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ARTICLE

Effects of *SLCO1B1* and *GATM* gene variants on rosuvastatin-induced myopathy are unrelated to high plasma exposure of rosuvastatin and its metabolites

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Myotoxicity is a significant factor contributing to the poor adherence and reduced effectiveness in the treatment of statins. Genetic variations and high drug plasma exposure are considered as critique causes for statin-induced myopathy (SIM). This study aims to explore the sequential influences of rosuvastatin (RST) pharmacokinetic and myopathy-related single-nucleotide polymorphisms (SNPs) on the plasma exposure to RST and its metabolites: rosuvastatin lactone (RSTL) and *N*-desmethyl rosuvastatin (DM-RST), and further on RST-induced myopathy. A total of 758 Chinese patients with coronary artery disease were enrolled and followed up SIM incidents for 2 years. The plasma concentrations of RST and its metabolites were determined through a validated ultra-performance liquid chromatography mass spectrometry method. Nine SNPs in six genes were genotyped by using the Sequenom MassArray iPlex platform. Results revealed that *ABCG2* rs2231142 variations were highly associated with the plasma concentrations of RST, RSTL, and DM-RST ($P_{adj} < 0.01$, FDR < 0.05). *CYP2C9* rs1057910 significantly affected the DM-RST concentration ($P_{adj} < 0.01$, FDR < 0.05). *SLCO1B1* rs4149056 variant allele was significantly associated with high SIM risk (OR: 1.741, 95% CI: 1.180–2.568, $P = 0.0052$, FDR = 0.0468). Glycine amidinotransferase (*GATM*) rs9806699 was marginally associated with SIM incidents (OR: 0.617, 95% CI: 0.406–0.939, $P = 0.0240$, FDR = 0.0960). The plasma concentrations of RST and its metabolites were not significantly different between the SIM ($n = 51$) and control groups ($n = 707$) (all $P > 0.05$). In conclusion, *SLCO1B1* and *GATM* genetic variants are potential biomarkers for predicting RST-induced myopathy, and their effects on SIM are unrelated to the high plasma exposure of RST and its metabolites.

Keywords: genetic polymorphism; rosuvastatin; metabolites; plasma concentration; myopathy

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INTRODUCTION

As a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, rosuvastatin (RST) is one of the most globally popular cholesterol-reducing drugs [1]. RST can decrease the risk of cardiovascular disease during primary and secondary prevention [2]. However, RST induces a class-wide adverse myotoxicity effect, which has become the leading factor for treatment discontinuation, switching, or non-adherence [3–5]. High plasma exposure of statin or its metabolites and genetic factor play crucial roles in statin-induced myopathy (SIM) [6–8].

Patients with SIM presented significantly higher circulation exposure to atorvastatin metabolites than patients without SIM [8]. Atorvastatin lactone is one of metabolites, which has potential toxicity for skeletal muscle cells in vitro [9]. RST-5S lactone (RSTL) and N-desmethyl RST (DM-RST) are two known metabolites, and DM-RST is biotransformed by the isoenzyme CYP2C9 [10]. The effects of these two metabolites and *CYP2C9* gene on RST-induced myopathy remain unclear.

Transporters play an important role in the disposition of RST owing to its low passive membrane permeability [11]. Many studies have revealed that *SLCO1B1* 521T>C (rs4149056) contributed to SIM [12–14]. This variant allele is also associated with the increased plasma exposure of statins [15, 16], which is considered a causative factor of SIM [7, 16, 17]. However, most studies focused on simvastatin, atorvastatin, or a combination of statins, and only few were specifically conducted for RST. Compared with that of *SLCO1B1* 521T>C, the variation of *ABCG2* is a potentially crucial heredity factor in the pharmacokinetic of RST for East Asians because of the differences in drug property and patients' race [18–20]. Therefore, further investigation is needed to confirm whether or not the genic variations of

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SLCO1B1, ABCG2, or both influence the RST-induced myotopathy in Chinese people.

Glycine amidinotransferase (*GATM*) gene is functionally correlated with SIM [21]. *GATM* G>A (rs9806699) was discovered by a genome-wide association study as a possible genetic marker for the decreased risk of SIM [21]. However, this single-nucleotide polymorphism (SNP) failed to replicate this association in an independent sample and a case–control study [22, 23]. Nonetheless, these studies were performed on Caucasians who received statins. The influence of this locus on SIM in Asians has not been reported.

