Marked Alternation of Rosuvastatin Pharmacokinetics in Healthy Chinese
with ABCG2 34G > A and 421C > A Homozygote or Compound Heterozygote

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Running Title: ABCG2 Haplotypes on Rosuvastatin Pharmacokinetics

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Abbreviations: ABCG2, ATP-binding cassette G2; CYP2C9, Cytochrome P450 2C9; HPLC, high performance liquid chromatography; LDL-C, low-density lipoprotein cholesterol; MS, mass spectrometry; PCR, polymerase chain reaction; SLCO1B1, solute carrier organic anion transporter family member 1B1; SNP, single nucleotide polymorphism; WT, wild type.
Abstract

Rosuvastatin, a HMG-CoA reductase inhibitor used to lower blood LDL-C, is a substrate of the membrane ABCG2 exporter. ABCG2 variants have been shown to alter rosuvastatin disposition. The objective of this study is to determine the impact of ABCG2 34/421 compound haplotypes on rosuvastatin pharmacokinetics in healthy Chinese volunteer subjects. Eight-hundred healthy Chinese males were genotyped by PCR-Pyrosequencing for ABCG2 34G>A, ABCG2 421C>A, SLCO1B1 521T>C and CYP2C9*3 variants. Sixty-two male subjects with wild-type SLCO1B1 c.521TT and CYP2C9*3 were recruited for this pharmacokinetic study of rosuvastatin. A single oral dose of 10 mg rosuvastatin was administrated to each subject, and blood samples were collected before and at various time points after drug administration. Plasma concentration of rosuvastatin was determined by HPLC-MS/MS, and pharmacokinetic analysis was carried out using WinNonlin program. In Chinese males, high allele frequency of ABCG2 c.34G>A (0.275) and c.421C>A (0.282) was observed, resulting in a considerable portion (23.3%) of subjects being ABCG2 34/421 compound heterozygotes. Compared to subjects with ABCG2 wild-type (c.34GG/421CC), plasma rosuvastatin $C_{\text{max}}$ and $AUC_{0-\infty}$ were significantly higher, while CL/F was significantly lower in subjects with c.34AA, c.421AA, and c.34GA/421CA genotypes. Both $T_{1/2}$ and $T_{\text{max}}$ were similar among subjects with different genotypes. A high frequency of ABCG2 c.34G>A and c.421C>A variants were present in Chinese males, and the disposition of rosuvastatin was significantly affected by both variants. This data suggests that it is advisable to genotype these variants when prescribing rosuvastatin to Chinese subjects, leading to a precise dose for each individual.
Introduction

Statins, through reduction of plasma levels of low-density lipoprotein cholesterol (LDL-C), are widely used for the treatment of hyperlipidemia, and subsequently for the prevention of coronary heart disease. Rosuvastatin, a synthetic statin, is a stronger HMG-CoA reductase inhibitor, and more effective in lowering LDL-C than other statins such as atorvastatin and pravastatin (Jones et al., 2003). However, there is considerable inter-individual variability in the efficacy and toxicity of rosuvastatin. Clinical trial data suggests that Asian-Americans may have higher plasma drug levels and be at greater risk for rosuvastatin-produced side effects than the general population (US Food and Drug Administration: FDA Provides Updated Patient and Healthcare Provider Information Concerning Crestor, 2005). As a result, the FDA has recommended lower rosuvastatin doses in Asians (US Food and Drug Administration: FDA Public Health Advisory on Crestor (rosuvastatin), 2005). This variability in rosuvastatin efficacy and toxicity is partially due to genetic variation in the membrane influx and efflux transporters.

