

# Effect of *CYP2C9* and *SLCO1B1* polymorphisms on the pharmacokinetics and pharmacodynamics of nateglinide in healthy Chinese male volunteers

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## Abstract

**Purpose** Nateglinide is commonly used in the treatment of patients with type 2 diabetes mellitus. Our objective was to assess the association between *CYP2C9* and *SLCO1B1* polymorphisms and the metabolism of nateglinide in healthy Chinese male volunteers.

**Methods** A total of 35 healthy Chinese male volunteers with different *CYP2C9* and *SLCO1B1* genotypes were given a single oral dose of 120 mg nateglinide. Plasma concentrations of nateglinide and blood glucose level were measured up to 8 h.

**Results** In subjects with the *CYP2C9*\*1/\*3 & 521TT, *CYP2C9*\*1/\*1 & 521TC/CC and *CYP2C9*\*1/\*3 & 521TC genotype, AUC<sub>0-∞</sub> of nateglinide was 56 %, 34 % and 56 % higher ( $P=0.002$ ,  $P=0.041$  and  $P=0.013$ , respectively), and the CL/F of nateglinide was 35 %, 11 % and 36 % lower ( $P=0.000$ ,  $P=0.003$  and  $P=0.002$ , respectively) than that in the reference group. When only considering 521 T>C polymorphism, it had no significant association with the pharmacokinetics of nateglinide. *CYP2C9*\*3 and 521 T>C polymorphisms were the significant predictors of the AUC<sub>0-∞</sub> and CL/F of nateglinide (adjusted multiple  $R^2=34$  % and 43 %, respectively) according to multiple linear regression analyses, but they have no significant association with changes in the blood glucose-lowering effect of nateglinide.

**Conclusions** Both *SLCO1B1* 521 T>C and the *CYP2C9*\*3 polymorphisms can significantly affect the pharmacokinetics of nateglinide, but they could only partially explain the inter-individual variability of plasma concentration of nateglinide.

Moreover, 521 T>C and the *CYP2C9*\*3 polymorphisms have no effect on pharmacodynamics of nateglinide in healthy Chinese male subjects.

**Keywords** Nateglinide · *CYP2C9* polymorphism · *SLCO1B1* polymorphism · OATP1B1 · Pharmacogenetics

## Introduction

In the year 2007-2008, of Chinese adults who were 20 years of age or older, there were 92.4 million adults (9.7 % of the adult population) with diabetes, and 148.2 million adults (15.5 %) with prediabetes, which is an important risk factor for the development of diabetes and cardiovascular disease [1]. Nateglinide, as a novel mealtime glucose regulator, is a non-sulfonylurea insulinotropic agent for treatment of type 2 diabetes mellitus (T2DM) [2]. Predominantly stimulating early insulin secretion, leaving the late phase of insulin secretion and fasting insulin unaffected, nateglinide mainly reduces postprandial hyperglycaemia and may confer a relatively lower risk of subsequent hypoglycaemia [3].

Only approximately 16 % of an oral dose of nateglinide is recovered unchanged in the urine, indicating that metabolism plays an important role in nateglinide clearance [4]. According to in vitro experiments with human liver microsomes and cytochrome P450 (CYP) isozymes, *CYP2C9* (70 %) and a smaller fraction by *CYP3A4* (30 %) were the principal enzymes catalyzing the biotransformation of nateglinide [2, 4]. A novel allelic variant *CYP2C9*\*3 (rs1057910) can markedly decrease the catalytic activity of *CYP2C9* enzyme through affecting the substrate binding site in exon 7 [5]. Kirchheiner et al. [6] performed research in healthy Caucasian subjects and found that oral nateglinide clearance was significantly reduced in carriers of *CYP2C9*\*3 alleles but not in *CYP2C9*\*2 carriers, and this only partly explained the

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interindividual variability in the pharmacokinetics of nateglinide. No significant differences in plasma glucose, insulin and glucagon were detected in subjects with different *CYP2C9*\*2 or \*3 genotype. Organic anion transporting polypeptide 1B1 (OATP1B1, previously known as OATP-C, OATP2 and LST-1) is encoded by solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene [7, 8]. *SLCO1B1* 521 T>C (rs4149056) is an important single nucleotide polymorphism (SNP) with reduced transporting activity of OATP1B1 predominantly deriving from an alteration in membrane sorting [9–11]. Plasma concentrations of nateglinide were significantly increased in subjects with the 521TC or 521CC genotype in healthy Chinese male subjects. However, a shortcoming was mentioned that they ignored the genotype of *CYP2C9* [12]. Another study carried out by Kalliokoski *et al.* [13] in healthy Caucasians identified that *SLCO1B1* 521 T>C polymorphism had no significant association with the pharmacokinetics or pharmacodynamics of nateglinide after controlling for *CYP2C9* polymorphisms.