In the present study, we assessed the effects of pharmacokinetic-related and *GATM* SNPs on the systemic exposure to RST and its metabolites, and subsequently on the RST-related myopathy in Chinese people receiving RST therapy.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Medical Ethical Review Committee of Guangdong General Hospital and conducted in accordance with the Declaration of Helsinki. Informed consents were obtained from all participants in this study.

Study design

Nine polymorphisms were selected from five candidate genes that were potentially associated with the pharmacokinetic of RST and a functional SNP associated with SIM. These SNPs were: *ABCG2* 421C>A (rs2231142), *ABCG2* rs2199936, *ABCB1* rs1045642, *SLCO1B1* 521T>C (rs4149056), *SLCO1B1* 388A>G (rs2306283), *SLCO1B1* rs4363657, *SLCO1B3* rs7311358, *CYP2C9* rs1057910, and *GATM* rs9806699.

As an index of RST and its metabolites, the Css/D (dose-adjusted steady-state plasma concentrations) was used as a dependent variable. This ratio was defined as the drug concentration per 10 mg daily dosage (ng/mL per 10 mg).

Patients

Chinese Han patients with coronary artery disease who have ingested RST for more than 1 week were prospectively recruited between January 2010 and December 2013 from Guangdong General Hospital. Patients' baseline information, including demographics, medical history, biochemical measurements, and medication was obtained from the database of the hospital.

The exclusion criteria were as follows: (1) pretreatment with other statins in 2 weeks; (2) age ≥80 years; (3) renal insufficiency (defined as serum CREA concentration is thrice greater than the normal upper limit (345 μmol/L), renal transplantation, or dialysis); (4) liver insufficiency (defined as serum alanine aminotransferase (ALT) concentration is thrice greater the normal upper limit (120 U/L), or a cirrhosis diagnosis); (5) advanced cancer.

Blood samples were obtained from each eligible patient in the morning at 10–12 h after taking RST and collected in EDTA-coated tubes. The plasma and the blood cells were separated in 2 h by centrifugation at 3000 r/min for 10 min at 4 °C and then stored at −80 °C until usage.

Endpoints

Follow-up information was collected through inpatient and outpatient hospital visits and telephone contacts with the patients until December 2015. During each follow-up assessment (every 6 months), the participants were questioned about any uncomfortable muscle symptoms, including myalgia, weakness, stiffness, spasms, or twitches; their RST medication; and possible interference factors of SIM assessment. These data were recorded for all of the enrolled patients.

SIM was defined based on the patients' subjective sense of muscular pain and creatine kinase (CK) elevation. The considered musculoskeletal effects were new and inexplicable muscle-related symptoms, including myalgia, weakness, stiffness, spasms, or twitches (irrespective of CK values), rhabdomyolysis, and inexplicable CK elevations of over four times than normal upper limit (irrespective of symptoms) [24, 25].

Among the 975 patients, the following were excluded: 51 patients who were older than 80 years, 10 patients who experienced renal insufficiency, 18 patients who had liver insufficiency, 41 patients whose plasma concentrations were lower than the limit of detection, and 97 patients who provided incomplete information on SIM events during follow up. Finally, 758 eligible patients were included in the analysis.

Determination of RST and its metabolites concentrations in plasma

A sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay was developed and validated for the simultaneous quantification of RST, RSTL, and DM-RST in human plasma. Liquid–liquid extraction by using ethyl acetate was adopted to extract three analytes and the internal standard (carbamazepine) from 200 μL buffered plasma (adding 100 μL ammonium acetate of pH = 4.0–100 μL human plasma). The analytes were chromagraphically separated by an Acquity UPLC HSS T3 column (3.0 mm × 100 mm, 1.8 μm) with 0.1% formic acid and a gradient of 30%–85% acetonitrile at a flow rate of 0.30 mL/min. Mass detection was performed with a Waters Xevo TQ-S triple–quadrupole mass spectrometer under positive electrospray ionization mode. The responses of RST, RSTL, and DM-RST were optimized at m/z 482.1 → 258.1, m/z 464.1 → 270.1, m/z 468.0 → 258.0, respectively.

Genotyping

DNA was extracted by using a DNA automatic extractor (TGuide M16 Systems, Tiangen, China). The quality control of the DNA was based on the absorbance ratio (A_{260}/A_{280}: 1.8–2.0) and the concentrations were determined by using NanoDrop 2000. Nine SNPs were genotyped by using the Sequenom MassArray technology platform (Sequenom, CA, USA).

