RESEARCH ARTICLE

Mixed effects of OATP1B1, BCRP and NTCP polymorphisms on the population pharmacokinetics of pravastatin in healthy volunteers

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Abstract

1. Pravastatin is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor used for the treatment of hyperlipidaemia. This study aims to investigate the effects of genetic polymorphisms in OATP1B1, BCRP and NTCP on pravastatin population pharmacokinetics in healthy Chinese volunteers using a non-linear mixed-effect modelling (NONMEM) approach. A two-compartment model with a first-order absorption and elimination described plasma pravastatin concentrations well.

2. Genetic polymorphisms of rs4149056 (OATP1B1) and rs2306283 (OATP1B1) were found to be associated with a significant \( p < 0.01 \) decrease in the apparent clearance from the central compartment \( (CL/F) \), while rs2296651 (NTCP) increased \( CL/F \) to a significant degree \( (p < 0.01) \). The combination of these three polymorphisms reduced the inter-individual variability of \( CL/F \) by 78.8%.

3. There was minimal effect of rs2231137 (BCRP) and rs2231142 (BCRP) on pravastatin pharmacokinetics \( (0.01 < p < 0.05) \), whereas rs11045819 (OATP1B1), rs1061018 (BCRP) and rs61745930 (NTCP) genotypes do not appear to be associated with pravastatin pharmacokinetics based on the population model \( (p > 0.05) \).

4. The current data suggest that the combination of rs4149056, rs2306283 and rs2296651 polymorphisms is an important determinant of pravastatin pharmacokinetics.

Keywords

BCRP, genetic polymorphisms, NTCP, OATP1B1, population pharmacokinetics, pravastatin

Introduction

Pravastatin is a member of the drug class known as statins, which are used primarily for lowering blood cholesterol and for the prevention of events associated with cardiovascular disease. Similar to other statins, pravastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in the production of cholesterol in the body. Pravastatin has demonstrated to competitively inhibit the biosynthesis of cholesterol in cells, promote the expression of low-density lipoprotein–cholesterol (LDL-C) receptors on cell surface and accelerate the removal of LDL-C in the circulating blood (Hedman et al., 2003; Keogh et al., 2000; Kietsiriroje & Leelawattana, 2015; Schachter, 2005; Sponseller et al., 2014; van de Pas et al., 2014).

Pravastatin is relatively hydrophilic, which shows greater hepatoselectivity than lipophilic statins (Schachter, 2005). Pharmacokinetic (PK) assessments in patients and healthy volunteers have shown that the absolute oral bioavailability of pravastatin is approximately 18%, with an average time to reach the peak concentration (peak time, \( t_{\text{max}} \)) of 1 to 2 h (Escobar et al., 2005; Neuvonen et al., 2008; Pan et al., 1993; Sigurbjörnsdóttir et al., 1998). The elimination half-life \( (t_{1/2}) \) of pravastatin ranges from 1 to 3 h in clinical studies (Escobar et al., 2005; Pan et al., 1993; Sigurbjörnsdóttir et al., 1998). Pravastatin is also characterised by moderate plasma protein binding (~50%) and significantly higher concentrations in hepatocytes than in other tissue cells (Hatanaka, 2000). Pravastatin is minimally metabolised by hepatic enzymes. Most of the bioavailable fraction of an oral dose is excreted unchanged through both hepatic and renal routes (Hatanaka, 2000; Ogawa et al., 2003).

