

# Screening study for genetic polymorphisms affecting pharmacokinetics of simvastatin

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Simvastatin reduces plasma cholesterol by inhibiting HMG-CoA reductase (HMGR) and is widely used in the treatment of hypercholesterolemia. To screening the possible genetic factors affecting the pharmacokinetics (PK) of simvastatin, 35 male Korean volunteers were enrolled from two separate bioequivalence studies. Each subject was administered 20 mg simvastatin and reference drug PK parameters were used. We used Illumina Human610Quad v1.0 DNA Analysis BeadChip for whole genome SNPs analysis and whole genome genotyping data was processed by linear regression analysis for PK parameters of drug metabolizing enzymes and transporters. We found 145 significant SNPs ( $P < 0.01$ ) in  $C_{max}$ , 135 significant SNPs ( $P < 0.01$ ) in  $T_{max}$  and 85 significant SNPs ( $P < 0.01$ ) in  $AUC_{inf}$  from whole genome analysis. In particular, we found that the *ABCC2* gene had a significant effect on  $C_{max}$  and  $AUC_{inf}$ . These results could provide information of possible candidate genes for personalized simvastatin therapy.

## Introduction

There is wide interindividual variation in the pharmacokinetics (PK) and pharmacodynamics (PD) of administered drugs, as well as in their effectiveness and the appearance rates of adverse effects. This variation can result from genetic, environmental, physiological, and pathophysiological factors such as age, gender, circadian rhythm, diet, and concomitantly administered drugs and health foods.[1,2]

Single nucleotide polymorphisms (SNPs) in drug-metabolizing enzymes are known to be associated with these individual differences.[1] SNPs are unique genetic markers between individuals that contribute in significant ways to the determination of human variation such as personality, behavior and disease susceptibility. SNPs can also significantly influence responses to pharmacotherapy and the production of any adverse drug reactions.[3] SNPs are the most important source of variation in genetic differences[4] and are frequently used as relevant markers of PK.[5] After a common dose, variations in drug efficacy and toxicity can be observed depending on the polymorphisms.[6] The genotyping of drug-metabolizing enzymes prior to

drug administration would help to predict individual reactivity to drugs and possible adverse reactions that may occur, which is essential for realizing tailor-made therapy for individual patients.[7] Recent advances in molecular research have revealed that many of the genes that encode drug-metabolizing enzymes demonstrate genetic polymorphisms.

Simvastatin specifically and competitively inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which is an early rate-limiting step in cholesterol biosynthesis in the body.[8] These agents are highly effective in reducing total cholesterol and low-density lipoprotein levels in several forms of hypercholesterolemia.[9-12]

The purpose of this study is to identify possible candidate genes affecting PK of simvastatin. A total of 35 male Korean volunteers were recruited from two bioequivalence studies after approval by the Institutional Review Board (IRB) of Kyung Hee University Hospital. In this study, DNA from each subject was analyzed using Illumina Human610Quad v1.0 DNA Analysis BeadChip. Linear regression analysis was performed for significant SNPs against the PK parameters, such as maximum measured plasma concentration ( $C_{max}$ ), time of the maximum measured plasma concentration ( $T_{max}$ ) and area under the plasma concentration-time curve from zero to infinity ( $AUC_{inf}$ )[13] from drug metabolizing enzymes and transporters.

## Methods

### Subjects

Volunteers were healthy Korean males who participated in two simvastatin bioequivalence tests (1st beginning April 15, 2008; 2nd beginning August 16, 2008) at Kyung Hee Clinical Medical Research Institute of Kyung Hee University Hospital. The clinical protocol was approved by the IRB of Kyung Hee University Hospital and volunteers were recruited by direct call. Thirty-five out of 61 subjects in two simvastatin bioequivalence studies participated in this pharmacogenetics study after giving written informed consent. The demographic characteristics of the volunteers are summarized in Table 1. They ranged in age from 20 to 40 years ( $26 \pm 4.0$  years), in weight from 55.0 to 93.0 kg ( $69.7 \pm 9.6$  kg) and in height from 163.0 to 188.0 cm ( $175.6 \pm 5.6$  cm) (Table 1).

### Previous bioequivalence studies

Previous studies were based on two simvastatin bioequivalence tests. Each bioequivalence study of two 20 mg simvastatin formulations (reference drug, Zocor 20 mg, MSD Co., Ltd., Seoul, Korea) was conducted in healthy male Korean volunteers after a single dose administration in a randomized cross-over study with a washout period of at least one week. The subjects were hospitalized (Kyung Hee University Hospital, Seoul, Korea) and fasted overnight (10 h) and until 4 h after each drug administration. The doses were administered at 8.00 a.m. of each dosing day along with 240 ml of tap water. No food was allowed until 4 h after dose administration. Approximately 7 ml of blood for simvastatin and active metabolite assays were drawn into heparinized tubes through indwelling cannula before (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24 and 36 h after dosing. Blood samples were centrifuged at 3000 rpm for 10 min; plasma was separated and kept frozen at  $-70^{\circ}\text{C}$  until assayed. Plasma was analyzed for simvastatin concentration using a validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

### Pharmacokinetics analysis

PK parameters ( $C_{\max}$ ,  $T_{\max}$ ,  $AUC_t$  and  $AUC_{\text{inf}}$ ) were calculated by noncompartmental models in WinNonlin v5.2 (Table 1).

### DNA extraction

From November 2008 to January 2009, blood samples were obtained from 35 participants. After obtaining informed consent, approximately 10 ml of whole blood was collected from each participant. Blood samples were drawn into sterile tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at  $-70^{\circ}\text{C}$  until the isolation of genomic DNA. Genomic DNA was isolated from the blood sample by a standard phenol chloroform extraction method.

### Genotyping

SNPs of 35 healthy male volunteers were analyzed by Standard Illumina procedures using Illumina BeadStation 500G (Illumina Human610Quad v1.0 DNA Analysis BeadChip) according to the Illumina manual (Illumina, San Diego, CA, USA). Intensity files (\*.idat) were processed by BeadStudio GT module 3.3.4 with default analysis settings. Each SNP was analyzed independently to cluster and identify genotypes. Genotype calls were generated by comparing experimental data with those in the supplied cluster file (\*.egt). The SNP set was filtered on the basis of genotype call rates ( $\geq 95\%$ ), and minor allele frequency (MAF  $\geq 0.05$ ).