Statistical analysis

All data analyses were performed by using SAS 9.4 (SAS Institute, Cary, NC, USA). Categorical data were presented as percentages, and continuous variables were expressed as mean ± SD. The concentrations of RST, RSTL, DM-RST, serum ALT, aspartate aminotransferas (AST), triglyceride, and lipoprotein(a) levels were logarithmically transformed to normalize their distributions. Haploview 4.2 was used to determine the deviation from the Hardy–Weinberg equilibrium. Linkage disequilibrium analysis was performed online by using SHEsis (http://analysis.bio-x.cn). Linear regression analysis was adopted to evaluate the influences of the genotype and clinical baseline characteristics on the plasma exposure of RST and its two metabolites. Logistic regression analysis was used to assess the effects of the baseline features and genotypes on SIM incidents. P values <0.05 were considered statistically significant. The FDR was controlled by using the SAS PROC MULTTEST with the FDR option to correct multiple comparisons in the genetic factors analysis. P values of <0.05 were considered statistically significant, whereas FDR was controlled at 0.05.

RESULTS

Effects of genotypes on the plasma exposure of RST and its metabolites

First, we analyzed the clinical baseline characteristics and their influences on the Css/D of RST and its metabolites. Univariate linear regression analysis revealed the following: (1) plasma AST levels and combination with angiotensin-converting enzyme

Table 1. Genotypes and their effects on the plasma exposure of RST and its metabolites

Gene	SNP	Genotype	*n* (%)	Plasma RST concentration (ng/mL per 10 mg)				Plasma RSTL concentration (ng/mL per 10 mg)				Plasma DM-RST concentration (ng/mL per 10 mg)			
				Concentration	*P* value	*P* value[a]	FDR	Concentration	*P* value	*P* value[b]	FDR	Concentration	*P* value	*P* value[c]	FDR
ABCG2	rs2231142	CC	359 (47.55)	2.78 ± 3.59	1.0×10^{-4}	1.2×10^{-4}	0.0010	0.38 ± 0.49	1.3×10^{-5}	1.0×10^{-5}	<0.0001	0.38 ± 0.63	4.0×10^{-6}	6.0×10^{-6}	<0.0001
		AC	324 (42.91)	3.89 ± 4.03				0.50 ± 0.51				0.52 ± 0.67			
		AA	72 (9.54)	4.73 ± 3.88				0.67 ± 0.64				0.62 ± 0.63			
ABCB1	rs1045642	AA	108 (14.30)	2.89 ± 2.98	0.1959	0.1471	0.2354	0.40 ± 0.42	0.8348	0.7974	0.8399	0.36 ± 0.37	0.3702	0.5224	0.8536
		AG	363 (48.08)	3.78 ± 4.26				0.48 ± 0.58				0.52 ± 0.77			
		GG	284 (37.62)	3.22 ± 3.58				0.44 ± 0.48				0.43 ± 0.57			
SLCO1B1	rs4149056	TT	568 (75.53)	3.21 ± 3.77	0.0184	0.0247	0.0988	0.45 ± 0.53	0.3891	0.5621	0.8253	0.45 ± 0.58	0.4932	0.5763	0.8536
		CT	130 (17.29)	4.45 ± 4.39				0.48 ± 0.51				0.55 ± 0.94			
		CC	54 (7.18)	3.59 ± 3.92				0.46 ± 0.44				0.42 ± 0.56			
	rs2306283	AA	46 (6.07)	3.71 ± 4.16	0.8213	0.8517	0.8517	0.70 ± 0.97	0.2268	0.2436	0.6496	0.54 ± 0.75	0.7242	0.8617	0.8617
		AG	262 (34.89)	3.64 ± 4.06				0.47 ± 0.49				0.52 ± 0.71			
		GG	444 (59.03)	3.57 ± 3.66				0.46 ± 0.47				0.47 ± 0.65			
	rs4363657	TT	214 (28.46)	3.53 ± 4.14	0.3482	0.3386	0.3870	0.52 ± 0.64	0.3985	0.4838	0.8253	0.51 ± 0.73	0.7104	0.6678	0.8536
		TC	358 (47.59)	3.52 ± 3.83				0.47 ± 0.47				0.49 ± 0.61			
		CC	180 (23.95)	3.78 ± 3.44				0.45 ± 0.47				0.45 ± 0.74			
SLCO1B3	rs7311358	GG	68 (9.09)	4.12 ± 3.95	0.0729	0.0713	0.1426	0.52 ± 0.57	0.7608	0.8399	0.8399	0.63 ± 0.81	0.6945	0.7469	0.8536
		AG	299 (39.97)	3.69 ± 3.99				0.46 ± 0.52				0.47 ± 0.76			
		AA	381 (50.94)	3.46 ± 3.69				0.49 ± 0.52				0.48 ± 0.57			
CYP2C9	rs1057910	AA	706 (93.51)	3.48 ± 3.92	0.2865	0.2225	0.2967	0.46 ± 0.53	0.7224	0.6190	0.8253	0.47 ± 0.67	9.0×10^{-4}	9.0×10^{-4}	0.0036
		CA + CC	49 (6.49)	2.79 ± 2.82				0.40 ± 0.44				0.29 ± 0.39			
GATM	rs9806699	GG	71 (9.39)	3.78 ± 4.23	0.1302	0.0652	0.1426	0.54 ± 0.62	0.2075	0.1512	0.6048	0.48 ± 0.60	0.1797	0.1275	0.3400
		AG	298 (39.42)	3.40 ± 3.79				0.45 ± 0.54				0.48 ± 0.64			
		AA	387 (51.19)	3.74 ± 3.84				0.49 ± 0.49				0.50 ± 0.71			