ATP-binding cassette G2 protein (ABCG2, also known as breast cancer resistance protein) is a membrane efflux transporter which is expressed in various normal tissues such as the small intestine, colon, liver, and kidney, and plays a significant role in the disposition of various drugs (Gradhand and Kim, 2008; Hardwick et al., 2007). Currently, more than 50 ABCG2 single nucleotide polymorphisms (SNPs) have been reported in various ethnic populations (Iida et al., 2002; Zamber et al., 2003; Kobayashi et al., 2005; Bäckström et al., 2003). Previous studies have shown a significantly higher frequency of ABCG2 c.34G > A (rs2231137) and c.421C > A (rs2231142) variants in Asians when compared to Caucasians and African-Americans (Kim et al.,
The ABCG2 c.421C>A polymorphism has been reported to markedly affect the pharmacokinetics, efficacy and toxicity of rosuvastatin (Zhang et al., 2006; Keskitalo et al., 2009a). However, the importance of ABCG2 c.34G>A polymorphism in rosuvastatin disposition has not been studied in humans. Thus, the objective of this study is to determine the influence of ABCG2 c.34G>A SNP in rosuvastatin pharmacokinetics in healthy Chinese volunteers. As ABCG2 c.34G>A and c.421C>A variants are not in linkage disequilibrium (Kwan et al., 2011) and the reports of c.421CA heterozygotes on rosuvastatin disposition are inconsistent (Zhang et al., 2006; Keskitalo et al., 2009a), the effect of ABCG2 34GA/421CA compound heterozygotes on rosuvastatin pharmacokinetics was also analyzed.
Materials and Methods

1. Genomic DNA extraction and Determination of SLCO1B1 c.521T>C, ABCG2 c.421C>A and ABCG2 c.34G>A polymorphism

Genomic DNA from 800 healthy Chinese men was extracted from whole-blood samples using the QuickGene DNA whole blood kit (Kurabo, Osaka, Japan), and the DNA concentration was determined using a Nanodrop 2000. To determine the c.521T>C polymorphism of the SLCO1B1 gene, a 119 bp fragment was amplified by PCR using a specific pair of primers (Table 1) under the following conditions: one cycle pre-denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and elongation at 72°C for 30 s, and one cycle of post-elongation at 72°C for 5 min. The ABCG2 c.34G>A and c.421C>A polymorphic regions were amplified by PCR using the primer pairs indicated in Table 1 using the same conditions as SLCO1B1. The single strand sequencing templates were purified using Pyromark ID instrument (Qiagen, Germany). The single-nucleotide polymorphism (SNP) was determined by pyrosequencing on a Pyromark Q96 ID platform (Qiagen, Germany) using the PyroMark Gold® Q96 Reagents (Qiagen, Germany) with specific primers indicated in Table 1 following the manufacturer’s instructions. The resulting polymorphisms were identified automatically by PyroMark ID software (Qiagen, Germany), and verified by manual analysis. The CYP2C9*3 polymorphism was determined by PCR-pyrosequencing using specific primers (Table 1) as previously described (Zhao et al., 2009). Three to five samples for each polymorphism were randomly selected and directly sequenced using Sanger chain-termination method to verify the accuracy of pyrosequencing method, and the results were completely in agreement with pyrosequencing (Wan et al., 2012a & 2012b).
2. Subjects and pharmacokinetic study design

A total of 62 healthy Chinese men of Han ethnicity, between ages 18-24, were recruited for the pharmacokinetic study. These subjects had a BMI ranging from 18 to 24 and they were considered healthy as ascertained by physical examination. All subjects were non-smokers, were free of drugs, and abstained from coffee, tea and alcohol for at least 1 week before participating in the study.

These subjects were divided into six groups according to the ABCG2 c.34G>A(421C>A) haplotypes as indicated in Tables 2 and 3. All subjects have a SLCO1B1 c.521TT genotype and a CYP2C9 wild type. After an overnight fast, they received a single oral dose of 10 mg rosuvastatin calcium tablet (AstraZeneca, Wuxi, Jiangsu, China) taken with 200 ml of water. Meals were allowed 6 hours later after rosuvastatin administration. Venous blood was collected before rosuvastatin administration and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 hour intervals afterwards. The plasma was separated by centrifugation and stored in polypropylene tubes at -40°C until analysis.