In addition to *SLCO1B1* 521 T>C polymorphism, *SLCO1B1* 388A>G (rs2306283) is another common variant with the allele frequency of 62–84 % in Chinese [14]. The effect of *SLCO1B1* 388A>G on drug metabolism was not consistent in all studies that it was associated with increased plasma concentration of pitavastatin, reduced plasma concentration of pravastatin and repaglinide, and had no effect on pharmacokinetics of nateglinide except time to the maximum concentration ( $t_{max}$ ) [15–17]. Whether *SLCO1B1* 521 T>C polymorphism has significant association with interindividual variability of plasma concentration of nateglinide were contradictory in studies performed in different ethnic populations. The influence of *CYP2C9* polymorphism on pharmacokinetics of nateglinide was only performed in Caucasian. The aim of the present study was to assess the effect of *CYP2C9*\*3 and *SLCO1B1* 521 T>C polymorphisms on the pharmacokinetics and pharmacodynamics of nateglinide in a prospective genotype panel study controlling for the *SLCO1B1* 388A>G polymorphism in healthy Chinese male volunteers.

## Methods

### Participants

From a pool of pharmacogenetically characterized volunteers, 35 healthy Chinese male subjects were recruited into the study after giving their written informed consents. Only carriers of 388GG homozygote were recruited. The participants were allocated into one of four groups according to the *CYP2C9* and *SLCO1B1* 521 T>C genotypes. The reference group included nine participants with *CYP2C9*\*1/\*1 and 521TT genotype. The second group comprised nine

participants with *CYP2C9*\*1/\*3 and 521TT genotype. The third group consisted of 13 participants with *CYP2C9*\*1/\*1 and 521TC/CC genotype (nine participants with 521TC and four participants with 521CC). The fourth group had four participants with *CYP2C9*\*1/\*3 and 521TC genotype. No statistically significant differences existed in the age, weight, or height of subjects among four groups. Each of the participants was ascertained to be healthy by medical history, physical examination and routine laboratory tests. All participants were nonsmokers, abstained from other drugs for at least 2 weeks, and did not take any coffee, alcohol or grapefruit products for 1 week before the study.

### Study design

The study protocol was approved by the ethics committee of Central South University, Xiangya School of Medicine, and was registered online through the Chinese Clinical Trial Registry website ([www.chictr.org](http://www.chictr.org), Registration number: ChiCTR-ONC-11001810). Following an overnight fast, the participants ingested a single 120-mg dose of nateglinide (one Starlix 120 mg tablet, Novartis, Beijing, China) with 150 mL water at 08:00 h. They remained seated for 3 h, received a standardized breakfast 15 min after nateglinide intake and a standardized warm meal after 4 h. Glucose for oral and intravenous use were available in case of severe hypoglycemia, but they were not needed under the supervision of physician and nurses. Venous blood samples (5 mL) were collected into EDTA tubes before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h after the administration of nateglinide. Blood glucose was measured immediately after each blood sampling with the glucose oxidase method with the Precision G Blood Glucose Testing System (Medisense, Bedford, Massachusetts, USA). Plasma was separated within 30 min and stored at –20 °C until analysis.

### Genotyping

All subjects were genotyped for the 388A>G in exon 4 and the 521 T>C in exon 5 of the *SLCO1B1* gene and *CYP2C9*\*3 in exon 7 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), in a Perkin-Elmer DNA Model PJ2000 Thermal Cycler (Foster City, CA). The primers used for 521 T>C genotyping were 5'-AAAGGAATCTGGGTCATACATGTGGATATACG-3' (forward) and 5'-TTCAAAGTAGACAAAGGGAAAGTGATCATA-3' (reverse). The PCR conditions involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 20 s with a final extension at 72 °C for 5 min. The PCR product was digested with restriction enzyme Mlu I (TaKaRa, Dalian, China) and analyzed on a 2.5 % agarose gel. Wild type 521TT yielded a fragment of 196 bp, the variant 521CC

homozygotes had two fragments of 166 bp and 30 bp, while the heterozygotes were three fragments of 196 bp, 166 bp and 30 bp. The validity of the method was confirmed by sequencing. Genotyping methods for *CYP2C9\*3* and *SLCO1B1* 388A>G alleles were described as previously [18, 19].