ACEIs angiotensin-converting enzyme inhibitors, *AST* aspartate aminotransferase, *CREA* creatinine, *DM-RST* N-desmethyl rosuvastatin, *GATM* glycine amidinotransferase, *RST* rosuvastatin, *RSTL* rosuvastatin lactone, *SNPs* polymorphisms

[a]*P* value was adjusted by lnAST levels and conmination of ACEIs

[b]*P* value was adjusted by CREA levels

[c]*P* value was adjusted by age and heart failure

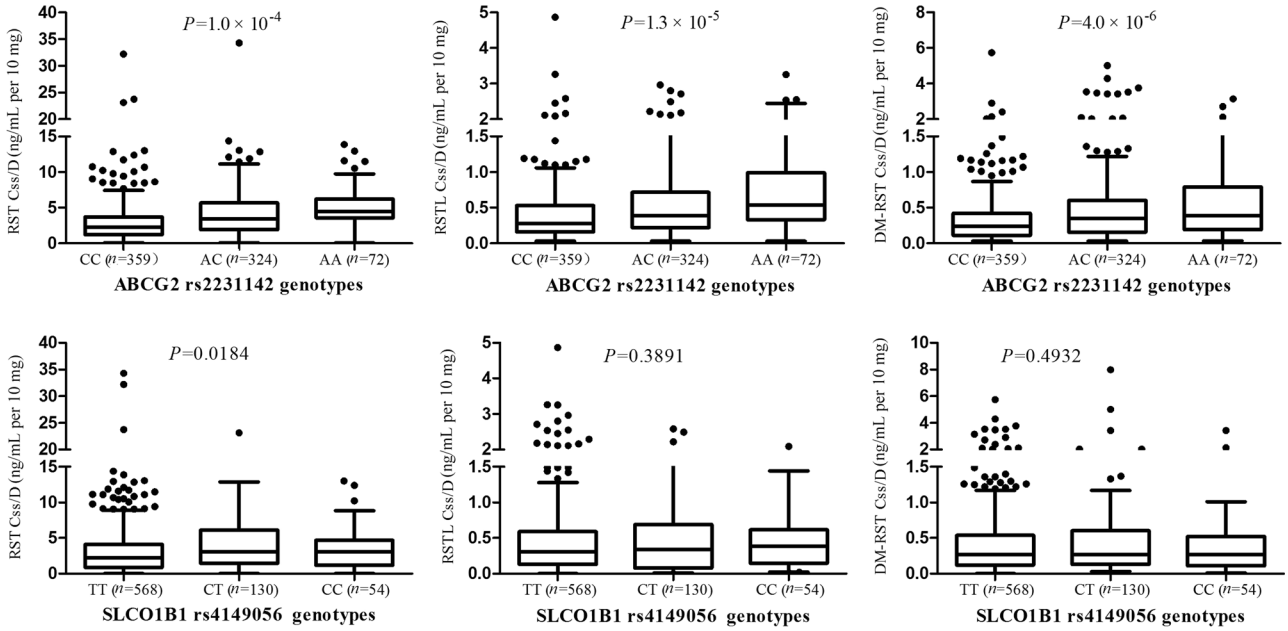

Fig. 1 Effects of *ABCG2* rs2231142 and *SLCO1B1* rs4149056 genetic polymorphisms on the plasma exposure to RST, RSTL, and DM-RST

inhibitors were associated with the Css/D of RST; (2) a high creatinine (CREA) level was correlated with a higher exposure to RSTL; and (3) age and heart failure could significantly affect the Css/D of DM-RST (Table S1). To exclude the effects of these clinical baseline features on the Css/D of RST, RSTL, and DM-RST, we used the factors that significantly influenced the drug concentrations in the univariate linear regression analysis as the covariates in the following analysis.