This study was approved by the Ethics Committee of Xiangya Medical College, Central South University (Changsha, China), and registered in the Chinese Clinical Trial Registry (ChiCTR-RCH-12002706). Written informed consent was obtained from each participant. This study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

3. The determination of plasma rosuvastatin concentration and pharmacokinetic analysis
The plasma concentration of rosuvastatin was determined using the API4000 HPLC-MS/MS system with an electrospray ionization (ESI) source system and Analyte version 1.4 software. A Hypurity C18 column (Thermo Fisher Scientific Inc. Waltham, MA, USA) and a mobile phase consisting of 2 mM ammonium acetate in 0.2% formic acid and acetonitrile (1:1, v/v) at a flow rate of 0.3 ml/min were applied. A plasma sample of 450 μL was mixed with 50 μL of 57.78 ng/ml atorvastatin (I.S. Toronto Research Chemicals, North York, Canada), 50 μL of 10% acetic acid and 2 mL of diethyl ether, vortexed for 10 min, and then centrifuged at 4,000 rpm for 10 min. The supernatant was removed and evaporated to dryness at 42°C under a gentle stream of nitrogen. The residue was reconstituted in 150 μL of the mobile phase, and 10 μL of the sample was injected into the LC–MS/MS system for analysis. The mass spectrometer was operated in an ESI positive ion mode. The multiple reaction monitoring (MRM) transitions were performed at m/z 482.1 to 258.1 for rosuvastatin and m/z 559.4 to 440.3 for atorvastatin. The limit of quantification was 0.369 ng/ml for rosuvastatin and the linear range of the assay was 0.369-88.5 ng/ml. Inter-day accuracies for rosuvastatin were within 85~115 % and total imprecision was <15%.

Pharmacokinetic analysis of rosuvastatin was performed using WinNonlin version 4.1 program (Pharsight Corporation, Mountain View, California). C_{max} and T_{max} were determined directly from the plasma concentration–time data, and the half-life (T_{1/2}) was calculated from the slope obtained by log-linear regression of the terminal plasma concentration–time data. AUC was determined by the linear trapezoidal method. The apparent oral clearance (CL/F) was calculated as follows: CL/F = dose (10 mg) /AUC_{(0-∞)} (ng·h/ml).
4. Statistical analysis

Pharmacokinetic data is expressed as mean ± SD in Table 3 and mean ± SEM in Figures. Genetic equilibrium was tested according to Hardy-Weinberg formula using the chi-squared test. Statistical analysis of pharmacokinetic data was carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL). Differences among multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Student-Newman-Keuls test. A p value less than 0.05 was considered statistically significant.
Results

1. Frequency of the ABCG2 c.421C>A and c.34G>A polymorphisms in the Chinese population

The ABCG2 c.421C>A and c.34G>A polymorphisms were simultaneously determined in 800 healthy Chinese men by PCR-Pyrosequencing. As shown in Table 2, the allele frequencies of c.34G>A and c.421C>A were 0.275 and 0.282 in this population, respectively. The allele frequency of c.421C>A in this population met the Hardy–Weinberg equilibrium ($\chi^2 = 0.714$, $p=0.398$), while c.34G>A was not in Hardy–Weinberg equilibrium ($\chi^2 = 51.58$, $p<0.001$). The 34GG and 34AA homozygotes at c.34G>A accounted for 57.6% and 12.6% of allele frequency, and the 421CC and 421AA at c.421C>A for 50.9% and 7.4% of allele frequency, respectively. Six haplotypes were detected and their frequencies are presented in Table 2. Subjects with c.[34GG;421CC] haplotype, which is considered the reference wild-type, accounted for 31.7% of this population. The c.[34AA;421CC] and c.[34GG;421AA] haplotypes were 12.6% and 7.4%, respectively. Interestingly, a very high percentage (23.3%) of compound heterozygotes of c.[34GA;421CA] variants was determined in this population.

The frequency of the SLCO1B1 c.521T>C SNP was also determined in this population, and the frequencies of 521TT, 521TC and 521CC genotype were 80%, 19% and 1%, respectively, which was in Hardy-Weinberg equilibrium ($\chi^2 = 0.095$, $p=0.758$).

2. Effect of ABCG2 421C>A and 34G>A polymorphisms on rosuvastatin pharmacokinetics

Sixty-two subjects selected from 800 healthy Chinese volunteers participated in the study of
rosuvastatin pharmacokinetics. All 62 subjects had a SLCO1B1 c.521TT genotype and a CYP2C9 wild type. Of the 62 individuals, 10 subjects had a haplotype of c.[34GG;421CC], 9 had a haplotype of c.[34GA;421CC], 9 had a haplotype of c.[34AA;421CC], 8 had a haplotype of c.[34GG;421CA], 10 had a haplotype of c.[34GG;421AA] and 16 had a haplotype of c.[34GA;421CA].