### Statistical analysis & data analysis

Hardy Weinberg equilibrium (HWE) was calculated for individual SNPs using an exact test. All of the SNPs reported in this manuscript have HWE  $P$ -values  $> 0.001$ . To calculate the degree (Beta), 95% confidence intervals (CI) and  $P$ -value while controlling for age, height and weight as covariants in a linear regression analysis with significant SNPs on individual PK parameters of simvastatin, unadjusted genotypic association with additive, dominant and recessive models were tested by calculating the Beta and  $P$ -value using PLINK version 1.06 (Shaun Purcell, USA). When the additive model was used, each genotype was independently coded as 0, 1, or 2 and analyzed. In the dominant model, a homozygote major allele and another two genotypes were coded as 0 and 1 and analyzed. In the recessive model, a homozygote minor allele and another two genotypes were coded as 0 and 1 and analyzed. After filtering, SNPs were analyzed on chromosome 1 through chromosome 22 for each group.

Significant SNPs were identified from each PK parameter ( $C_{\max}$ ,  $T_{\max}$  and  $AUC_{\text{inf}}$ ). Among these, SNPs of phase I and II enzymes, transporters and non-cytochrome metabolic enzymes were selected from the DMET Plus Marker List in Additional Support, DMET™ (Drug-Metabolizing Enzymes and Transporters), Affymetrix, Inc (AFFX) ([http://www.affymetrix.com/products\\_services/arrays/specific/dmet.affx#1\\_4](http://www.affymetrix.com/products_services/arrays/specific/dmet.affx#1_4)).

## Results

### Pharmacokinetics analysis

The PK parameters of simvastatin are shown in Table 1.  $C_{\max}$  is  $7.5 \pm 4.8$  ng/ml,  $T_{\max}$  is  $1.9 \pm 1.2$  h,  $AUC_t$  is  $29.7 \pm 16.8$  ng·h/ml and  $AUC_{\text{inf}}$  is  $34.8 \pm 20.0$  ng·h/ml. These data were used for linear regression analysis.

### SNPs analysis

PK parameters of  $C_{\max}$ ,  $T_{\max}$  and  $AUC_{\text{inf}}$  were analyzed by linear regression analysis. For clinical pharmacological purposes, significant genes encoding phase I and II enzymes, transporters and noncytochrome metabolic enzymes were selected for further analysis. These analyses were adjusted for age, height

**Table 1.** Demographic characteristics and reference simvastatin pharmacokinetics parameters of volunteers

Subject No.	Age (year)	Sex (M/F)	Weight (kg)	Height (cm)	Zocor 40 mg (two tablets of 20 mg simvastatin, MSD Co., Ltd., Seoul, Korea)			
					$C_{max}$ (ng/ml)	$T_{max}$ (h)	$AUC_t$ (ng·h/ml)	$AUC_{inf}$ (ng·h/ml)
1	27	M	84.0	176.0	4.80	3.50	17.983	19.700
2	26	M	76.4	180.6	0.97	1.00	13.499	18.390
3	26	M	55.0	163.0	4.49	5.00	42.589	46.237
4	33	M	67.6	176.9	8.20	0.67	38.992	46.317
5	30	M	75.8	180.2	2.68	1.33	7.561	8.224
6	31	M	64.0	180.0	9.96	0.67	30.355	32.879
7	31	M	77.7	181.7	12.61	2.00	43.494	45.780
8	20	M	63.0	167.8	7.30	1.67	26.874	27.686
9	27	M	57.0	167.5	9.98	0.67	28.825	32.290
10	21	M	68.0	177.0	16.34	3.00	61.885	63.780
11	24	M	93.0	188.0	6.61	1.00	15.617	18.476
12	25	M	69.5	172.2	4.97	0.67	11.166	15.134
13	25	M	68.3	183.0	3.93	2.50	17.679	18.479
14	23	M	87.0	181.6	17.25	1.67	21.184	21.635
15	25	M	68.0	175.4	4.68	1.67	43.160	45.254
16	26	M	65.0	177.4	6.81	1.33	35.282	90.582
17	24	M	66.0	170.0	4.63	3.00	48.621	54.339
18	20	M	75.2	173.8	2.14	1.00	6.787	9.535
19	27	M	79.0	184.0	5.42	1.67	15.221	15.958
20	27	M	61.0	174.0	9.12	1.67	40.288	48.106
21	28	M	60.0	173.0	5.06	0.67	13.200	19.015
22	28	M	85.2	179.9	9.19	1.67	32.320	34.484
23	23	M	68.8	169.4	9.53	0.67	31.386	37.412
24	22	M	65.0	169.7	6.52	3.00	31.262	33.912
25	20	M	55.0	176.3	3.65	3.50	9.345	10.303
26	25	M	66.2	174.4	17.66	1.00	39.767	42.814
27	24	M	75.2	185.1	1.25	0.67	8.561	23.638
28	25	M	59.8	170.2	2.40	3.00	27.549	31.360
29	20	M	56.0	170.0	5.95	5.00	33.259	39.373
30	24	M	59.3	169.1	3.91	2.00	19.060	20.786
31	27	M	83.0	171.6	21.33	1.67	76.230	77.471
32	26	M	70.0	176.0	6.27	1.33	29.589	31.321
33	27	M	75.4	177.4	10.12	1.67	31.061	32.953
34	25	M	70.6	177.6	7.21	1.00	21.359	23.410
35	40	M	71.0	177.0	10.29	3.50	67.206	80.128
Mean ± SD	26 ± 4.0		69.7 ± 9.6	175.6 ± 5.6	7.5 ± 4.8	1.9 ± 1.2	29.7 ± 16.8	34.8 ± 20.0

Abbreviation; M: male, F: female,  $C_{max}$ : maximum measured plasma concentration,  $T_{max}$ : time of the maximum measured plasma concentration,  $AUC_t$ : area under the plasma concentration-time curve from time zero to time of last measurable concentration,  $AUC_{inf}$ : area under the plasma concentration-time curve from zero to infinity, SD: standard deviation.

and weight. We found 145 possible candidate SNPs ( $P < 0.01$ ) including 7 highly possible candidate SNPs ( $P < 0.00001$ ) in  $C_{\max}$ , 135 possible candidate SNPs ( $P < 0.01$ ) including 2 highly possible candidate SNPs ( $P < 0.00001$ ) in  $T_{\max}$ , and 85 possible candidate SNPs ( $P < 0.01$ ) including 3 highly possible candidate SNPs ( $P < 0.00001$ ) in  $AUC_{\text{inf}}$  from whole genome analysis (Table 2).