All allelic distributions in our study conformed to the Hardy–Weinberg equilibrium ($P > 0.05$). All of the allelic frequencies were close to the reference data of 1000G population (http://www.ncbi.nlm.nih.gov/snp/). *ABCG2* rs2199936 was highly linked with rs2231142 ($D' = 0.996$, $r^2 = 0.993$). Then only the locus of *ABCG2* rs2231142 was included in following analysis. The mean Css/D of RST and its metabolites were significantly higher in the subjects carrying the *ABCG2* 421A than in non-carriers of this allele. The effects of this allele remained significant after being adjusted by the baseline characteristics and false discovery rate (FDR) ($P_{adj} < 0.01$, FDR < 0.05). The results are listed in Table 1 and Fig. 1.

SLCO1B1 521T>C (rs4149056) in patients with one or two copies of the variant allele had a significantly high plasma exposure to RST, whereas the significance was not found after multiple testing ($P_{adj} = 0.0247$, FDR = 0.0988), and a gene-dose effect was not observed in the three genotypes of this SNP. In addition, there were no obviously effects on the plasma concentrations of RSTL and DM-RST ($P_{adj} > 0.05$, FDR > 0.05; Table 1, Fig. 1). Plasma exposure to DM-RST of patients with one and two copies of the *CYP2C9* rs1057910 (CA and CC) was significantly low ($P_{adj} = 9.0 \times 10^{-4}$, FDR = 0.0036). However, the difference was not statistically significant in the Css/D of RST and RSTL. The other SNPs induced no significant effects on drug exposure ($P_{adj} > 0.05$, FDR > 0.05; Table 1, Figures S1 and S2).

Effects of plasma exposure to RST and its metabolites on SIM

Among the 758 eligible subjects, 51 patients manifested occurrences of SIM. The incidence of RST-induced myopathy was 6.73%. The clinical baseline characteristics of without SIM (control group, $n = 707$) and with SIM (SIM group, $n = 51$) were summarized in Table 2. Univariate logistic regression analyses revealed no significant differences in terms of age, sex, dosage, medical history, biochemical levels, and drug combinations between the control and SIM groups ($P > 0.05$). The plasma exposure of RST, RSTL, and DM-RST between control and SIM groups were 3.64 ± 4.21 vs 3.54 ± 4.75, 0.48 ± 0.54 vs 0.49 ± 0.53, 0.49 ± 0.86 vs 0.47 ± 0.71, respectively (Fig. 2). The SIM incidents were not significantly associated with the plasma concentrations of RST and its metabolites (all $P > 0.05$).

Effects of genetic variations on RST-induced myopathy

Among the nine SNPs in six genes, we observed two polymorphisms: *SLCO1B1* rs4149056 and *GATM* rs9806699, which were associated with SIM incidents (Table 3). Carriers of the C allele of *SLCO1B1* 521T>C (rs4149056) had a high risk of SIM (odds ratio (OR): 1.741, 95% confidence interval (CI): 1.180–2.568, $P = 0.0052$, FDR = 0.0416). The *GATM* rs9806699 mutant allele indicated a marginally protective effect on SIM (OR: 0.617, 95% CI: 0.406–0.939, $P = 0.024$, FDR = 0.0960). The other polymorphisms, including the SNPs that were significantly relevant to the pharmacokinetics of RST, i.e., *ABCG2* 421C>A (rs2231142) and *CYP2C9* rs1057910, did not induce significant effects on SIM.

Furthermore, we analyzed the myopathy risk of carriers or no-carriers with risk alleles of both *SLCO1B1* rs4149056 (C allele) and *GATM* rs9806699 (G allele). Results showed that SIM events occurred in nine patients among 249 individuals without any above risk allele of SIM. SIM incidence was 3.61%. However, 8 subjects had SIM in 76 patients with both risk alleles (rs4149056 CC or CT and rs9806699 GG or AG). SIM incidence was 10.53% and its risk was significantly higher (OR: 3.137, 95% CI: 1.166–8.441, $P = 0.024$) compared to patients without risk alleles of SIM. Only five patients simultaneously carried CC genotype of rs4149056 and GG genotype of rs9806699. Of them, SIM events occurred in one patient (OR: 6.667, 95% CI: 0.675–65.841, $P = 0.104$). The SIM incidence was 20.0%. No significant difference was observed because of small size sample. Results were also listed in Table 4.