Considering the c.421C>A SNP in subjects with an ABCG2 c.34GG homozygotes, subjects that were c.421AA homozygotes had a 227% and 200% higher mean $C_{\text{max}}$ of rosuvastatin than those with c.421CC and c.421CA genotypes (p<0.001) as shown in Table 3 and Figure 1. The mean AUC$_{(0-\infty)}$ of rosuvastatin was 164% and 154% greater and the mean AUC$_{(0-48h)}$ was 164% and 155% greater in the c.421AA homozygotes than those with the c.421CC and c.421CA genotypes (p<0.001). In contrast, the mean CL/F value in c.421AA homozygotes was 63% and 64% lower (p<0.001) than those in subjects with c.421CC and c.421CA genotypes (Table 3). However, there were no statistically significant differences in AUC$_{(0-\infty)}$, AUC$_{(0-48h)}$, and $C_{\text{max}}$ between c.421CC homozygotes and c.421CA heterozygotes or in T$_{1/2}$ and T$_{\text{max}}$ of rosuvastatin among the six studied haplotypes (Table 3).

Like the c.421C>A SNP, the c.34G>A SNP in the c.421CC homozygous background also had a significant influence on rosuvastatin pharmacokinetics as shown in Table 3 and Figure 2. Subjects with the c.34AA genotype had a significant higher $C_{\text{max}}$, AUC$_{(0-\infty)}$ and AUC$_{(0-48h)}$ with a lower CL/F than those with the c.34GG and c.34GA genotypes (p<0.01). However, the alteration in rosuvastatin pharmacokinetics in subjects with c.34AA homozygote was significantly less compared to those with c.421AA homozygotes (see Table 3 and Figure 3). The AUC$_{(0-\infty)}$, AUC$_{(0-48h)}$ and $C_{\text{max}}$ of rosuvastatin was 64%, 64% and 57% greater, while the CL/F was 44% lower in
subjects with c.421AA homozygotes than in those with c.34AA homozygotes (p<0.05). The $C_{max}$, $AUC_{(0-\infty)}$ and $AUC_{(0-48h)}$ were not statistically different between c.34GG homozygotes and c.34GA heterozygotes.

Although neither c.421CA nor c.34GA heterozygotes had significant influence on rosuvastatin pharmacokinetics, the $AUC_{0-\infty}$, $AUC_{0-48h}$ and $C_{max}$ of rosuvastatin in the compound c.[34GA;421CA] heterozygotes were 69%, 67% and 74% higher, while the CL/F was 40% lower than those with c.[34GG;421CC] wild-type reference group (p<0.01) (Table 3 and Figure 3). However, there were no statistically significant differences in $T_{1/2}$ and $T_{max}$ between these two groups.
Discussion

Our present study has analyzed the frequency of ABCG2 c.34G>A and c.421C>A variants and their influence on rosuvastatin pharmacokinetics in a large population of healthy Chinese males. For the first time, we have demonstrated that both ABCG2 c.34G>A and c.421C>A variants play a significant role in rosuvastatin pharmacokinetics in humans. In agreement with previous studies (Zhang et al., 2006; Keskitalo et al., 2009a; Lee et al., 2013), the ABCG2 c.421C>A variant has a significant influence on rosuvastatin pharmacokinetics. The plasma C<sub>max</sub> and AUCs of rosuvastatin in c.421AA homozygotes were about 200% greater than those with c.421CC homozygotes (see Table 3), similar to previous reports (Zhang et al., 2006; Keskitalo et al., 2009a; Lee et al., 2013). Moreover, in c.34AA homozygotes, the plasma C<sub>max</sub> and AUCs of rosuvastatin were significantly higher than those with c.34GG homozygotes, indicating that the c.34G>A variant also plays a significant role in rosuvastatin pharmacokinetics. Recently, Kim et al (Kim et al., 2015) have reported that the ABCG2 c.421C>A variant significantly influenced the pharmacokinetics of bicalutamide in a gene dose-dependent manner, but c.34G>A did not have such an effect in healthy Korean male subjects. It is unclear whether the lack of change in bicalutamide pharmacokinetics in subjects with c.34AA genotype is due to the small sample size of the study or a drug specific action of ABCG2 genetic variants. The reason remains to be elucidated.