In the  $C_{\max}$  group, we found several highly possible candidate SNPs among drug-metabolizing enzymes and transporters (Table 3). For example, *SLC44A3* (rs696620) showed statistically significant changes by genotype in a recessive model ( $P = 2.580 \times 10^{-4}$ ). *SLC4A5* (rs10182348, rs10211612, rs2421844, rs3755441, rs3771740, rs3821303, rs7567768, rs7592599 and rs9789404) showed statistically possible changes by genotype with LD blocks in a recessive model ( $P = 1.604 \times 10^{-4}$ ). *SLC2A12* (rs2811675) showed statistically possible changes by genotype in additive, dominant and recessive models ( $P = 3.621 \times 10^{-4}$ ;  $P = 0.006$ ;  $P = 0.003$ , respectively). Other possible candidate SNPs are presented in Table 3. Also, SNPs of simvastatin PK pathways in the  $C_{\max}$  group were analyzed. Certain SNPs, such as *CYP3A5*, *ABCC2* and *SLCO1B3*, showed statistically possible changes by genotype (Supplementary table 1).

In the  $T_{\max}$  group, we found several highly possible candidate SNPs among drug-metabolizing enzymes and transporters (Table 4). For example, *ABCC4* (rs1059751, rs3742106,

rs4148549, rs4148553 and rs7330196) showed statistically possible changes by genotype with LD blocks in additive and recessive models ( $P = 0.001$ ;  $P = 9.468 \times 10^{-4}$ , respectively). *CYP2F1* (rs305968) showed statistically possible changes by genotype in additive, dominant and recessive models ( $P = 5.876 \times 10^{-4}$ ;  $P = 5.239 \times 10^{-4}$ ;  $P = 0.032$ , respectively). Other possible candidate SNPs are presented in Table 4. Also, SNPs of simvastatin PK pathways in the  $T_{\max}$  group were analyzed. Certain SNPs, such as *UGT2B7* and *ABCG2*, showed statistically possible changes by genotype (Supplementary table 2).

In the  $AUC_{\text{inf}}$  group, we found several highly possible candidate SNPs among drug-metabolizing enzymes and transporters (Table 5). For example, *SLC2A12* (rs2811675) showed statistically possible changes by genotype in additive and dominant models ( $P = 0.002$ ;  $P = 0.001$ , respectively). *SLC18A1* (rs4922132) showed statistically possible changes by genotype in additive, dominant and recessive models ( $P = 3.414 \times 10^{-4}$ ;  $P = 0.004$ ;  $P = 0.010$ , respectively). Other significant SNPs are presented in Table 5. Also, SNPs of simvastatin PK pathways in  $AUC_{\text{inf}}$  group were analyzed. Certain SNPs, such as *ABCC2*, showed statistically possible changes by genotype (Supplementary table 3).

Although minor genotypes showed very low frequencies in some SNPs, their effects on PK parameters were great. For example, two volunteers who had the GG genotype of rs11183395 (*SLC38A1*) and the TT genotype of rs10483482 (*SLC25A21*) exhibited two-fold increases in  $C_{\max}$  (Table 3). In the  $T_{\max}$  group, two volunteers who had the CC genotype of rs4646715 (*ALDH1L1*) showed delayed absorption of simvastatin, while nine volunteers who had the AA genotype of rs305968 (*CYP2F1*) showed accelerated absorption of simvastatin (Table 4). Also, in the  $AUC_{\text{inf}}$  group, two volunteers who had the GG genotype of rs7830129 (*SLC25A37*) exhibited two-fold increases in simvastatin absorption (Table 5). These results suggest that genotype can affect simvastatin PK.

## Discussion

Simvastatin is widely used in the treatment of hypercholesterolemia. It reduces low density lipoprotein (LDL) cholesterol concentrations by inhibiting cholesterol synthesis in the liver.[14] Many clinical trials have shown that simvastatin reduces the risk of death and other complications in patients with chronic heart disease.[15-17] Recently, many studies have reported a relationship between genetic polymorphisms and simvastatin PK.[18-21]

**Table 2.** Summary of linear regression analysis

PK parameters	P-value	SNP number		Model number		
		Drug metabolizing enzyme	ADD	DOM	REC	
$C_{\max}$	$P < 0.00001$	7		7		
	$< 0.0001$	7		7		
	$< 0.001$	33	10	9	23	
	$< 0.01$	145	91	75	63	
	$< 0.05$		100	89	69	
$T_{\max}$	$P < 0.00001$	2			2	
	$< 0.0001$	4			4	
	$< 0.001$	25	3	3	20	
	$< 0.01$	135	63	37	73	
	$< 0.05$		67	49	92	
$AUC_{\text{inf}}$	$P < 0.00001$					
	$< 0.0001$	3			3	
	$< 0.001$	12	2	1	9	
	$< 0.01$	85	32	20	56	
	$< 0.05$		32	20	56	

Abbreviation; ADD: additive model, DOM: dominant model, REC: recessive model.

**Table 3.** Highly significant SNPs of drug metabolizing enzymes in C<sub>max</sub> group from linear regression analysis