It is reported that there is considerable interindividual variability (IIV) in pravastatin PKs, and differences in the activity of influx/efflux transporters are likely to be responsible for the majority of the variation (Ide et al., 2009; Ieiri et al., 2009; Neuvonen et al., 2008; Ogawa et al., 2003; Watanabe et al., 2009). Many previous studies focused on the effects of the uptake transporter OATP1B1 (encoded by SLCO1B1 gene) polymorphisms on pravastatin PKs (Deng et al., 2008; Hedman et al., 2006; Niemi et al., 2004, 2006). An in vivo study indicated that the SLCO1B1 c.521T > C (rs4149056) polymorphism was associated with increased plasma concentrations of pravastatin (Niemi et al., 2004). Another clinical study also demonstrated that the polymorphism of SLCO1B1 c.521T > C was associated with higher
exposure to pravastatin (Niemi et al., 2006). However, there are conflicting suggestions on whether c.521T>C and c.388A>G (rs2306283) polymorphisms influence pravastatin PKs. Niemi et al. indicated that both the polymorphisms of SLCO1B1 c.512T>C and c.388A>G were associated with the reduced hepatic uptake of pravastatin (Niemi et al., 2004). While Deng et al. demonstrated that pravastatin PKs was significantly affected by OATP1B1 c.521T>C, but not significantly affected by c.388A>G (Deng et al., 2008). In addition, Hedman et al. showed that the c.512T>C polymorphism may be associated with decreased plasma concentrations of pravastatin in children with heterozygous familial hypercholesterolaemia and in paediatriac cardiac transplant recipients receiving immunosuppressive medication (Hedman et al., 2006). However, as the numbers of these enrolled subjects were small, large-scale studies are needed for further confirmation of these conclusions.

The breast cancer resistance protein (BCRP, encoded by ABCG2 gene) functions as a xenobiotic transporter which may play a role in multidrug resistance to therapeutic agents, including mitoxantrone, camptothecin, rosuvastatin and atorvastatin (Assaraf, 2006; Birmingham et al., 2015; Hua et al., 2012; Keskitalo et al., 2009; Lee et al., 2015). Several studies have been reported on the effects of genetic polymorphisms of BCRP on statins disposition or pharmacologic action. A previous study with 62 healthy Chinese volunteers demonstrated that both the ABCG2 c.421C>A (rs2231137) and c.34G>A (rs2231142) polymorphisms markedly affected the pharmacokinetics of rosuvastatin (Wang et al., 2015). Another small-scale PK study with 32 Finnish volunteers also showed the ABCG2 c.421C>A polymorphism contributed toward between-population exposure differences of atorvastatin PKs, potentially affecting the efficacy and toxicity of statin therapy (Kunze et al., 2014). However, the effect of ABCG2 polymorphisms on pravastatin PKs has not been well evaluated.

The Na+-taurocholate cotransporting polypeptide (NTCP), which is also known as the sodium/bile acid co-transporter or liver bile acid transporter (LBAT), is a protein that in humans is encoded by the SLCL10A1 gene (Hagenbuch & Meier, 1994; Yan et al., 2012). Several previous in vivo investigations have shown significant correlations between statins PKs and NTCP polymorphisms (Bi et al., 2013; Lou et al., 2014; Pan et al., 2011). Lou et al. reported SLCL10A1 c.800C>T (rs2296651) polymorphism played a critical role in the individual variability of rosuvastatin pharmacokinetics in Chinese healthy males after excluding the impact of OATP1B1 c.521T>C and BCRP c.421C>A polymorphisms (Lou et al., 2014). Pan et al. also indicated that the stably transfected NTCP-A64T variant showed significantly decreased uptake of rosuvastatin compared with wild-type NTCP, while the NTCP-S267F variant showed significantly increased rosuvastatin uptake (Pan et al., 2011). Bi et al. also reported NTCP-mediated uptake contributed significantly to active hepatic uptake in hepatocyte models for pitavastatin (Bi et al., 2013). To the best of our knowledge, no study on the influence of NTCP genetic polymorphisms on pravastatin PKs has been reported. As the importance of NTCP in pravastatin uptake clearance is not yet clarified, the effect of NTCP genetic polymorphisms on the PKs of pravastatin still needs to be well evaluated.

Therefore, we studied the impact of genetic variations for a total of eight alleles in OATP1B1, BCRP and NTCP, as well as demographic and biochemical variables on the pravastatin PKs in healthy Chinese volunteers, and on the IIV in the disposition of orally administered pravastatin utilising a nonlinear mixed-effect modelling (NONMEM) approach.