DISCUSSION

Our study revealed that RST-induced myopathy was significantly affected by *SLCO1B1* 521T>C (rs4149056) and marginally affected by *GATM* (rs9806699). However, their influences on SIM were not associated with the high systemic exposure of RST and its metabolites. Our study identified for the first time the relationship

Table 2. Patients' characteristics and their effects on SIM

Characteristics		Control group	SIM group	Univariate logistic regression	
		n (%) or mean ± SD	*n* (%) or mean ± SD	OR (95% CI)	*P* value
Demographic data					
Total no.		707	51		
Age		63.19 ± 10.33	61.40 ± 10.93	0.984 (0.958–1.011)	0.2356
Sex	Female	167 (23.62)	15 (29.41)	0.740 (0.396–1.386)	0.3473
	Male	540 (76.38)	36 (70.59)		
Dosage (mg)	5	13 (1.84)	1 (1.96)	1.011 (0.928–1.103)	0.7955
	10	619 (87.55)	44 (86.27)		
	20	75 (10.61)	6 (11.76)		
Medical history					
Arrhythmia	No	657 (92.93)	49 (96.08)	0.546 (0.129–2.311)	0.4107
	Yes	50 (7.07)	2 (3.92)		
Heart failure	No	654 (92.50)	51 (100)	1.536 (0.344–6.851)	0.5736
	Yes	53 (7.50)	0 (0)		
Hypertension	No	322 (45.54)	24 (47.06)	0.937 (0.530–1.657)	0.8242
	Yes	385 (54.46)	27 (52.94)		
Hyperlipidemia	No	627(88.68)	46 (90.20)	0.860 (0.332–2.228)	0.7561
	Yes	80 (11.32)	5 (9.80)		
Biochemical measurements					
ALT, U/L		32.77 ± 27.72	29.74 ± 18.53	0.995 (0.981–1.009)	0.4669
AST, U/L		32.47 ± 30.03	35.25 ± 38.19	1.075 (0.611–1.893)	0.0636
CREA, μmol/L		88.19 ± 33.26	83.17 ± 34.28	0.994 (0.984–1.005)	0.2991
CK, U/L		133.96 ± 239.30	174.21 ± 371.57	1.000 (1.000–1.001)	0.2977
APOA, g/L		1.08 ± 0.27	1.02 ± 0.21	0.418 (0.119–1.464)	0.1724
CHOL, mmol/L		4.45 ± 1.29	4.61 ± 1.48	1.086 (0.890–1.325)	0.4159
CKMB, U/L		8.03 ± 9/94	8.57 ± 11.29	1.004 (0.980–1.029)	1.0040
HDLC, mmol/L		1.01 ± 0.26	0.98 ± 0.16	0.563 (0.170–1.870)	0.3484
LDLC, mmol/L		2.73 ± 1.04	2.87 ± 1.25	1.125 (0.874–1.446)	0.3604
Lpa, mg/L		274.04 ± 309.39	246.49 ± 219.43	1.000 (0.999–1.001)	0.5173
TRIG, mmol/L		1.58 ± 1.06	1.63 ± 1.02	1.041 (0.805–1.345)	0.7602
Medication					
β-blockers	No	85 (12.02)	7 (13.73)	0.863 (0.377–1.978)	0.1211
	Yes	622 (87.98)	44 (86.27)		
ACEIs	No	294 (41.58)	22 (43.14)	0.934 (0.526–1.659)	0.8164
	Yes	413 (58.42)	29 (56.86)		
CCBs	No	506 (71.57)	40 (78.43)	0.693 (0.349–1.378)	0.2960
	Yes	201 (28.43)	11(21.57)		
PPIs	No	338(47.81)	23 (45.10)	1.105 (0.624–1.956)	0.7313
	Yes	369 (52.19)	28(54.90)		
Clopidogrel	No	13 (1.84)	1 (1.96)	0.941 (0.121–7.337)	0.9535
	Yes	694 (98.16)	50 (98.04)		
Aspirin	No	25 (3.11)	2 (3.92)	0.790 (0.181–3.458)	0.7545
	Yes	682 (96.89)	49 (96.08)		
Plasma concentrations					
RST		3.64 ± 4.21	3.54 ± 4.75	0.900 (0.789–1.027)	0.1177
RSTL		0.48 ± 0.54	0.49 ± 0.53	0.971 (0.799–1.178)	0.7634
DM-RST		0.49 ± 0.86	0.47 ± 0.71	0.889 (0.695–1.136)	0.3469