#### Determination of plasma nateglinide concentration

Plasma concentrations of nateglinide were quantified by an Agilent 1100 HPLC system (California, USA) containing a HP 1100 pump, an automatic sampler and a Variable Wavelength Detector (VWD). The Chem Station software (Agilent) was used for data analysis and processing. Nateglinide was used as an external standard. After protein precipitation of 200  $\mu$ L of plasma with 400  $\mu$ L of acetonitrile, the supernatants were separated on a diamonsil C18 column (250 mm  $\times$  4.6 mm, 5  $\mu$ m particle size; Dikma, Beijing, China) with a SecurityGuard<sup>TM</sup> guard column (Phenomenex, California, USA) and monitored by UV absorption at 215 nm. The mobile phase was composed of 0.01 M ammonium formate solution (with 0.2 % formic acid): acetonitrile (25: 75 v: v), and was delivered at a flow rate of 1.0 mL/min. The limit of quantification was 93.75 ng/mL for nateglinide. The interday coefficients of variation (CV) were 6.5 % at 0.75  $\mu$ g/mL, 9.1 % at 3  $\mu$ g/mL and 3.2 % at 12  $\mu$ g/mL ( $n=4$ ).

#### Pharmacokinetics and pharmacodynamics analysis

The pharmacokinetics of nateglinide was characterized by the maximum concentration ( $C_{\max}$ ), time to  $C_{\max}$  ( $t_{\max}$ ), elimination half-life ( $t_{1/2}$ ), area under the plasma concentration-time curve from 0 h to 8 h ( $AUC_{0-8\text{ h}}$ ) and from 0 h to infinity ( $AUC_{0-\infty}$ ), and the total oral clearance (CL/F).  $C_{\max}$  and  $t_{\max}$  were directly observed from the concentration-time curve.  $AUC_{0-8\text{ h}}$  was calculated by the linear trapezoidal rule from 0 to 8 h. The  $t_{1/2}$ ,  $AUC_{0-\infty}$  and CL/F were calculated by the following equations:  $t_{1/2} = \ln 2 / k_e$ ,  $AUC_{0-\infty} = AUC_{0-8\text{ h}} + C_{p(8\text{ h})} / k_e$ , CL/F = dose /  $AUC_{0-\infty}$ . The elimination rate constant ( $k_e$ ) was determined by linear regression analysis of the log-linear part of the concentration-time curve,  $C_{p(8\text{ h})}$  was the last measured concentration at 8 h.

The blood glucose response to nateglinide was characterized by the maximum decrease and maximum increase in blood glucose concentration. Decremental area above the plasma glucose-time curve from 0 to 3 h ( $AAC_{0-3\text{ h}}$ ) was calculated by the linear trapezoidal rule.

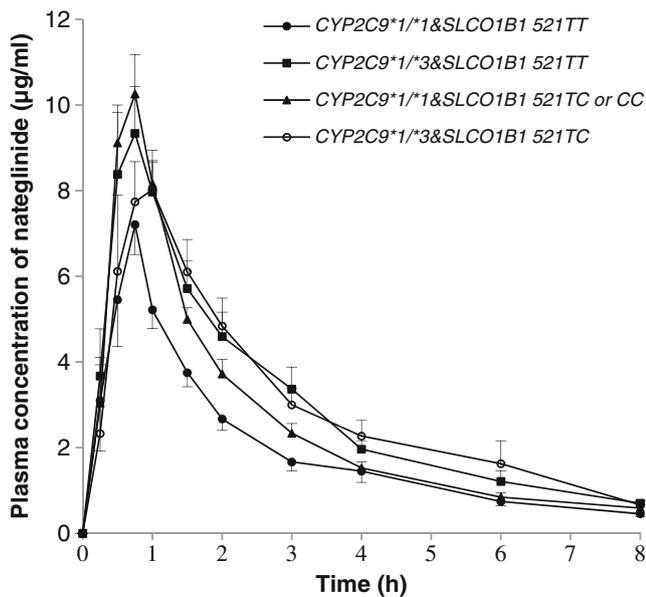
#### Statistical analysis

Results are expressed as mean  $\pm$  SD. Statistical comparisons of pharmacokinetic and pharmacodynamic parameters