Methods

Chemical and reagents

Pravastatin tablets (80 mg specification) were obtained from Sino American Shanghai Squibb Pharmaceutical Co., Ltd (Shanghai, China). Pravastatin standard (purity 100.1%) was purchased from North China Pharmaceutical Co., Ltd (Shijiazhuang, Hebei, China). Rosuvastatin standard (purity 99.8%) was obtained from Sigma Chemical Co. (St. Louis, MO). Acetonitrile of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ). Distilled water was prepared from demineralised water throughout the study. Other chemicals were of analytical grade.

Study design and population

In this retrospective analysis, data on subjects from five independent pravastatin PK studies, which used the same protocol were assembled. A total of 98 adult volunteers participated in this study, which was performed in accordance with the Declaration of Helsinki and the ICH Guidelines for Good Clinical Practise, and approved by the Ethics Committee of the Second Affiliated Hospital of Jilin University, China. Written informed consent was obtained from volunteers or legally authorised representatives before participation in the study. Each subject in trial received a single oral dose of 10, 20 or 40 mg pravastatin. A full PK profile of 13 blood samples was collected from each volunteer in ethylenediaminetetraacetic acid (EDTA) tubes predose and at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10 and 12 hours after dose. Blood samples were immediately separated for plasma by centrifugation at 3000 g for 5 min and then stored at -80°C until further analysis.

Pravastatin assay

The quantitative analytical method of pravastatin in plasma is established based on high performance liquid chromatography equipped with tandem mass spectrometry (HPLC-MS/MS, Waters Co., Milford, MA) method. Briefly, chromatographic separation was performed using a Thermo Hypurity C18, 5 μm particle size (150 × 2.1 mm, internal diameter, Thermo Fisher Scientific, Waltham, MA) column kept at 40°C with a constant flow rate of 0.2 mL/min. The mobile phase consisted of acetonitrile:formic acid (80: 20, v/v). Tandem mass spectrometry detection was performed in the positive ion, multiple reaction monitoring (MRM) mode following the transition m/z 447.8 → 327.6 for pravastatin, and the transition m/z 482.4 → 259.2 for internal standard (IS, rosuvastatin). The IS stock solution was prepared at a concentration of 50.4 μg/mL and further diluted to achieve a final concentration of 25.2 ng/mL with acetonitrile. A volume of 100 μL IS solution and 100 μL of ammonium formate solution (0.10% w/v) were added into 500 μL of plasma.
This mixture was extracted with a volume of 5 mL MTBE, vortex-mixed for 3 min and centrifuged at 3000rpm for 10 min. The upper extract was then evaporated to dryness at 30°C under nitrogen stream. The residue was reconstituted with a 150 μL aliquot of the mobile phase, and a 10 μL aliquot was injected directly onto the HPLC-MS/MS system.

The retention times for pravastatin and IS were approximately 1.92 and 2.11 min, respectively. No interference from any endogenous substances was observed in human plasma. The method was linear over the concentration range of 0.957–510 ng/mL with a lower limit of quantitation (LLOQ) of 0.957 ng/mL. The method showed good intra-assay precision and accuracy with relative standard deviation (% RSD) values from 5.31% to 10.4% and mean relative error (% MRE) from −0.321 to 5.90%, as well as good interassay precision and accuracy with % RSD from 9.17% to 11.6%.

The detailed assay validation results are listed in the Supplementary Table S1–S4.

Genotyping

DNA was isolated from ethylenediamine tetraacetic acid (EDTA) blood collected from the volunteers. The polymerase chain reaction (PCR) method was used to amplify the eight fragments (rs4149056, rs2306283, rs11045819, rs22231137, rs2231142, rs1061018, rs2296651 and rs61745930), and the restriction fragment length polymorphism (RFLP) analysis was used for genotyping assays.

The total volume of the PCR was 50 μL, consisting of 10 pmol of each primer pair, 2.5 nmol of dNTPs, 5 μL of 10 × PCR buffer (containing MgCl₂), 2.5 U of Taq DNA polymerase and 100 ng of DNA. Cycle conditions were initial denaturation at 95–98°C for 5 min, followed by 35 cycles of denaturation at 95–98°C for 30 s, annealing at 53°C–58°C for 30 s, initial extension at 72°C for 30 s, and final extension at 72°C for 5 min.