ALT alanine aminotransferase, *APOA* apolipoprotein a, *CCBs* calcium channel blockers, *CHOL* cholesterol, *CK* creatine kinase, *CKMB* creatine kinase MB, *HDLC* high-density lipoprotein cholesterol, *LDLC* low-density lipoprotein cholesterol, *Lpa* lipoprotein (a), *PPIs* proton pump inhibitors, *TRIG* triglyceride

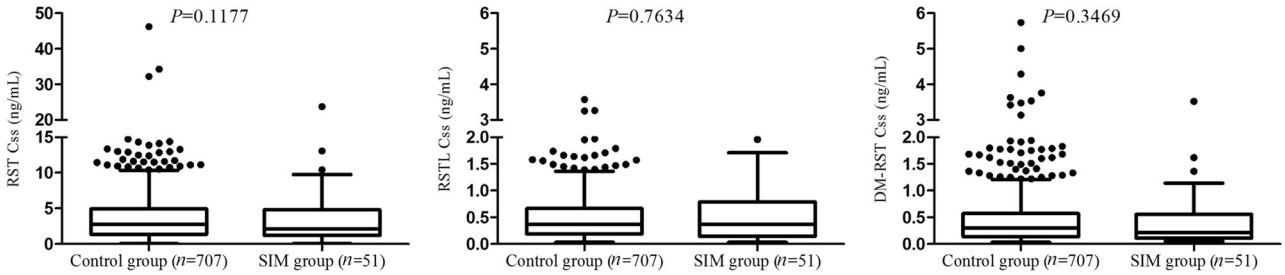

Fig. 2 Comparisons of plasma exposure to RST, RSTL, and DM-RST between control and SIM groups

Table 3. Genotypes and their effects on RST-induced myopathy

Gene	SNP	Genotype	Control group	SIM group	Univariate logistic regression		FDR
			Number of carriers (%)	Number of carriers (%)	OR (95% CI)	*P* value	
ABCG2	rs2231142	CC	329 (46.73)	30 (58.82)	0.707 (0.444–1.127)	0.1447	0.3859
		AC	307 (43.61)	17 (33.33)			
		AA	68 (9.66)	4 (7.84)			
ABCB1	rs1045642	AA	95 (13.53)	11 (21.57)	0.885 (0.585–1.337)	0.5611	0.7459
		AG	343 (48.86)	20 (39.22)			
		GG	264 (37.61)	20(39.22)			
SLCO1B1	rs4149056	TT	537 (76.60)	31 (60.78)	1.741 (1.180–2.568)	0.0052	0.0416
		CT	118 (16.83)	12 (23.53)			
		CC	46 (6.56)	8 (15.69)			
	rs2306283	AA	42 (5.99)	4 (7.84)	1.015 (0.610–1.691)	0.9531	0.9531
		AG	245 (34.95)	17 (33.33)			
		GG	414 (59.06)	30 (58.82)			
	rs4363657	TT	200 (28.53)	14 (27.45)	0.906 (0.590–1.392)	0.6527	0.7459
		TC	331 (47.22)	27 (52.94)			
		CC	170 (24.25)	10 (19.61)			
SLCO1B3	rs7311358	GG	63 (9.04)	5 (9.80)	1.201 (0.733–1.967)	0.4676	0.7459
		AG	282 (40.46)	17 (33.33)			
		AA	352 (50.50)	29 (56.86)			
CYP2C9	rs1057910	AA	657 (93.32)	49 (96.08)	0.581 (0.137–2.466)	0.4617	0.7459
		CA + CC	47 (6.68)	2 (3.92)			
GATM	rs9806699	GG	59 (8.37)	12 (23.53)	0.617 (0.406–0.939)	0.0240	0.0960
		AG	281 (39.86)	17 (33.33)			
		AA	365 (51.77)	22 (43.14)			

SIM statin-induced myopathy

between RST metabolites and SIM incidents, and clarified the effect of *GATM* on SIM in Asians.