Most importantly, we demonstrated in the present study that the ABCG2 c.34G>A and c.421C>A compound heterozygotes, but not the heterozygotes of either c.34GA or c.421CA, had a significant impact on rosuvastatin disposition resulting in elevated plasma C<sub>max</sub> and AUCs (see Table 3). This demonstration in compound heterozygotes may explain, at least in part, the
inconsistent reports on rosvastatin pharmacokinetics in c.421CA heterozygotes. Zhang et al (Zhang et al., 2006) and Lee et al (Lee et al., 2013) reported significant differences in plasma C<sub>max</sub> and AUCs of rosvastatin in the c.421CA heterozygotes when compared to c.421CC homozygotes of healthy Chinese males. These changes were not observed in the c.421CA heterozygous Caucasians (Keskitalo et al., 2009a). This discrepancy is most likely due to the high frequency of compound heterozygotes in Chinese populations, accounting for over 55% of c.421CA heterozygotes (see Table 2). In contrast, both the c.34A and c.421A alleles are quite low in Caucasian populations (Kim et al., 2010). Taken together, this data indicates that genetic variants of ABCG2 efflux transporter play a significant role in the disposition of rosvastatin in humans.

Changes in rosvastatin pharmacokinetics could subsequently result in a significant impact on rosvastatin efficacy and toxicity. Tomlinson et al have found that in Chinese patients with hypercholesterolemia, subjects with the c.421AA variant had a significantly greater reduction in LDL-C level than those with c.421CC variant after a daily 10 mg rosvastatin regimen, and this reduction was equivalent to the effect obtained by doubling the dose of rosvastatin (Tomlinson et al., 2010). Lee et al have reported in Chinese patients with hypercholesterolemia that subjects with the ABCG2 c.421AA genotype had a higher plasma concentration of rosvastatin and a greater reduction in low-density lipoprotein cholesterol than those with the c.421CC genotype when they were treated with the same rosvastatin regimen (Lee et al., 2013). Furthermore, the FDA has warned of a higher incidence of rosvastatin toxicity in Asian subjects compared to Caucasians when they used the same rosvastatin regimen (US Food and Drug Administration: FDA Provides Updated Patient and Healthcare Provider Information Concerning Crestor. 2005). It is therefore
imperative to personalize rosuvastatin treatment in patients, especially in Asians because of the high frequency of ABCG2 c.34A and c.421A alleles.

The elevated plasma concentration of rosuvastatin in c.421AA homozygotes is consistent with previous demonstrations of a lower expression level of the ABCG2 efflux transporter protein and a reduced ability to export substrate for the c.421AA variant. This leads to increased drug absorption in the gastrointestinal track, and drug accumulation in hepatocytes and systemic circulation (Kondo et al., 2004; Urquhart et al., 2008; Morisaki et al., 2005). On the other hand, the ABCG2 c.34AA variant has been shown to possess decreased efflux activity due to disturbed protein membrane localization, which could also result in increased drug absorption in the gastrointestinal track and drug accumulation in systemic circulation (Mizuarai et al., 2004). However, the molecular mechanism of how the compound c.34GA/421CA heterozygote affects rosuvastatin pharmacokinetics remains to be investigated.

The disposition of rosuvastatin may also be influenced by other genetic variations in addition to ABCG2 variants. It has been shown that the SLCO1B1 c.521T>C variant was able to alter rosuvastatin disposition in humans (Bolego et al., 2002; Pasanen et al., 2007). To minimize the influence of SLCO1B1 c.521T>C in rosuvastatin pharmacokinetics, we genotyped the SLCO1B1 c.521T>C variant in subjects and only those subjects with SLCO1B1 c.521TT genotype were included in the study. Furthermore, since approximately 10% of rosuvastatin is metabolized by CYP2C9 (Bolego et al., 2002), we genotyped CYP2C9*3 in the volunteers and confirmed all 62 participants possessed CYP2C9 wild-type genotype (data not shown). Although it has been shown that rosuvastatin is a substrate of ABCB1 and ABCC2 transporters, pharmacokinetic analysis indicates that the genetic variations of ABCB1 and ABCC2 had no
significant impact on rosuvastatin disposition in humans (Keskitalo et al., 2009b; Kitamura et al., 2008). Taken together, this data suggests that the observed variation in rosuvastatin disposition is mainly due to genetic variants of the ABCG2 gene.