The PCR amplification products were then digested by restriction enzymes (Table S5 in the Supplementary Information). The total reaction system consisted of 10 μL of PCR amplification products, 1 μL of restriction enzyme, 2 μL 10 × H buffer and 7 μL of purified water. This mixture was incubated for 6–12 h at 37°C. The resulting restriction fragments were then separated according to their lengths by 2%–4% agarose gel electrophoresis.

As quality control of genotype analyses, 5% of samples were genotyped in duplicate, and the validation of RFLP was conducted using the quality control samples by pyrosequencing analysis, and no inconsistencies were observed.

Modelling methodology

Development of the base model

The population pharmacokinetic (PPK) model was built using nonlinear mixed-effect modelling in the computer programme NONMEM (version 7.3, Icon Development Solutions, Ellicott City, MD). The first-order conditional estimation with interaction (FOCEI) method within NONMEM was employed throughout the model-building procedure. Development of the PPK model started with construction of the base model, including the structural PK model and models for the interindividual and residual variabilities. Once the base model was developed, covariate (COV) models were developed to explain the interindividual and residual variabilities. The likelihood ratio test (LRT) was used for statistical hypothesis testing to discriminate among alternative base and COV models.

Several structural PK models, including the one-, two- and three-compartment models, with and without lag times, were tested during model development. Three residual error models were assessed: the additive error model, the proportional error model and the proportional plus additive error model (the combined error model). Individual PK parameters were assumed to be log-normally distributed, and the IIV in PK parameters was modelled as an exponential error model.

Development of the covariate model

The influence of demographic and biochemical COVs was assessed by visual inspection of the COV and IIV plots. For continuous COVs, the linear models, the exponential models and the power models centred on a median value were tested for age, body weight (BW), body mass index (BMI) and the liver function markers (albumin, total bilirubin, blood urea nitrogen, aspartate aminotransferase and alanine aminotransferase). Creatinine clearance (CLCR) was investigated as a potential continuous COV and was calculated using the Cockcroft–Gault equation (Cockcroft & Gault, 1976). As the linear models were enough to describe the correlation between the PK parameters and the categorical COVs (sex and the single-nucleotide polymorphisms (SNPs)), only the linear models were tested for the categorical COVs.

Potential COVs were incorporated sequentially into the model in order of significance, whereby the inclusion of the COV was based on a significant drop in the objective function value (OFV), biological plausibility and a drop in the IIV values. COVs selected to enter the intermediate model had to decrease the OFV significantly (ΔOFV > 3.84 or p value < 0.05) from the COV-free model. Addition of COVs to the base model was conducted in a stepwise manner. Finally, in the backward-deletion step, each COV was independently removed from the full-covariate model to confirm its relevance. If the increase in the OFV was not statistically significant (ΔOFV < 6.63 or p value > 0.01), the relationship between this COV and the corresponding PK parameter was not taken into account in the final model.

Model qualification

All diagnostic graphs and statistical analyses were conducted utilising Matlab software (version 8.2.0.701, MathWorks Corporation, Natick, MA).

The performance of the final model was evaluated using Bayesian forecasting. Bayesian estimates of individual PK parameters were calculated for each subject, using as priors the mean or median values and interindividual variabilities of the parameters previously estimated with the best model retained with NONMEM. Goodness-of-fit of the population models was assessed by comparing the observed and Bayesian estimated pravastatin plasma concentrations.

The robustness of the final model estimates was assessed using bootstrap analysis, in which subjects were randomly
sampled with replacement from the dataset that was used in model development to obtain 1000 datasets that have the same number of subjects as the original dataset. The mean and variability (as measured in coefficient of variation, %CV) of the parameter estimates from the 1000 runs were then calculated and compared with the point estimates (mean values) obtained with the original dataset.

The adequacy of the final model was appraised by a visual predictive check (VPC). A total of 1000 datasets were simulated using the original dataset and calculated with the final model. Plots of the median values and 90% confidence interval (CI: 5–95%) of the simulated concentration-time profiles were generated to check that the observed profiles were reasonably contained within this CI.