between subjects with different genotypes of *SLCO1B1* 521 T>C and *CYP2C9\*3* were investigated by ANOVA test, followed by a posteriori testing with the Dunnett test (equal variances) or the Games-Howell test (unequal variances). The data of  $t_{\max}$  were analyzed by the Kruskal-Wallis H test and posteriori testing with the Mann-Whitney U test. The contribution of these two SNPs in the pharmacokinetic and pharmacodynamic parameters of nateglinide was done by use of forward, stepwise multiple linear regression analysis. The different SNPs were put into the model as independent variables valued as 0, 1 and 2 which represented carriers of none, one and two mutant alleles, respectively. Relationships between the pharmacokinetic and pharmacodynamics parameters of nateglinide were analyzed by Pearson correlation coefficient. Statistical analyses were performed by the SPSS software for Windows (Version 18.0, SPSS Inc., Chicago, IL). Differences were considered statistically significant when  $P$  value was less than 0.05.

#### Results

According to *CYP2C9* and *SLCO1B1* genotype, all 35 healthy Chinese male subjects were divided into four groups, *CYP2C9\*1\*1* & 521TT group ( $n=9$ ), *CYP2C9\*1\*3* & 521TT group ( $n=9$ ), *CYP2C9\*1\*1* & 521TC/CC group ( $n=13$ ) and *CYP2C9\*1\*3* & 521TC group ( $n=4$ ).  $AUC_{0-8\text{ h}}$ ,  $AUC_{0-\infty}$  and CL/F of nateglinide were statistically different among four groups (Fig. 1, Table 1). Compared to the reference group, the  $AUC_{0-\infty}$  of nateglinide was 56 %, 34 %, and 56 % higher ( $P=0.002$ ,  $P=0.041$  and  $P=0.013$ , respectively) (Fig. 2), and the CL/F was 35 %, 11 % and 36 % lower ( $P=0.000$ ,  $P=0.003$  and  $P=0.002$ , respectively) in carriers of *CYP2C9\*3* allele, *SLCO1B1* 521 C allele, and both *CYP2C9\*3* and *SLCO1B1* 521 C alleles. The  $C_{\max}$  of nateglinide in *CYP2C9\*1\*1* & 521TC/CC subjects was 39 % higher than that in the reference group ( $P=0.049$ ). The differences of  $t_{1/2}$  among the four genotypic groups were not statistically significant. In a stepwise forward multiple regression analysis, heterozygosity for the *CYP2C9\*3* allele ( $P=0.000$ ) and heterozygosity and homozygosity for *SLCO1B1* 521 T>C SNP ( $P=0.028$ ) independently predicted the  $AUC_{0-\infty}$  of nateglinide (adjusted multiple  $R^2=34\%$ ), the standardized coefficient of *CYP2C9\*3* polymorphism ( $b=0.587$ ) was larger than that of *SLCO1B1* 521 T>C polymorphism ( $b=0.349$ ). The CL/F was predicted by heterozygosity for the *CYP2C9\*3* allele ( $P=0.000$ ,  $b=-0.637$ ) and heterozygosity and homozygosity for *SLCO1B1* 521 T>C SNP ( $P=0.004$ ,  $b=-0.444$ ) (adjusted multiple  $R^2=43\%$ ). When only considering *CYP2C9\*3* or *SLCO1B1* 521 T>C SNP in all 35 healthy Chinese male subjects, no significant association was found between the *SLCO1B1*



**Fig. 1** The plasma concentration-time curve of nateglinide in 35 healthy subjects with different *SLCO1B1* and *CYP2C9* genotypes after a single oral dose of 120 mg nateglinide. Solid circles represent *CYP2C9*\*1/\*1 and *SLCO1B1* 521TT genotype group ( $n=9$ ) (●), solid squares represent *CYP2C9*\*1/\*3 and *SLCO1B1* 521TT genotype group ( $n=9$ ) (■), solid triangles represent *CYP2C9*\*1/\*1 and *SLCO1B1* 521TC/CC genotype group ( $n=13$ ) (▲), open circles represent *CYP2C9*\*1/\*3 and *SLCO1B1* 521TC genotype group ( $n=4$ ) (○). Data are expressed as mean  $\pm$  SEM

521 T>C SNP and the pharmacokinetics of nateglinide. The  $AUC_{0-\infty}$  of nateglinide were  $21.11 \pm 6.79$   $\mu\text{g}\cdot\text{h}/\text{mL}$  in the subjects with 521TT genotype ( $n=18$ ),  $21.89 \pm 4.42$   $\mu\text{g}\cdot\text{h}/\text{mL}$  in the subjects with 521TC genotype ( $n=13$ ), and  $26.51 \pm 6.74$   $\mu\text{g}\cdot\text{h}/\text{mL}$  in the subjects with 521CC genotype ( $n=4$ ) ( $P=0.279$ ).