Finally, to the best of our knowledge, the present study is the largest study of the frequency of ABCG2 c.34G>A and c.421C>A variants in healthy Asian males with a sample size of 800. In agreement with previous studies (Kim et al., 2010), we confirmed that the Chinese population, like other Asian populations, has the highest incidence of ABCG2 c.34G>A and c.421C>A variants with a allele A frequency of 0.275 and 0.282, respectively (see Table 2), compared to a frequency of 0.175 and 0.103, and 0.10 and 0.023 in Caucasian and African-American populations (Kim et al., 2010). It is obvious that this high allele frequency is an important factor to be considered in the administration of rosuvastatin in Asian populations. Additionally, a deviation of the Hardy-Weinberg equilibrium for the c.34G>A, but not c.421C>A variant was observed in this population. Although disequilibrium may arise from inbreeding, we are not aware of any consanguinity within the population as we recruited the subjects from a large urban area. As previously suggested, a deviation could be a consequence of the recruiting strategy despite the fact that the other tested polymorphism did not deviate from Hardy-Weinberg equilibrium (Kim et al., 2010; Böger et al., 2005; Böger et al., 2007). A potential explanation is a sampling bias since all subjects were male. Further study is necessary to confirm this hypothesis.

**Conclusion**

We have demonstrated that both ABCG2 c.34G>A and c.421C>A variants play a significant role in rosuvastatin pharmacokinetics, almost doubling the $C_{\text{max}}$ and AUCs of rosuvastatin in
c.34AA and c.421AA homozygotes and in c.34GA/c.421CA compound heterozygotes compared to the reference wild-type group. Considering the high allele A frequencies of c.34G>A and c.421C>A variants in Chinese and other Asian populations, it is advisable to personalize the dosage of rosuvastatin in the treatment of hypercholesterolemia through genotyping genetic variations, such as ABCG2, in the patients.
Acknowledgment

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Authorship Contribution

Participated in study design: Wang, Zhu

Conducted experiments: Wan, Wang, Li, Xu, Pei, Peng, Sun, Cheng, Zeng

Contributed new reagents or analysis tool: Pei, Yang

Performed data analysis: Wan, Wang, Zhu

Wrote or contributed to the writing of the manuscript: Wan, Wang, Zhu
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Urquhart BL, Ware JA, Tirona RG, Ho RH, Leake BF, Schwarz UI, Zaher H, Palandra J, Gregor JC, Dresser GK, and Kim RB (2008) Breast cancer resistance protein (ABCG2) and drug

US Food and Drug Administration: FDA Provides Updated Patient and Healthcare Provider Information Concerning Crestor. Media release 2 March 2005

US Food and Drug Administration: FDA Public Health Advisory on Crestor (rosuvastatin). Media release 2 March 2005


Footnote

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The authors declare no conflicts of interest.

Z.W. and G.W. contributed equally to this work.
Legends for Figures

Figure 1. The time-concentration curve of rosuvastatin in healthy Chinese subjects with the ABCG2 c.421C>A SNP after a single oral dose of 10 mg rosuvastatin. Open circles indicate subjects with the c.421CC (n=10); open up-triangles, those with the c.421CA heterozygotes (n=8); and open diamonds, those with the c.421AA homozygotes (n=10).

Figure 2. The time-concentration curve of rosuvastatin in healthy Chinese subjects with the ABCG2 c.34G>A SNP after a single oral dose of 10 mg rosuvastatin. Open circles indicate subjects with the c.34GG homozygotes (n=10); open squares, those with the c.34GA heterozygotes (n=9); and open down-triangles, those with the c.34AA homozygotes (n=9).