**Results**

**Subject characteristics**

A total of 1176 plasma pravastatin concentrations were included in the analysis. The demographic and clinical characteristics of the volunteers, including the distribution of the continuous COVs assessed in this study, are summarised in Table 1.

Observed SNP frequencies are listed in Table 2. Haplotype profiles were generated to check that the observed profiles were statistically well fitted with the observed profiles.

Table 1. Summary of volunteers’ characteristics at baseline.

| Parameter (unit) | Mean (SD) | Median | Range |
|------------------|-----------|--------|-------|
| Demographics     |           |        |       |
| Age (years)      | 24.0 (3.63)| 23.0   | 19.0, 29.0 |
| Body weight (kg) | 64.7 (6.94)| 63.0   | 47.0, 80.0 |
| Body mass index (kg/m²) | 20.5 (3.70) | 21.5 | 18.5, 22.6 |
| Sex              |           |        |       |
| Male             | 78 (79.6%)a |        |       |
| Female           | 20 (20.4%)a |        |       |
| Clinical         |           |        |       |
| Alanine aminotransferase (U/L) | 18.3 (8.66) | 16.0 | 3.00, 47.0 |
| Albumin (g/L)    | 3.90 (0.416)| 3.70  | 2.90, 4.90 |
| Aspartate aminotransferase (U/L) | 21.5 (5.55) | 20.0 | 13.0, 38.0 |
| Blood urea nitrogen (mmol/L) | 4.85 (1.07) | 4.80 | 3.00, 8.00 |
| Creatinine (umol/L) | 79.2 (9.43) | 81.0 | 55.0, 91.0 |
| Creatinine clearance (mL/min) | 98.0 (34.3) | 99.0 | 27.0, 194 |
| Total bilirubin (mg/dL) | 0.800 (0.921) | 0.800 | 0.100, 4.20 |

SD: standard deviation.
aData are presented as number (percentage).

Table 2. The list of eight single nucleotide polymorphisms (SNPs) of the OATP1B1, BCRP and NTCP investigated in volunteers.

| Transporter enzyme | SNPs | Allele change | Amino acid variation | WW | WV | VV |
|--------------------|------|---------------|----------------------|----|----|----|
| OATP1B1            | rs4149056 | T → C         | V174A                | 78 (79.6) | 18 (18.4) | 2 (2.0) |
|                    | rs2306283 | C → T         | N130D                | 45 (45.9) | 39 (39.8) | 14 (14.3) |
|                    | rs11045819 | C → A         | P155T                | 87 (88.8) | 10 (10.2) | 1 (1.0) |
| BCRP               | rs2231137 | G → A         | V12M                 | 49 (50.0) | 43 (43.9) | 6 (6.10) |
|                    | rs2231142 | C → A         | Q141K                | 60 (61.2) | 32 (32.7) | 6 (6.10) |
|                    | rs1061018 | T → C         | F208S                | 93 (94.9) | 5 (5.10)  | 0 (0)   |
| NTCP               | rs2296651 | G → A         | S267F                | 91 (92.9) | 7 (7.10)  | 0 (0)   |
|                    | rs61745930 | A → G         | I223T                | 92 (93.9) | 6 (6.10)  | 0 (0)   |

The wild-type alleles were designated as W and the variants as V. WW, WV and VV represent individuals of homozygous for the wild type allele, heterozygous for the variant allele, and homozygous for the variant allele, respectively.
(OATP1B1), rs2306283 (OATP1B1), rs2231137 (BCRP), rs2231142 (BCRP) and rs2296651 (NTCP) polymorphisms had significant \((p < 0.05)\) effects on CL/F. Other COVs, such as age, sex, BW, CL\(_{\text{CR}}\), rs11045819 (OATP1B1), rs1061018 (BCRP) and rs61745930 (NTCP) polymorphisms did not appear to improve the goodness-of-fit (data not shown). Furthermore, a combination of rs4149056, rs2306283 and rs2296651 was found to be associated with a statistically significant difference \((p < 0.01)\) in CL/F (Table 3). Removal of any of the three polymorphisms in the stepwise backward elimination was associated with a significant increase in the OFV \((p < 0.01)\). Hence, the rs4149056, rs2306283 and rs2296651 genotype effects on CL/F were enrolled in the final model. Notably, removal of the rs2231137 and rs2231142 genotype effects on CL/F in the stepwise backward elimination was associated with a non-significant increase in OFV \((p > 0.01)\). Hence, these genotype effects were removed from the model.