Gene	SNP	Chr	Location	Genotype	n	Mean	SD	Model	$\beta$	95% CI		P-value	Sig
										LCL	UCL		
SLC44A3	rs696620	1	intron	CC	13	7.22	4.06	Additive	1.61	-1.30	4.52	0.287	
				CT	20	6.52	3.80	Dominant	-0.71	-4.15	2.74	0.689	
				TT	2	19.49	2.60	<b>Recessive</b>	<b>11.80</b>	<b>6.19</b>	<b>17.41</b>	<b>2.580x10<sup>-4</sup></b>	<b>***</b>
SLC4A5	rs10182348	2	intron	CC	15	8.01	3.82	Additive					
				CT	16	5.02	2.44	Dominant					
				TT	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs10211612	2	intron	GG	15	8.01	3.82	Additive					
				GA	16	5.02	2.44	Dominant					
				AA	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs2421844	2	intron	GG	15	8.01	3.82	Additive					
				GA	16	5.02	2.44	Dominant					
				AA	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs3755441	2	intron	TT	14	8.41	3.63	Additive					
				TC	17	4.87	2.44	Dominant					
				CC	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs3771740	2	intron	AA	15	8.01	3.82	Additive					
				AT	16	5.02	2.44	Dominant					
				TT	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs3821303	2	intron	GG	15	8.01	3.82	Additive					
				GA	16	5.02	2.44	Dominant					
				AA	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs7567768	2	intron	TT	15	8.01	3.82	Additive					
				TC	16	5.02	2.44	Dominant					
				CC	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs7592599	2	intron	TT	14	8.41	3.63	Additive					
				TC	17	4.87	2.44	Dominant					
				CC	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
	rs9789404	2	intron	CC	15	8.01	3.82	Additive	1.63	-0.73	3.98	0.185	
				CT	16	5.02	2.44	Dominant	-0.52	-3.73	2.70	0.755	
				TT	4	15.69	6.38	<b>Recessive</b>	<b>9.20</b>	<b>5.00</b>	<b>13.41</b>	<b>1.604x10<sup>-4</sup></b>	<b>***</b>
SLC2A12	rs2811675	6	intron	AA	17	5.24	2.80	<b>Additive</b>	<b>4.23</b>	<b>2.16</b>	<b>6.30</b>	<b>3.621x10<sup>-4</sup></b>	<b>***</b>
				AG	15	8.37	4.39	<b>Dominant</b>	<b>4.23</b>	<b>1.45</b>	<b>7.00</b>	<b>0.006</b>	<b>**</b>
				GG	3	16.23	5.67	<b>Recessive</b>	<b>9.11</b>	<b>3.61</b>	<b>14.62</b>	<b>0.003</b>	<b>**</b>
ALDH3B1	rs15518	11	3UTR	TT	25	5.51	2.71	<b>Additive</b>	<b>3.84</b>	<b>1.91</b>	<b>5.77</b>	<b>4.881x10<sup>-4</sup></b>	<b>***</b>
				TC	6	13.39	5.86	<b>Dominant</b>	<b>6.93</b>	<b>4.36</b>	<b>9.51</b>	<b>9.759x10<sup>-4</sup></b>	<b>****</b>
				CC	4	11.31	4.95	Recessive	3.57	-1.32	8.46	0.162	
	rs2286164	11	intron	TT	25	5.51	2.71	<b>Additive</b>	<b>4.16</b>	<b>2.10</b>	<b>6.22</b>	<b>4.120x10<sup>-4</sup></b>	<b>***</b>
				TC	7	12.90	5.51	<b>Dominant</b>	<b>6.93</b>	<b>4.36</b>	<b>9.51</b>	<b>9.759x10<sup>-6</sup></b>	<b>*****</b>
				CC	3	11.76	5.96	Recessive	3.35	-2.31	9.02	0.255	

Table 3. continued

Gene	SNP	Chr	Location	Genotype	n	Mean	SD	Model	$\beta$	95% CI		P-value	Sig
										LCL	UCL		
ALDH3B1	rs2286168	11	missense	GG	25	5.51	2.71	Additive	4.16	2.10	6.22	4.120x10 <sup>-4</sup>	***
				GA	7	12.90	5.51	Dominant	6.93	4.36	9.51	9.759x10 <sup>-6</sup>	*****
				AA	3	11.76	5.96	Recessive	3.35	-2.31	9.02	0.255	
	rs3133268	11	3flanking	GG	25	5.51	2.71	Additive	3.84	1.91	5.77	4.881x10 <sup>-4</sup>	***
				GC	6	13.39	5.86	Dominant	6.93	4.36	9.51	9.759x10 <sup>-6</sup>	*****
				CC	4	11.31	4.95	Recessive	3.57	-1.32	8.46	0.162	
	rs886701	11	intron	GG	25	5.51	2.71	Additive	4.16	2.10	6.22	4.881x10 <sup>-4</sup>	***
				GA	7	12.90	5.51	Dominant	6.93	4.36	9.51	9.759x10 <sup>-6</sup>	*****
				AA	3	11.76	5.96	Recessive	3.35	-2.31	9.02	0.255	
SLC38A1	rs11183395	12	intron	AA	14	6.00	3.85	Additive	3.40	0.95	5.85	0.011	**
				AG	19	7.64	4.60	Dominant	2.37	-0.85	5.59	0.159	
				GG	2	17.00	0.93	Recessive	11.45	5.90	17.01	3.280x10 <sup>-4</sup>	***
ABCC4	rs1729741	13	intron	AA	24	6.79	4.23	Additive	2.90	0.68	5.13	0.016	**
				AG	8	6.43	2.77	Dominant	2.27	-1.02	5.55	0.186	
				GG	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup>	***
	rs2892716	13	intron	CC	17	5.20	3.70	Additive	3.97	2.06	5.87	2.941x10 <sup>-4</sup>	***
				CT	14	8.19	2.30	Dominant	4.18	1.32	7.05	0.008	**
				TT	4	15.04	7.63	Recessive	8.02	3.77	12.28	8.436x10 <sup>-4</sup>	***
	rs4148436	13	intron	TT	17	5.20	3.70	Additive	3.97	5.06	5.87	2.941x10 <sup>-4</sup>	***
				TC	14	8.19	2.30	Dominant	4.18	1.32	7.05	0.008	**
				CC	4	15.04	7.63	Recessive	8.02	3.77	12.28	8.436x10 <sup>-4</sup>	***
	rs4148440	13	intron	AA	10	5.77	4.52	Additive	2.99	0.98	4.99	0.007	**
				AG	18	6.68	2.94	Dominant	2.19	-1.20	5.58	0.215	
				GG	7	12.17	6.59	Recessive	6.23	2.95	9.50	7.833x10 <sup>-4</sup>	***
SLC25A21	rs10483482	14	intron	CC	20	6.35	3.87	Additive	3.36	1.00	5.73	0.009	**
				CT	13	7.49	3.88	Dominant	2.46	-0.64	5.55	0.130	
				TT	2	19.49	2.60	Recessive	11.80	6.19	17.41	2.580x10 <sup>-4</sup>	***
	rs1884777	14	intron	CC	22	6.63	4.42	Additive	3.01	0.78	5.24	0.013	**
				CT	10	6.83	2.45	Dominant	2.34	-0.91	2.59	0.169	
				TT	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup>	***
	rs1955761	14	intron	AA	22	6.63	4.42	Additive	3.01	0.78	5.24	0.013	**
				AT	10	6.83	2.45	Dominant	2.34	-0.91	5.59	0.169	
				TT	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup>	***
	rs1956424	14	intron	CC	22	6.51	4.37	Additive	3.06	0.85	5.28	0.011	**
				CT	10	7.11	2.60	Dominant	2.42	-0.79	5.62	0.149	
				TT	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup>	***
	rs2078246	14	intron	GG	21	6.89	4.36	Additive	2.58	0.30	4.86	0.034	**
				GA	11	6.33	2.87	Dominant	1.54	-1.71	4.78	0.360	
				AA	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup>	***