Figure 3. The time-concentration curve of rosuvastatin between subjects with ABCG2 34/421 compound haplotypes after a single oral dose of 10 mg rosuvastatin. Open circles indicate subjects with the c.421CC/34GG homozygotes (n=10); open diamonds, those with the c.421AA/34GG homozygotes (n=10); open down-triangles, those with the c.421CC/34AA homozygotes (n=9); and semi-filled squares, those with the c.421CA/34GA compound heterozygotes (n=16).
Table 1. Primers Used in PCR-Pyrosequencing Analysis

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<th>Name of Primer</th>
<th>Primer Sequences (5’-3’ )</th>
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<tr>
<td>SLCO1B1 521-F</td>
<td>GTTGGTTAAAGGAATCTGGGTCAT (5’-Biotin labeled)</td>
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<tr>
<td>SLCO1B1 521-R</td>
<td>AGCGAAATCATCAATGTAAGAAAG</td>
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<tr>
<td>SLCO1B1 521-S</td>
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<td>ATGTCGAAAGTHTHTATCC</td>
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Table 2. Frequency of the ABCG2 c.34G>A and c.421C>A Variants in 800 Healthy Chinese Men

<table>
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<th>SNPs</th>
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<td>452</td>
<td>28.2%</td>
</tr>
<tr>
<td>34/421 Haplotype</td>
<td>GG/CC</td>
<td>254</td>
<td>31.7%</td>
</tr>
<tr>
<td></td>
<td>AA/CC</td>
<td>101</td>
<td>12.6%</td>
</tr>
<tr>
<td></td>
<td>GA/CC</td>
<td>52</td>
<td>6.5%</td>
</tr>
<tr>
<td></td>
<td>GG/AA</td>
<td>59</td>
<td>7.4%</td>
</tr>
<tr>
<td></td>
<td>GG/CA</td>
<td>148</td>
<td>18.5%</td>
</tr>
<tr>
<td></td>
<td>GA/CA</td>
<td>186</td>
<td>23.3%</td>
</tr>
<tr>
<td></td>
<td>GA/AA</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>AA/AA</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>AA/CA</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>
Table 3. Pharmacokinetic Variables of a Single Dose of 10 mg Rosuvastatin in 62 Healthy Chinese Subjects with ABCG2 c.34G>A and c.421C>A Variants

<table>
<thead>
<tr>
<th>Haplotype Variables</th>
<th>34GG/421CC (n=10)</th>
<th>34GA/421CC (n=9)</th>
<th>34AA/421CC (n=9)</th>
<th>34GG/421AA (n=10)</th>
<th>34GG/421CA (n=8)</th>
<th>34GA/421CA (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>8.1±1.8</td>
<td>8.1±1.3</td>
<td>16.9±6.0&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>26.5±12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.8±4.2</td>
<td>14.1±5.2&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-48h)&lt;/sub&gt; (ng·h/ml)</td>
<td>95.5±26.2</td>
<td>81.6±15.9</td>
<td>153.6±57.1&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>252.2±66.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.8±42.8</td>
<td>159.8±57.4&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-∞)&lt;/sub&gt; (ng·h/ml)</td>
<td>97.7±26.9</td>
<td>84.2±18.5</td>
<td>157.9±58.4&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>258.4±66.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.4±42.9</td>
<td>165.0±58.7&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>111.1±39.1</td>
<td>125.4±30.9</td>
<td>74.1±34.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.4±12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114.7±47.8</td>
<td>66.9±20.0&lt;sup&gt;i,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>12.7±2.1</td>
<td>14.3±3.0</td>
<td>13.6±2.4</td>
<td>13.2±3.1</td>
<td>14.3±1.9</td>
<td>14.4±2.5</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>3.8±2.4</td>
<td>3.4±1.3</td>
<td>2.4±1.0</td>
<td>3.6±1.6</td>
<td>3.5±1.8</td>
<td>3.4±1.4</td>
</tr>
</tbody>
</table>

Notes: a: p<0.01, and b: p<0.001 compared to the 34GG/421CC, 34GA/421CC and 34GG/421CA groups; and c: p<0.05 compared to the 34GG/421AA group (Student-Newman-Keuls test).