**Final model**

The estimation result from the final model showed all PK parameters and corresponding IIVs were precisely estimated, with a relative standard error \((\% \text{ RSE})\) of 12.7% or less (Table 4). When comparing the basic and the final model, the IIV in CL/F was reduced by 78.8% after adding rs4149056, rs2306283 and rs2296651 WV/VV genotypes as model COVs. The effects of genetic polymorphisms on pravastatin PK parameters are shown in Figure 1. The final equation for CL/F is presented as follows:

\[
\frac{CL}{F}(L/h) = 69.5 \times (1 + G_{1,i} \times \theta_1) \times (1 + G_{2,j} \times \theta_2,3) \times (1 + G_{3,k} \times \theta_4)
\]

where \(G_1 (i = 0 \text{ and } 1)\), \(G_2 (j = 0, 1 \text{ and } 2)\) and \(G_3 (k = 0 \text{ and } 1)\) represent the index variables for rs4149056, rs2306283 and rs2296651 polymorphisms, respectively. \(G_{1,0}, G_{2,0}\) and \(G_{3,0}\) \((\text{value 0})\) represent the WW genotype; \(G_{1,1}, G_{2,1}\) and \(G_{3,1}\) \((\text{value 1})\) represent the WV genotype; \(G_{2,2}\) \((\text{value 1})\) represents the VV genotype. The estimated \(\theta_s\) are presented in Table 4.

### Table 3. Summary of the covariate model-building steps.

| Effect of covariates | Parameter variation | \(\Delta IIV\) (%) | \(\Delta OFV\) |
|----------------------|---------------------|-----------------|----------------|
| **Forward selection** |                     |                 |                |
| rs4149056 on CL/F    |                    | -43.9           | -56.7          |
| rs2306283 on CL/F    |                    | -66.4           | -107           |
| rs2231137 on CL/F    |                    | -5.27           | -5.30          |
| rs2231142 on CL/F    |                    | -6.57           | -6.66          |
| rs2296651 on CL/F    |                    | -23.9           | -26.8          |
| **Backward elimination** |                  |                 |                |
| rs4149056 on CL/F    |                    | 8.98            | 27.3           |
| rs2306283 on CL/F    |                    | 28.6            | 69.2           |
| rs2296651 on CL/F    |                    | 2.92            | 10.8           |
| **Final model**      |                     | -78.8           | -152           |

\(\Delta IIV\): variation of inter-individual variability; \(\Delta OFV\): variation of objective function value; CL/F: apparent clearance from the central compartment; F: bioavailability.

### Table 4. Final estimates of the population pharmacokinetic parameters obtained using NONMEM and bootstrap analysis of the final model.

| Parameter | Estimate (% RSE) | Bootstrap result | CI (5%, 95%) |
|-----------|------------------|------------------|--------------|
| \(k_s (h^{-1})\) | 1.26 (0.00172) | 1.28 | 1.25, 1.29 |
| \(Q_{CL}/F (L/h)\) | 11.4 (0.00197) | 11.5 | 11.2, 11.6 |
| \(V_{F} (L)\) | 318 (0.00199) | 323 | 301, 332 |
| CL/F (L/h) | 69.5 (1.27) | 69.5 | 69.2, 70.2 |
| \(V_{F} (L)\) | 80.5 (0.00176) | 81.2 | 80.3, 82.2 |

**Effect of rs4149056**

- WV on CL/F (\(\theta_1\)): -0.134 (12.7) -0.135 -0.137, -0.133
- VV on CL/F (\(\theta_1\)): -0.164 (9.66) -0.163 -0.164, -0.162
- Proportional error (%): 30.0 30.0 30.0, 30.1

**Mixed effects of SNPs on pravastatin PPKs**

The predictive performance (goodness-of-fit) of the final population model was assessed graphically (Figure 2). Population-predicted (PRED) concentrations were calculated using population parameter estimates (mean values) and COVs information, while individual-predicted (IPRED) concentrations were based on post hoc empiric Bayesian estimates of the PK parameters (Table 4). The PRED and IPRED concentrations versus the observations were distributed randomly across the line of unity, indicating the final model adequately described the observed concentrations over the entire pravastatin concentration range. The conditional weighted residuals (CWRES) plots showed symmetrical distribution and no concentration- or time-related trends.