The prevalence of SIM ranges from 7% to 29% in patients treated with statins in clinical practice [26]. In our study, the incidence of RST-induced myopathy was 6.73%. This value was similar to previous reports that SIM occurred in 2.5%–10% of subjects receiving 5–80 mg/day of oral RST in randomized controlled trials [27]. The physiological mechanism of SIM has yet to be elucidated. Our results indicated that RST-induced myopathy was not correlated with the high plasma concentrations of RST and its metabolites. This finding was consistent with a previous report that SIM was present in plasma concentrations of statins at an acceptable normal range [28].

For all SNPs in our study, the loci that significantly affected SIM were inconsistent with that of the drug plasma concentrations. *SLCO1B1* 521T>C was significantly correlated with RST-induced myopathy, although this SNP was not significantly associated with the drug concentration after correction for multiple testing (FDR > 0.05). Previous studies have demonstrated the hydrophilic property of RST and hepatic bile acid uptake transporter were involved in the disposition of RST [29], which diminished the role of *SLCO1B1* in the pharmacokinetic of RST [30]. The polymorphisms of *ABCG2* 421C>A (rs2231142) played important roles in the systemic exposure to both RST and its metabolites in our study ($P_{adj} < 0.01$, FDR < 0.05). Moreover, the mutation frequency of *ABCG2* 421C>A was obviously higher in East Asians (allele frequency ~35%) than that in Caucasians (14%) [20, 31]. Thus, the variation of *ABCG2* was likely to be the primary genetic determinant of the pharmacokinetic difference of RST in Chinese population. This result is consistent with previous studies on Chinese people [18, 19, 32]. However, variant of *ABCG2* were not associated with elevated SIM risk.

Pharmacokinetic conditions result in statin accumulation in the muscle, while the molecular determinants of SIM development is

Table 4. SIM incident of patients with or without risk alleles

SLCO1B1 rs4149056	*GATM* rs9806699	Number of carriers	SIM incident (%)	Univariate logistic regression	
				OR (95% CI)	*P* value
CC + CT	GG + AG	76	8 (10.53)	3.137 (1.166–8.441)	0.024
CC	GG	5	1 (20.00)	6.667 (0.675–65.841)	0.104
TT	AA	249	9 (3.61)		

statin distribution into myocytes and exerting its negative function in myocytes. Maybe they are not pharmacokinetic-related transporters (OATP1B1 (organic anion-transporting polypeptide 1B1, encoded by *SLCO1B1*) or BCRP2 (breast cancer resistant protein 2, encoded by *ABCG2*)) primarily involving in transferring RST into/out myocytes. Study have indicated that OATP2B1 and multidrug resistance protein (MRP)1, MRP4, and MRP5 played roles for statins transporting into myocytes [33].

A decreased risk was observed on patients with *GATM* rs9806699 G>A in our study. This correlation was replicated for the first time since the study of Mangravite et al [21–23]. As a functional gene, *GATM* was related to the energy metabolism of skeletal muscles. It encodes *GATM*, which is responsible for the synthesis of creatine. Creatine is a major source of energy in skeletal muscles. The A allele of *GATM* rs9806699 leads to a greater decrease in GATM RNA expression [21]. This effect of *GATM* on SIM found in our study should be validated by future studies on a large sample.

Our study has two limitations. First, we did not measure the exact plasma exposure to RST and its metabolites at the time of myotoxicity, and we did not conduct serial monitoring during the follow up. The drug concentration was considered to remain at a steady-state level. Second, we did not detect the CK level in the follow-up period. However, the international expert workshop on SIM proposed that the elevation of serum CK might be not a necessary sign of SIM; any of the SIM relevant symptoms with or without an elevation of CK were considered as SIM [24, 25].

CONCLUSIONS

The variant allele of *SLCO1B1* 521T>C (rs4149056) and *GATM* rs9806699 were potential biomarkers for predicting RST-induced myopathy, and their influences on SIM were unrelated to the high plasma exposure of RST and its metabolites. These findings offer further insight into the mechanism underlying RST-induced myopathy.

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AUTHOR CONTRIBUTIONS

In this project, X.B. and B.Z. contributed to the study design, experiment performance, follow up, and manuscript writing. S.-L.Z. and M.H. contributed to study design and management, manuscript revision. P.W., G.-l.W., J.-l.L., and H.-s.S. were involved in patient recruitment and draft revision. D.-s.W., X.-z.L., and Y.-b.L. performed the sample collection, follow up, and data analysis.

ADDITIONAL INFORMATION

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