The *CYP2C9*\*3 and *SLCO1B1* 521 T>C polymorphisms did not result in statistically significant differences in plasma glucose variables after a single oral dose of 120 mg nateglinide shown in Table 2, including maximum increase, maximum decrease and decremental  $AAC_{0-3\text{ h}}$ . In a stepwise, forward multiple regression analysis, heterozygosity and homozygosity for *SLCO1B1* 521 T>C SNP independently predicted the decremental  $AAC_{0-3\text{ h}}$  (adjusted multiple  $R^2=22\%$ ,  $P=0.024$ ). The maximum decrease in plasma glucose concentration was predicted by heterozygosity for the *CYP2C9*\*3 allele (adjusted multiple  $R^2=15\%$ ,  $P=0.038$ ).

## Discussion

In this study, we have provided the evidence that the *SLCO1B1* 521 T>C allele and the *CYP2C9*\*3 SNP are significant predictors of the pharmacokinetics of nateglinide in healthy Chinese male subjects with *SLCO1B1* 388GG genotype after a single 120-mg dose of nateglinide. The

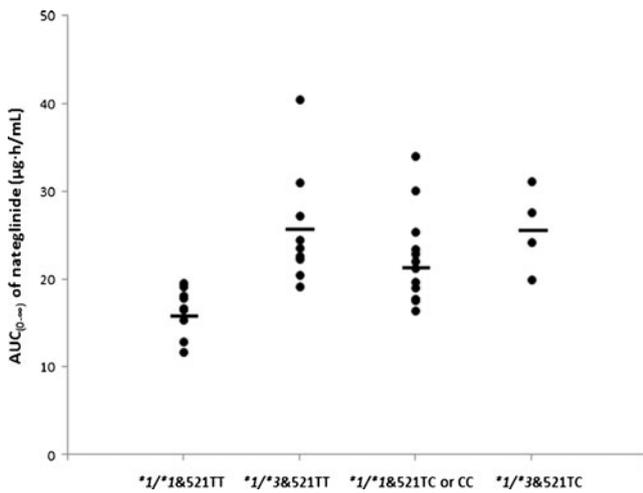
**Table 1** Pharmacokinetic parameters of a single dose of 120 mg nateglinide for different *SLCO1B1* and *CYP2C9* genotypes

<i>CYP2C9</i> & <i>SLCO1B1</i> Genotype	$C_{\text{max}}$ ( $\mu\text{g}/\text{mL}$ ) (95 % CI)	$t_{1/2}$ (h) (95 % CI)	$AUC_{(0-8\text{h})}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ) (95 % CI)	$AUC_{(0-\infty)}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ) (95 % CI)	CL/F (L/h) (95 % CI)
*1/*1&521TT ( $n=9$ )	$7.75 \pm 2.2$ (6.05, 9.44)	$1.92 \pm 0.36$ (1.64, 2.19)	$15.16 \pm 2.36$ (13.34, 16.98)	$16.5 \pm 2.72$ (14.41, 18.59)	$7.48 \pm 1.41$ (6.4, 8.56)
*1/*3&521TT ( $n=9$ )	$10.1 \pm 3.27$ (7.58, 12.61)	$2.02 \pm 0.39$ (1.72, 2.31)	$23.61 \pm 6.89^*$ (18.31, 28.91)	$25.72 \pm 6.53^*$ (20.71, 30.74)	$4.89 \pm 1.01^{**}$ (4.11, 5.66)
*1/*1&521TC or CC ( $n=13$ )	$10.79 \pm 3.15^*$ (8.88, 12.7)	$1.8 \pm 0.41$ (1.55, 2.05)	$20.46 \pm 4.48^*$ (17.76, 23.17)	$22.13 \pm 5.23^*$ (18.97, 25.29)	$5.67 \pm 1.15^{**}$ (4.97, 6.36)
*1/*3&521TC ( $n=4$ )	$9.88 \pm 1$ (8.29, 11.47)	$2.06 \pm 0.3$ (1.59, 2.54)	$23.75 \pm 4.35^*$ (18.32, 22.24)	$25.75 \pm 4.76^*$ (18.17, 33.32)	$4.79 \pm 0.92^{**}$ (3.32, 6.25)
ANOVA $P$ value	0.114	0.491	0.004	0.003	0.000