Table 3. continued

Gene	SNP	Chr	Location	Genotype	n	Mean	SD	Model	$\beta$	95% CI		P-value	Sig
										LCL	UCL		
SLC25A21	rs2415388	14	intron	GG	21	6.89	4.36	Additive	2.58	0.30	4.86	0.034 **	
				GA	11	6.33	2.87	Dominant	1.54	-1.71	4.78	0.360	
				AA	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup> ***	
	rs8008478	14	intron	AA	22	6.63	4.42	Additive	3.01	0.78	5.24	0.013 **	
				AG	10	6.83	2.45	Dominant	2.34	-0.91	5.59	0.169	
				GG	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup> ***	
	rs8019489	14	intron	CC	23	6.64	4.32	Additive	3.01	0.76	5.25	0.013 **	
				CT	9	6.84	2.60	Dominant	2.35	-0.96	5.66	0.173	
				TT	3	16.32	5.79	Recessive	9.06	4.41	13.70	5.973x10 <sup>-4</sup> ***	

Abbreviation; Chr: chromosome, n: number, SD: standard deviation, CI: confidence intervals, LCL: lower confidence limit, UCL: upper confidence limit, Sig: significant. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

However, there has been no study concerning PK of simvastatin and whole genome SNPs analysis. In this study, we conducted a whole genome SNPs analysis to investigate possible candidate genes for simvastatin PK. We analyzed three PK parameters ( $C_{max}$ ,  $T_{max}$  and  $AUC_{inf}$ ). For clinical pharmacological purposes, candidate genes which showed statistically possible differences among phase I and II enzymes, transporters and non-cytochrome metabolic enzymes were selected for further analysis. These analyses were adjusted for age, height and weight. Several SNPs showed possible differences in each group by linear regression analysis. Among the significant SNPs, although their minor allele frequency was relatively very small ( $n = 2$  to 4), some SNPs showed great changes in PK parameters.

These results are closely related with rare extensive or poor metabolizers in drug metabolism. For example, the *CYP3A5* genotype is related to genetic variation in the pharmacokinetics of tacrolimus, and *CYP3A5* genotype (\*1/\*1, \*1/\*3, \*3/\*3) is related to levels of tacrolimus in living-donor liver transplant recipients. The trough blood levels ( $C_0$ ) of tacrolimus were substantially lower in patients receiving livers with *CYP3A5*\*1/\*3 genotype (*CYP3A5* non-expressers) than *CYP3A5*\*3/\*3 genotype (*CYP3A5* expressers), which may hinder a stronger effect of the donor's genotype on tacrolimus dose requirements.[22]

Based on the current study, genetic polymorphisms, especially SNPs, could have effects on simvastatin concentration and PK parameters. In the  $AUC_{inf}$  group (Table 5), *ARNT* and five SLC transporters including *SLC45A2* showed statistically strong differences ( $P < 0.001$ ). The aryl hydrocarbon receptor nuclear translocator (*ARNT*) is a member of the basic helix-loop-helix/PERARNT-SIM (bHLH/PAS) family of proteins.[23] In this study, minor allele homozygotes (G) of rs2134688 of *ARNT* might be associated ( $P = 3.188 \times 10^{-4}$ , in recessive model; Table 5) with increased absorption of simvastatin. The *SLC45A2* gene,

which encodes a transporter protein involved in melanin synthesis, is considered one of the most important genes affecting human pigmentation.[24] Our results showed that minor allele homozygotes (A) of rs35390 of *SLC45A2* might be associated ( $P = 0.007$  and  $P = 8.877 \times 10^{-5}$ , in additive and recessive models, respectively; Table 5) with an increased absorption of simvastatin. *SLC25A37* (Mitoferrin-1; *Mfrn1*), a member of the solute carrier family localized in the mitochondrial inner membrane, functions as an essential iron importer for the synthesis of mitochondrial heme and iron-sulfur clusters in erythroblasts.[25] There have been no studies about genetic polymorphisms of *SLC25A37* to date. In this study, minor allele homozygotes (G) of rs7830129 of *SLC25A37* might be associated ( $P = 4.141 \times 10^{-4}$ , in recessive model; Table 5) with increased absorption of simvastatin. We suggest that the results of this study could be used for further study to predict simvastatin PK parameters.

In the  $C_{max}$  group (Table 3), *ALDH3B1*, *ABCC4* transporter, and five SLC transporters including *SLC44A3* showed statistically strong differences ( $P$ -value  $< 0.001$ ). Recent studies have linked SNPs at the *ALDH3B1* locus to the development of paranoid schizophrenia.[26-28] In this study, major allele homozygotes (G) of rs2286168 of *ALDH3B1* might be associated ( $P = 4.120 \times 10^{-4}$  and  $P = 9.759 \times 10^{-6}$ , in additive and dominant models, respectively; Table 3) with decreased absorption of simvastatin. *ABCC4* encodes MRP4, a member of the ATP-binding cassette family of membrane transporters involved in the efflux of endogenous and xenobiotic molecules.[29] Although *ABCC4* is a highly polymorphic gene,[30] little data is available concerning the function of its variants. Recent studies have shown the functional effects of several *ABCC4* SNPs on drug disposition. Anderson et al., showed a 20% increase in lamivudine-triphosphate intracellular concentrations in patients carrying the 4131T>G (rs3742106) variant, while the 3724G>A (rs11568695) variant

