui K, *et al.* Sensitive detection of EGFR mutations using a competitive probe to suppress background in the SMart Amplification Process. *Biologicals*. 2008;36(4):234–8.
10. Watanabe J, Mitani Y, Kawai Y, Kikuchi T, Kogo Y, Oguchi-Katayama A, *et al.* Use of a competitive probe in assay design for genotyping of the UGT1A1 *28 microsatellite polymorphism by the smart amplification process. *Biotechniques*. 2007;43(4):479–84.
11. 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(38):35669–75.
12. Nozawa T, Nakajima M, Tamai I, Noda K, Nezu J, 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(2):804–13.
13. Op den Buijsch RA, Wijnen PA, van Dieijen-Visser MP, de Vries JE, Bekers O. Rapid genotyping of the OATP1B1 polymorphisms A388G and T521C with real-time PCR FRET assays. *Pharmacogenomics*. 2005;6(4):393–7.
14. Lezhava A, Ishida T, Ishizu Y, Naito K, Hanami T, Katayama A, *et al.* Exciton primer-mediated SNP detection in SmartAmp2 reactions. *Hum Mutat*. 2010;31(2):208–17.