In order to evaluate the precision of estimated PK parameters, the bootstrap analysis was performed and it showed narrow confidence intervals for all parameters. The mean, median, 5th and 95th percentiles of the parameter estimates from the fit of the final model to the bootstrap samples are summarised in Table 4. The asymptotic estimates obtained from the original dataset showed close agreement with the medians and were all included in the 5th and 95th percentiles of the bootstrap values, indicating model stability.

The mean and nonparametric 90% CI \((5-95\%)\) of the simulated concentrations were overlaid on the observed concentrations and visual inspection was performed to determine whether the observed data were reasonably contained within this CI. About 55.8% of the original dataset fit within the 5th and 95th percentiles of the simulated data, and the median of the PRED concentrations adequately described the central tendency in the observed data (Figure 3).

Parameters were estimated based on the dose-normalised (based on 10 mg) pravastatin concentrations. CI: confidence interval; CL/F: apparent clearance from the central compartment; CV: coefficient of variation; F: bioavailability; IIV: interindividual variability; \(k_s\): absorption rate constant; \(Q_{CL}/F\): apparent inter-compartmental clearance; RSE: relative standard error; \(V_{F}/F\): apparent volume of the peripheral compartment. WV and VV represent individuals of heterozygous for the variant allele and homozygous for the variant allele, respectively. \(\theta_i\) represent the fractional change in the PK parameters for the WV or VV groups.

*Based on 956/1000 successful runs.*
Overall, the final model provided an adequate and unbiased fit to the pravastatin PK data included in this analysis.

**Discussion**

This is the study to examine extensively the comprehensive effects of genetic variations in influx/efflux transporters, demographic and biochemical variables on the pravastatin population pharmacokinetics (PPKs). The most robust fit to the data was obtained using a simple two-compartment mamillary model with linear absorption and elimination. This population model identified the three factors rs4149056 (OATP1B1), rs2306283 (OATP1B1) and rs2296651 (NTCP) polymorphisms as major predictors of pravastatin plasma exposure in healthy Chinese volunteers.

The effect of rs4149056 (OATP1B1) and rs2306283 (OATP1B1) polymorphisms on the PK parameters of pravastatin was significant, and the estimated \( CL/F \) was lower in subjects with the WV/VV genotype than in those with the WW genotype. As only two subjects were found carrying rs4149056 WV genotype, the effect of the WV genotype was not evaluated for lack of statistical confidence. However, these findings are in line with the report of Niemi et al., which showed that pravastatin concentration was significantly associated with SLCO1B1 c. 512T > C and c.388 A > G (Niemi et al., 2004). When rs2296651 (NTCP) was evaluated, statistically significant differences in \( CL/F \) were observed among the WW and WV groups. In contrast to the effects of rs4149056 and rs2306283 polymorphisms on pravastatin PKs, subjects with rs2296651 WV genotype were characterised by significantly higher \( CL/F \) than the carriers with WW genotype. NTCP (Na⁺-taurocholate cotransporting polypeptide, encoded by the SLC10A1 gene) mainly expresses in the basolateral membranes of hepatocytes, which are integral membrane glycoproteins that participate in the enterohepatic circulation of bile acids (Bi et al., 2013; Hagenbuch & Meier, 1994; Yan et al., 2012). Its functional activity of uptake of various statins was confirmed by several reports, but the functional consequences of variants of NTCP may be different for various statins, depending on the substrate specificity of the NTCP transporters. Several previous *in vitro* investigations have shown controversial results regarding the affiliation between NTCP and other statins. Using *in vitro* hepatocyte models, Pan et al. concluded that NTCP-mediated uptake contributed significantly to active hepatic uptake for rosuvastatin (Pan et al., 2011). Bi et al. also found that NTCP-mediated uptake contributed significantly to active hepatic uptake in hepatocyte models for rosuvastatin (Bi et al., 2013); while Lee et al. reported a different result that the polymorphisms in SLC10A1 did not significantly affect oral rosuvastatin pharmacokinetics in Chinese patients with hypercholesterolaemia (Lee et al., 2013). To date, there was no previous report directly supporting our findings. As low allele frequency (0.0755) of the variant allele was detected based on the data in hand, more large scale studies are needed for further confirmation.