**Table 4.** Highly significant SNPs of drug-metabolizing enzymes in  $T_{\max}$  group from linear regression analysis

Gene	SNP	Chr	Location	Genotype	n	Mean	SD	Model	$\beta$	95% CI		P-value	Sig
										LCL	UCL		
DPYD	rs12725266	1	intron	CC	23	1.80	1.11	Additive	0.80	0.13	1.47	0.026 *	
				CT	10	1.62	0.95	Dominant	0.49	-0.39	1.36	0.283	
				TT	2	4.25	1.06	Recessive	3.43	1.89	4.97	1.317x10 <sup>-4</sup> ***	
ABCA4	rs1889404	1	intron	GG	24	1.84	1.13	Additive	0.52	-0.14	1.18	0.135	
				GA	9	1.48	0.80	Dominant	0.16	-0.71	1.02	0.721	
				AA	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs1889405	1	intron	GG	24	1.84	1.13	Additive	0.52	-0.14	1.18	0.135	
				GA	9	1.48	0.80	Dominant	0.16	-0.71	1.02	0.721	
				AA	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs3789439	1	intron	GG	25	1.71	1.08	Additive	0.82	0.17	1.47	0.019 *	
				GA	8	1.86	1.00	Dominant	0.64	-0.26	1.54	0.176	
				AA	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs3789442	1	intron	TT	25	1.71	1.08	Additive	0.82	0.17	1.47	0.019 *	
				TC	8	1.86	1.00	Dominant	0.64	-0.26	1.54	0.176	
				CC	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs3789443	1	intron	AA	25	1.71	1.08	Additive	0.82	0.17	1.47	0.019 *	
				AT	8	1.86	1.00	Dominant	0.64	-0.26	1.54	0.176	
				TT	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs3789444	1	intron	CC	25	1.71	1.08	Additive	0.82	0.17	1.47	0.019 *	
				CT	8	1.86	1.00	Dominant	0.64	-0.26	1.54	0.176	
				TT	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs4147807	1	intron	TT	27	1.75	1.11	Additive	0.85	0.19	1.51	0.017 *	
				TC	6	1.72	0.83	Dominant	0.69	-0.26	1.63	0.164	
				CC	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
	rs4147808	1	intron	CC	27	1.75	1.11	Additive	0.85	0.19	1.51	0.017 *	
				CT	6	1.72	0.83	Dominant	0.69	-0.26	1.63	0.164	
				TT	2	4.25	1.06	Recessive	2.73	1.27	4.19	9.115x10 <sup>-4</sup> ***	
ALDH1L1	rs11923466	3	intron	TT	15	1.54	1.01	Additive	0.83	0.26	1.40	0.008 **	
				TC	17	1.80	0.87	Dominant	0.63	-0.15	1.41	0.122	
				CC	3	4.17	1.44	Recessive	2.45	1.19	3.71	6.221x10 <sup>-4</sup> ***	
	rs2166766	3	intron	TT	15	1.54	2.80	Additive	0.83	0.26	1.40	0.008 **	
				TC	17	1.80	4.39	Dominant	0.63	-0.15	1.41	0.122	
				CC	3	4.17	5.67	Recessive	2.45	1.19	3.71	6.221x10 <sup>-4</sup> ***	
	rs4646715	3	intron	GG	15	0.91	2.71	Additive	0.91	0.30	1.51	0.006 **	
				GC	18	0.63	5.86	Dominant	0.63	-0.15	1.41	0.122	
				CC	2	3.25	4.95	Recessive	3.25	1.83	4.66	8.905x10 <sup>-5</sup> ****	
	rs4646717	3	intron	TT	15	0.91	2.71	Additive	0.91	0.30	1.51	0.006 **	
				TC	18	0.63	5.51	Dominant	0.63	-0.15	1.41	0.122	
				CC	2	3.25	5.96	Recessive	3.25	1.83	4.66	8.905x10 <sup>-5</sup> ****	



Table 4. continued

Gene	SNP	Chr	Location	Genotype	n	Mean	SD	Model	$\beta$	95% CI		P-value	Sig
										LCL	UCL		
SLC10A7	rs6537420	4	intron	GG	11	2.82	1.34	Additive	-0.68	-1.16	-0.19	0.011 *	
				GT	15	1.29	0.70	Dominant	-1.49	-2.19	-0.78	2.416x10 <sup>-4</sup> ***	
				TT	9	1.74	1.01	Recessive	-0.31	-1.24	0.62	0.516	
SLC2A12	rs2811675	6	intron	AA	17	1.22	0.63	Additive	0.92	0.35	1.49	0.003 **	
				AG	15	2.69	1.32	Dominant	1.36	0.70	2.01	2.952x10 <sup>-4</sup> ***	
				GG	3	1.67	0.00	Recessive	0.19	-1.44	1.82	0.822	
SLCO1B1	rs4149032	12	intron	TT	13	1.31	0.69	Additive	1.13	0.57	1.70	4.342x10 <sup>-4</sup> ***	
				TC	19	2.00	1.18	Dominant	1.01	0.24	1.78	0.015 *	
				CC	3	3.67	1.26	Recessive	2.37	1.04	3.70	0.001 **	
	rs4149034	12	intron	AA	13	1.31	0.69	Additive	1.13	0.57	1.70	4.342x10 <sup>-4</sup> ***	
				AG	19	2.00	1.18	Dominant	1.01	0.24	1.78	0.015 *	
				GG	3	3.67	1.26	Recessive	2.37	1.04	3.70	0.001 **	
ABCC4	rs1059751	13	3UTR	TT	10	1.34	0.75	Additive	0.88	0.39	1.38	0.001 **	
				TC	17	1.69	0.88	Dominant	0.84	-0.04	1.72	0.070	
				CC	8	3.00	1.56	Recessive	1.51	0.70	2.32	9.468x10 <sup>-4</sup> ***	
	rs3742106	13	3UTR	CC	10	1.34	0.75	Additive	0.88	0.39	1.38	0.001 *	
				CA	17	1.39	0.88	Dominant	0.84	-0.04	1.72	0.070	
				AA	8	3.00	1.56	Recessive	1.51	0.70	2.32	9.468x10 <sup>-4</sup> ***	
	rs4148549	13	intron	AA	10	1.34	0.75	Additive	0.88	0.39	1.38	0.001 *	
				AG	17	1.39	0.88	Dominant	0.84	-0.04	1.72	0.070	
				GG	18	3.00	1.56	Recessive	1.51	0.70	2.32	9.468x10 <sup>-4</sup> ***	
	rs4148553	13	3UTR	GG	10	1.34	0.75	Additive	0.88	0.39	1.38	0.001 *	
				GA	17	1.39	0.88	Dominant	0.84	-0.04	1.72	0.070	
				AA	8	3.00	1.56	Recessive	1.51	0.70	2.32	9.468x10 <sup>-4</sup> ***	
	rs7330196	13	intron	TT	10	1.34	0.75	Additive	0.88	0.39	1.38	0.001 *	
				TC	17	1.39	0.88	Dominant	0.84	-0.04	1.72	0.070	
				CC	8	3.00	1.56	Recessive	1.51	0.70	2.32	9.468x10 <sup>-4</sup> ***	
SLC39A11	rs2859523	17	intron	CC	14	1.81	0.86	Additive	0.62	0.00	1.24	0.058	
				CT	18	1.51	0.91	Dominant	0.06	-0.76	0.89	0.880	
				TT	3	4.50	0.87	Recessive	2.82	1.78	3.87	9.471x10 <sup>-6</sup> *****	
	rs2915446	17	intron	AA	12	1.75	0.85	Additive	0.71	0.08	1.34	0.035 *	
				AC	20	1.58	0.92	Dominant	0.14	-0.72	0.99	0.754	
				CC	3	4.50	0.87	Recessive	2.82	1.78	3.87	9.471x10 <sup>-6</sup> *****	
CYP2F1	rs305968	19	synony-mous	GG	12	2.79	1.14	Additive	-0.86	-1.30	-0.42	5.876x10 <sup>-4</sup> ***	
				GA	14	1.61	1.11	Dominant	-1.38	-2.07	-0.68	5.239x10 <sup>-4</sup> ***	
				AA	9	1.11	0.44	Recessive	-1.01	-1.89	-0.13	0.032 *	