Data are mean  $\pm$  SD; CI, confidence interval;  $C_{\text{max}}$ , peak plasma concentration;  $t_{1/2}$ , elimination half-life;  $AUC_{(0-8\text{h})}$ , area under the plasma concentration-time curve from 0 to 8 h;  $AUC_{(0-\infty)}$ , area under the plasma concentration-time curve from 0 h to infinity; CL/F, total oral clearance

\*  $P < 0.05$  vs. *CYP2C9*\*1/\*1 & *SLCO1B1* 521TT group

\*\*  $P < 0.01$  vs. *CYP2C9*\*1/\*1 & *SLCO1B1* 521TT group



**Fig. 2** Individual AUC<sub>0-∞</sub> values in healthy subjects with four different *SLCO1B1* and *CYP2C9* genotypes after a single oral dose of 120 mg nateglinide. Horizontal lines show mean value for all genotype groups

AUC<sub>0-∞</sub> and CL/F of nateglinide varied nearly four-fold among all the subjects. Subjects carried the *CYP2C9*\*1/\*3 & 521TT, *CYP2C9*\*1/\*1 & 521TC/CC or *CYP2C9*\*1/\*3 & 521TC genotype had significantly larger AUC<sub>0-∞</sub> and smaller CL/F than subjects with the reference genotype. Moreover, it firstly reported that despite remarkable alternations in AUC<sub>0-∞</sub> and CL/F, the elimination t<sub>1/2</sub> of nateglinide among subjects with different *SLCO1B1* and *CYP2C9* genotypes remained unchanged. Similar to nateglinide, the AUC<sub>0-∞</sub>, but not t<sub>1/2</sub> of repaglinide, pravastatin, atorvastatin and rosuvastatin was affected by *SLCO1B1* polymorphism [13, 20, 21]. The possible explanation is that the apparent volume of distribution (Vd) is decreased in the same proportion to reduced clearance of nateglinide, as t<sub>1/2</sub> equals to ln2 times Vd divided by clearance. According to the stepwise multiple linear regression analysis, the *SLCO1B1* 521 T>C and *CYP2C9*\*3 polymorphisms could not fully explain the interindividual differences on the pharmacokinetics of nateglinide (only approximately 30–40 %). The standardized coefficient of the *CYP2C9*\*3 polymorphism

was larger than that of the *SLCO1B1* 521 T>C polymorphism indicated that *CYP2C9*\*3 polymorphism seemed to be more powerful to explain the interindividual variability of nateglinide. Nateglinide is at least partially transported by OATP1B1 from blood into the liver, then eliminated primarily by *CYP2C9* (70 %) [4, 12]. This is the first study to investigate the interindividual variability in the pharmacokinetics of nateglinide by simultaneously considering the common polymorphisms of drug transporter and drug metabolic enzyme in healthy Chinese male subjects.

Nateglinide has an oral bioavailability of 72 %, and approximately 16 % is excreted unchanged in the urine [2]. Compared with the carriers of *CYP2C9*\*1/\*3 & 521TT genotype, no effect of *SLCO1B1* 521 T>C polymorphism on the pharmacokinetics of nateglinide was found in the subjects with *CYP2C9*\*1/\*3 & 521TC genotype. Furthermore, no significant difference on the pharmacokinetic variables of nateglinide was shown when only using the *SLCO1B1* 521 T>C polymorphism to group the whole 35 healthy Chinese male subjects. One possible reason is that the AUC<sub>0-∞</sub> and C<sub>max</sub> of nateglinide markedly increased in the *CYP2C9*\*1/\*1 & *SLCO1B1* 521TC/CC group compared with the reference group, whereas the t<sub>1/2</sub> remained unchanged, suggests that OATP1B1 is at least partially involved in the hepatic uptake of nateglinide. Besides OATP1B1, some other transporters may take part in the hepatic uptake, renal excretion and reabsorption of nateglinide. Another possibility is that a large amount of nateglinide is transported into hepatocyte after oral administration. Subjects carrying the *CYP2C9*\*3 alleles have distinct lower catalytic ability of nateglinide, leading to a markedly increased intrahepatic concentration of nateglinide which may inhibit the hepatic uptake of nateglinide or induce the unchanged nateglinide out of the hepatocytes.