The combination of rs4149056, rs2306283 and rs2296651 was also found to be associated with statistically significant differences in the pravastatin disposition, which indicates that pravastatin is a strong substrate of both OATP1B1 and NTCP. After including these genotypes in the model, the inter-individual variability of \( CL/F \) was decreased by more than 78%. Since previous reports suggested that the increased plasma concentrations of statins in patients with hypercholesterolaemia were associated with increased lipid-lowering effects (Hu et al., 2011; Lee et al., 2013), the difference in pravastatin PK parameters among different genotypes may significantly influence pravastatin pharmacodynamic effects. This suggests that it would be useful for the tailored medicine of pravastatin to analyse an individual’s genotypes of both OATP1B1 and NTCP. Moreover, some recent studies reported that OATP1B1 polymorphisms were associated with the
increased risk of statin-induced and dose-dependent musculoskeletal side effects (myalgia, rhabdomyolysis and creatine kinase elevations) (Martin et al., 2012; Santos et al., 2011; Voora et al., 2009). Therefore, our findings could have potential implications for clinical practise since the vast majority of patients who are intolerant to statins have mild symptoms without associated creatine kinase elevations (Voora et al., 2009).

The allele frequency of rs2306283 variation (34.2%) in our study population was close to that in other Asians and Caucasians, while the frequency of rs4149056 variation (11.2%) was similar to other Chinese, but a little lower than Caucasians (Hedman et al., 2006; Kerb, 2006; Lou et al., 2014). Therefore, the impact of rs4149056 polymorphism on pravastatin pharmacokinetics seems to be weaker in Asians than it does in Caucasians in the context...
of clinical therapy. Moreover, the prevalence of the rs2296651 allele in this study (3.57%) was found a little higher than other Chinese population (Lou et al., 2014). However, due to the low allele frequency, the significance of rs2296651 in clinical therapy needs further investigation.

In this study, we also investigated the association of rs11045819 (OATP1B1), rs2231137 (BCRP), rs2231142 (BCRP), rs1061018 (BCRP) and rs61745930 (NTCP) polymorphisms with the pravastatin PKs. However, these five polymorphisms were not enrolled in the final population model, indicating that these five SNPs may play no significant role in the disposition of pravastatin and then have no clinically relevant effect on the efficacy or risk of adverse reactions of pravastatin. In addition, other covariates such as age, sex, BW, BMI and biochemical markers also do not induce any obvious effects on the PK parameters. This may have occurred when the study was conducted only in healthy adult subjects. In sum, studies of more diverse population and genetic polymorphisms may allow a more meaningful examination of covariate influences on pravastatin pharmacokinetics.

It is important to account for interaction between pharmacogenetic covariates, but the identification of true covariate combinations may be difficult during a classical PK analysis. In order to allow for such combinations, we used the mixed-effect modelling approach in which the impact of specific genetic polymorphisms was initially investigated one by one, followed by the incorporation of all potentially interesting covariates in a full-covariate model.

These modelling processes were optimised processes to the current data. There was a wave pattern in the CWRES plot of goodness-of-fit and a little underestimation in VPC. In addition, the bootstrap analysis confirmed the predictive ability, model stability and precision of the parameter estimates. The use of mixed-effect modelling allowed the analysis and integration of multiple pharmacogenetic and demographic covariates in a pharmacokinetic/pharmacogenetic population model. These results mean that the changes of PK parameters due to genotype and cogenetic population model. These results mean that the contribution of transporting activity changes by SLCO1B1*15. Pharmacogenet Genomics 18:424–33.

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Supplementary materials available online