Abbreviation; Chr: chromosome, n: number, SD: standard deviation, CI: confidence intervals, LCL: lower confidence limit, UCL: upper confidence limit, Sig: significant. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\*\* $P < 0.0001$ .

**Table 5.** Highly significant SNPs of drug-metabolizing enzymes in  $AUC_{inf}$  group from linear regression analysis

Gene	SNP	Chr	Location	Genotype	n	Mean	SD	Model	$\beta$	95% CI		P-value	Sig
										LCL	UCL		
ARNT	rs2134688	1	intron	AA	19	34.71	19.36	Additive	7.32	-2.34	16.98	0.148	
				AG	13	25.94	10.67	Dominant	0.41	-12.57	13.40	0.951	
				GG	3	67.01	21.57	<b>Recessive</b>	<b>39.44</b>	<b>20.35</b>	<b>58.54</b>	<b>3.188x10<sup>-4</sup></b>	<b>***</b>
	rs3738483	1	intron	GG	19	34.71	19.36	Additive	7.32	-2.34	16.98	0.148	
				GA	13	25.94	10.67	Dominant	0.41	-12.57	13.40	0.951	
				AA	3	67.01	21.57	<b>Recessive</b>	<b>39.44</b>	<b>20.35</b>	<b>58.54</b>	<b>3.188x10<sup>-4</sup></b>	<b>***</b>
SLC45A2	rs35390	5	intron	CC	10	29.18	14.40	<b>Additive</b>	<b>12.22</b>	<b>3.93</b>	<b>20.51</b>	<b>0.007</b>	<b>**</b>
				CA	18	27.68	10.09	Dominant	6.09	-8.09	20.28	0.406	
				AA	7	58.24	27.42	<b>Recessive</b>	<b>28.88</b>	<b>16.31</b>	<b>41.45</b>	<b>8.877x10<sup>-5</sup></b>	<b>****</b>
	rs35391	5	intron	TT	10	29.18	14.40	<b>Additive</b>	<b>12.22</b>	<b>3.93</b>	<b>20.51</b>	<b>0.007</b>	<b>**</b>
				TC	18	27.68	10.09	Dominant	6.09	-8.09	20.28	0.406	
				CC	7	58.24	27.42	<b>Recessive</b>	<b>28.88</b>	<b>16.31</b>	<b>41.45</b>	<b>8.877x10<sup>-5</sup></b>	<b>****</b>
	rs35408	5	intron	CC	11	32.59	12.59	<b>Additive</b>	<b>10.69</b>	<b>2.41</b>	<b>18.97</b>	<b>0.017</b>	<b>*</b>
				CT	17	25.57	10.63	Dominant	3.91	-10.14	17.97	0.589	
				TT	7	57.80	27.63	<b>Recessive</b>	<b>28.64</b>	<b>16.01</b>	<b>41.27</b>	<b>1.047x10<sup>-4</sup></b>	<b>***</b>
	rs35412	5	intron	CC	11	32.59	12.59	<b>Additive</b>	<b>10.69</b>	<b>2.41</b>	<b>18.97</b>	<b>0.017</b>	<b>*</b>
				CG	17	25.57	10.63	Dominant	3.91	-10.14	17.97	0.589	
				GG	7	57.80	27.63	<b>Recessive</b>	<b>28.64</b>	<b>16.01</b>	<b>41.27</b>	<b>1.047x10<sup>-4</sup></b>	<b>***</b>
SLC25A46	rs6892259	5	intron	AA	15	25.20	12.14	<b>Additive</b>	<b>17.21</b>	<b>7.95</b>	<b>26.47</b>	<b>0.001</b>	<b>***</b>
				AC	18	39.00	19.22	<b>Dominant</b>	<b>17.16</b>	<b>5.57</b>	<b>28.75</b>	<b>0.007</b>	<b>**</b>
				CC	2	58.90	44.42	<b>Recessive</b>	<b>31.96</b>	<b>6.07</b>	<b>57.85</b>	<b>0.022</b>	<b>*</b>
SLC2A12	rs2811675	6	intron	AA	17	24.25	10.40	<b>Additive</b>	<b>15.58</b>	<b>6.67</b>	<b>24.49</b>	<b>0.002</b>	<b>**</b>
				AG	15	43.71	21.53	<b>Dominant</b>	<b>20.14</b>	<b>9.34</b>	<b>30.95</b>	<b>0.001</b>	<b>***</b>
				GG	3	43.27	28.79	Recessive	14.85	-10.77	40.47	0.265	
SLC25A37	rs2928686	8	intron	CC	25	31.48	14.10	<b>Additive</b>	<b>11.91</b>	<b>1.11</b>	<b>22.70</b>	<b>0.038</b>	<b>*</b>
				CT	8	30.21	20.54	Dominant	7.57	-6.63	21.77	0.304	
				TT	2	84.51	8.20	<b>Recessive</b>	<b>51.49</b>	<b>25.97</b>	<b>77.01</b>	<b>4.141x10<sup>-4</sup></b>	<b>***</b>
	rs2942194	8	missense	TT	23	31.48	14.72	<b>Additive</b>	<b>11.19</b>	<b>1.56</b>	<b>20.83</b>	<b>0.030</b>	<b>*</b>
				TC	9	26.86	14.94	Dominant	4.96	-8.87	18.79	0.487	
				CC	3	77.33	13.72	<b>Recessive</b>	<b>44.53</b>	<b>26.45</b>	<b>62.60</b>	<b>3.513x10<sup>-5</sup></b>	<b>****</b>
	rs7830129	8	intron	AA	23	31.41	14.73	Additive	10.68	-0.4422	21.8	0.069	
				AG	10	30.63	18.14	Dominant	4.912	-9.013	18.84	0.495	
				GG	2	84.51	8.20	<b>Recessive</b>	<b>51.49</b>	<b>25.97</b>	<b>77.01</b>	<b>4.141x10<sup>-4</sup></b>	<b>***</b>
SLC18A1	rs4922132	8	intron	CC	21	29.47	16.26	<b>Additive</b>	<b>19.92</b>	<b>10.22</b>	<b>29.62</b>	<b>3.414x10<sup>-4</sup></b>	<b>***</b>
				CT	12	37.57	21.78	<b>Dominant</b>	<b>20.33</b>	<b>7.635</b>	<b>33.02</b>	<b>0.004</b>	<b>**</b>
				TT	2	64.00	16.75	<b>Recessive</b>	<b>36.81</b>	<b>10.68</b>	<b>62.94</b>	<b>0.010</b>	<b>**</b>