As shown in Fig. 2, a considerable unexplained pharmacokinetic variability remained within the subgroups. The *CYP3A4*\*1 G is a common allele in a Chinese population with the allelic frequency of 22.1 % [22]. the *CYP3A4*\*1 G allele has been suggested to alter the *CYP3A* activity by increasing the lipid-lowering efficacy of atorvastatin [23],

**Table 2** Blood glucose variables before and after a single dose of 120 mg nateglinide in healthy participants with different *SLCO1B1* and *CYP2C9* genotypes

<i>CYP2C9</i> & <i>SLCO1B1</i> Genotype	Baseline concentration (mmol/L) (95 % CI)	Maximum decrease (mmol/L) (95 % CI)	Maximum increase (mmol/L) (95 % CI)	Decremental AAC <sub>(0-3h)</sub> (mmol·h/L) (95 % CI)
*1/*1&521TT (n=9)	4.9±0.3 (4.7, 5.1)	1±0.4 (0.4, 1.6)	1.6±0.6 (0.8, 2.5)	1±0.9 (1.7, 0.3)
*1/*3&521TT (n=9)	5±0.3 (4.8, 5.2)	1.4±0.6 (0.8, 2)	2.4±1 (1.2, 3.4)	1.3±0.7 (1.9, 0.7)
*1/*1&521TC or CC (n=13)	5.1±0.1 (5, 5.1)	1.1±0.4 (0.5, 1.7)	1.2±0.6 (0.3, 2.1)	0.7±0.6 (1.3, 0.5)
*1/*3&521TC (n=4)	4.9±0.2 (4.9, 5.1)	1.5±1 (0, 3)	2±0.4 (1.3, 2.7)	1.1±1.2 (3.5, 1.5)
ANOVA P value	0.551	0.237	0.195	0.780

AAC<sub>(0-3h)</sub>, area above the plasma glucose-time curve from 0 to 3 h.

increasing the plasma concentration of fentanyl and decreasing fentanyl consumption for postoperation pain control [24, 25]. All microsomal CYP450 enzymes must get electrons from NADPH transferred by cytochrome P450 oxidoreductase (POR) to perform their catalytic functions [26]. In vitro, the *POR\*28* variation may affect the activities of some CYP enzymes, such as enhancing CYP2C19 activity or decreasing CYP1A2 activity [27]. Compared with *CYP3A4*, *CYP3A5* and *CYP3A7* polymorphisms, the *POR\*28* polymorphism with the allelic frequency of 43.2 % in a Chinese population, was reported to be more sensitively reflected the variability of CYP3A catalytic activity in both Caucasian [28] and Chinese populations [29]. Moreover, some inevitable factors, e.g., differences in intestinal absorption, gastric emptying and plasma protein binding rate, could influence the pharmacokinetics of nateglinide. Further limitation of this study was that urine was not collected to detect the concentrations of nateglinide metabolites which could be used to calculate the metabolic ratio of the respective metabolite to the parent drug. Thus, whether CYP polymorphisms could play the role in the interindividual variability of plasma concentration of nateglinide will be more reliable by synthetically considered the pharmacokinetic parameters of parent drug and its metabolites.

After administration of 120 mg nateglinide, *SLCO1B1* 521 T>C and the *CYP2C9\*3* polymorphisms were not significantly associated with changes in the blood glucose-lowering effect of nateglinide. However, the differences of the maximum decrease in plasma glucose concentration and decremental  $AAC_{0-3h}$  were predicted by the *CYP2C9\*3* and *SLCO1B1* 521 T>C polymorphisms respectively. The possible mechanism is unclear. The detection of plasma insulin concentration could be a more sensitive indicator of the influence of polymorphisms on the therapeutic efficacy of nateglinide.

In conclusion, the *SLCO1B1* 521 T>C and the *CYP2C9\*3* polymorphisms could partially explain the interindividual variability of plasma concentration of nateglinide in healthy Chinese population. The *SLCO1B1* 521 T>C and the *CYP2C9\*3* polymorphisms have significant influence on the pharmacokinetics of nateglinide. Further studies are needed to find other genetic polymorphisms which can affect the pharmacokinetics and/or pharmacodynamics of nateglinide, especially in patients with T2DM.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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