Abbreviation; Chr: chromosome, n: number, SD: standard deviation, CI: confidence intervals, LCL: lower confidence limit, UCL: upper confidence limit, Sig: significant. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

was associated with a trend toward elevated AZT-triphosphate, suggesting reduced MRP4 efflux function.[31] Interestingly, the 4131T>G variant is in the 3'-untranslated region (UTR) of the gene, while the 3724G>A variant is synonymous; there is therefore no clear mechanism explaining these effects. In another study, no association was observed between two non-synonymous and seven synonymous *ABCC4* variants and tenofovir disoproxil fumarate-induced renal proximal tubulopathy.[32] Most recently, 74 genetic variants in *ABCC4* were shown to have no effect on MRP4 mRNA and protein expression in Caucasian cholestatic and non-cholestatic patients.[33] However, they reported that *ABCC4* genotype affected drug metabolism. In this study, minor allele homozygotes (C) of rs4148436 of *ABCC4* were significantly associated ( $P = 2.941 \times 10^{-4}$ ,  $P = 0.008$  and  $P = 8.436 \times 10^{-4}$ , in additive, dominant and recessive models, respectively; Table 3) with increased absorption of simvastatin. Based on these results, we suggest that these genes could be used for candidate genes for prediction of simvastatin pharmacogenomics study.

In the  $T_{\max}$  group (Table 4), *ALDH1L1*, *CYP2F1*, *ABCA4*, *ABCC4*, and four SLC transporters including *SLC39A11* showed statistically strong differences ( $P < 0.001$ ). Minor allele homozygotes (A) of rs305968 of *CYP2F1* might be associated ( $P = 5.786 \times 10^{-4}$ ,  $P = 5.239 \times 10^{-4}$  and  $P = 0.032$  in additive, dominant and recessive models, respectively; Table 4) with accelerated absorption of simvastatin. Also, minor allele homozygotes (C) of rs1059751 of *ABCC4* might be associated ( $P = 0.001$  and  $P = 9.468 \times 10^{-4}$  in additive and recessive models, respectively; Table 4) with delayed absorption of simvastatin. By linear regression analysis, the *ABCC4* gene has a strong effect on  $C_{\max}$  and  $T_{\max}$ , together.

We also found several significant SNPs affecting simvastatin PK pathways using whole-genome linear regression analysis. In the  $C_{\max}$  group, *CYP3A5*, three ABC transporters including *ABCB11*, and two SLC transporters including *SLCO2B1* showed relatively weak differences ( $P < 0.05$ ). Minor allele homozygotes (C) of rs10208831 of *ABCB11* might be associated ( $P = 0.004$ ,  $P = 0.013$  and  $P = 0.025$ , in additive, dominant and recessive models, respectively) with increased absorption of simvastatin (Supplementary Table 1). In the  $T_{\max}$  group, *UGT2B7*, three ABC transporters including *ABCG2*, and three SLC transporters including *SLCO1B1* showed relatively weak differences ( $P < 0.05$ ). Major allele homozygotes (C) of rs10028494 of *UGT2B7* might be associated ( $P = 0.013$  in recessive model) with increased absorption of simvastatin. Our results suggest that *UGT2B7* could have a clinically important effect on simvastatin absorption (Supplementary Table 2). Major allele homozygotes (T) of rs2306283 of *SLCO1B1* might be associated ( $P = 0.007$ ,  $P = 0.024$  and  $P = 0.036$  in additive, dominant and recessive models, respectively) with accelerated absorption of simvastatin. Hence, polymorphisms of *SLCO1B1* gene could be assessed in order to tailor simvastatin treatment (Supplementary Table 3). In the  $AUC_{\text{inf}}$  group, *ABCC2* and *SLC15A1* showed relatively

weak differences ( $P < 0.05$ ). Minor allele homozygotes (C) of rs3740065 of *ABCC2* might be associated ( $P = 0.005$  in recessive model) with increased absorption of simvastatin. *ABCC2*, the gene encoding MRP2, is expressed on the bile canalicular membrane and plays an important role in the biliary excretion of various substrates.[34] Genetic variations in the *ABCC2* gene have been reported to alter functional activity, thereby contributing to interindividual differences in drug disposition.[35] The *ABCC2* gene is also involved in the inhibition of biliary excretion HMGR.[34,36,37]

By whole genome linear regression analysis, we found several highly possible candidate SNPs which affecting PK parameters of simvastatin. Interestingly, except for few SNPs of phase I and phase II enzymes, such as *CYP3A5* ( $C_{\max}$ ) (Supplementary Table 1) and *CYP2F1* ( $T_{\max}$ ), most of the significant SNPs were found in transporters. Previous studies have been performed on drug-metabolizing enzymes, but our results showed that transporters can have great influences on the PK of drugs in PK study. For example from this study, we found that the *ABCC2* gene could be a candidate gene for personalized simvastatin therapy. The *ABCC2* gene has a strong effect on  $C_{\max}$  and  $AUC_{\text{inf}}$  together. Other SNPs also can be candidate biomarkers for clinical use in tailored simvastatin administration.

In this study, we performed linear regression analysis for PK parameters of simvastatin. The screened SNPs from this study might be related to the PK of simvastatin and used for tailored pharmacotherapy of simvastatin. But our study has significant limitations. Because of small sample size, Bonferroni correction cannot be adjusted. Although we have no SNPs which can be used for prediction of simvastatin pharmacogenomics because all significant SNPs showed p value above  $10^{-6}$ , SNPs from this study could be used for candidate genes for further study. So further studies for simvastatin pharmacogenomics using SNPs from this study are needed.

## Conflict of interest

The authors declare no conflict of interest.

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