



Research Article

Impact of *NR1I2*, adenosine triphosphate–binding cassette transporters genetic polymorphisms on the pharmacokinetics of ginsenoside compound K in healthy Chinese volunteers

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ABSTRACT

Background: Ginsenoside compound K (CK) is a promising drug candidate for rheumatoid arthritis. This study examined the impact of polymorphisms in *NR1I2*, adenosine triphosphate–binding cassette (ABC) transporter genes on the pharmacokinetics of CK in healthy Chinese individuals.

Methods: Forty-two targeted variants in seven genes were genotyped in 54 participants using Sequenom MassARRAY system to investigate their association with major pharmacokinetic parameters of CK and its metabolite 20(S)-protopanaxadiol (PPD). Subsequently, molecular docking was simulated using the AutoDock Vina program.

Results: *ABCC4* rs1751034 TT and rs1189437 TT were associated with increased exposure of CK and decreased exposure of 20(S)-PPD, whereas *CFTR* rs4148688 heterozygous carriers had the lowest maximum concentration (C_{max}) of CK. The area under the curve from zero to the time of the last quantifiable concentration (AUC_{last}) of CK was decreased in *NR1I2* rs1464602 and rs2472682 homozygous carriers, while C_{max} was significantly reduced only in rs2472682. *ABCC4* rs1151471 and *CFTR* rs2283054 influenced the pharmacokinetics of 20(S)-PPD. In addition, several variations in *ABCC2*, *ABCC4*, *CFTR*, and *NR1I2* had minor effects on the pharmacokinetics of CK. Quality of the best homology model of multidrug resistance protein 4 (MRP4) was assessed, and the ligand interaction plot showed the mode of interaction of CK with different MRP4 residues.

Conclusion: *ABCC4* rs1751034 and rs1189437 affected the pharmacokinetics of both CK and 20(S)-PPD. *NR1I2* rs1464602 and rs2472682 were only associated with the pharmacokinetics of CK. Thus, these hereditary variances could partly explain the interindividual differences in the pharmacokinetics of CK.

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1. Introduction

Ginseng is one of traditional medicinal plants widely used in Asia for its extensive therapeutic effects on delaying aging and maintaining physical vitality [1]. Modern medical research has also shown that ginseng has a positive impact on a variety of

diseases, including cancer [2], diabetes [3], neurodegeneration [4], inflammation [5], etc. The advantageous effects of ginseng are essentially benefited from the characteristics of ginsenosides, a group of triterpenoid saponins [6]. To exert various bioactive functions and be absorbed, ginsenosides must be converted into deglycosylated ginsenosides by the intestinal microflora [7].

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Ginsenoside compound K (20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol; also named G-CK, IH-901) belongs to the tetracyclic dammarane-type triterpenoid saponins and is absent naturally [8]. CK can be transformed from protopanaxadiol (PPD)-type ginsenosides by various methods [9] and disintegrated further into 20(S)-PPD in the intestines [10]. So far, researchers have mostly focused on the pharmacological activity of CK and methodologies for production. However, there are only few reports on its absorption, distribution, metabolism, and excretion, to date.

Ginsenoside Compound K Tablets are currently being tested as a candidate drug for rheumatoid arthritis by Hisun Pharmaceutical Co., Ltd. (Taizhou, Zhejiang, China). From the pharmacokinetic (PK) data in animals and healthy individuals [11,12], we found that CK is absorbed with atypical absorption kinetics, characterized by poor oral bioavailability and large interindividual variability. It is well known that both drug-metabolizing enzymes and transporters contribute enormously to the bioavailability of oral drugs. Therefore, we first evaluated the interactions between CK and cytochrome P450 enzymes (CYPs) *in vitro* (unpublished) and found that CYPs have minimal effects on CK. As a result, we hypothesized that the significant interindividual variability in the disposition of CK mainly results from differences in the absorption phase, mediated by intestinal transporters. Because the expression and functions of various types of transporters can differ significantly, it is considered that hereditary abnormalities in their coding genes could partly explain the interindividual variability in pharmacokinetics, therapeutic effects, and incidence of adverse reactions of their substrate drugs. Consequently, intestinal transporters may play a crucial role in the pharmacokinetics of CK, and a better understanding of these impacts would provide a more comprehensive theoretical basis for the clinical application of CK.

Atypical absorption kinetics of most drugs with poor absorption can be caused by interactions with intestinal adenosine triphosphate-binding cassette (ABC) transporters. The human ABC transporter family consists of 49 members divided into seven subfamilies, containing some transporters that play key roles in the disposition of exogenous substances, such as P-glycoprotein (P-gp, also named MDR1; HGNC name: *ABCB1*), multidrug resistance-associated proteins (MRPs: especially MRP2, MRP3, MRP4; HGNC name: *ABCC2*, *ABCC3*, *ABCC4*), and a breast cancer-resistance protein (BCRP; HGNC name: *ABCG2*). Many phytochemicals (PCs) can act as substrates, inducers, or inhibitors of the ABC transporters, which can severely influence their oral bioavailability [13–15]. For a typical ABC transporter, the spectrum of substrates is generally broad and partly overlaps with that of other ABC transporters [16]. *In vitro* investigations and studies in knock-out mice have indicated a significant influence of MDR1 on the disposition of CK [17–19], supporting the hypothesis that single nucleotide polymorphisms (SNPs) in *ABCB1* and other transporters could influence CK pharmacokinetics. In addition, the activation of drug-metabolizing enzymes and transporters induced by the pregnane X receptor (PXR; HGNC name: *NR1I2*) affects the pharmacokinetics of both xenobiotics and endobiotics [20]. Thus, genetic variations in PXR might also account for these interindividual differences mediated by drug transporters.

Moreover, *ABCC4* and cystic fibrosis transmembrane conductance regulator (*CFTR*) gene polymorphisms were evaluated to investigate the possible mechanism of adverse reactions occurring in the same sample of individuals. *CFTR* is a unique chloride ion channel protein, rather than an ABC transporter [21]. Considering the reported physical and functional coupling of MRP4 with *CFTR* in the intestines [22], 18 SNPs in these two genes were analyzed in this study.

The molecular docking approach can be available to simulate the interaction between a protein and a small molecule at the atomic level, allowing the characterization of small molecules based on the

binding site of their target proteins. It is believed that it is possible to identify the substrate of transporters by confirming the combination of a drug with specific transporter residues, using a molecular model [23]. As this method is more efficient than the classic methods of investigating the interaction between drugs and transporters, herein we performed a docking experiment to simulate the interaction between CK and MRP4, genetic variants of which were associated with the pharmacokinetics of CK.

In summary, 42 SNPs of seven genes, namely *ABCC2*, *ABCC3*, *ABCC4*, *ABCB1*, *ABCG*, *NR1I2*, and *CFTR*, were incorporated in this study. This work was aimed at investigating the effects of gene polymorphisms on the PK characteristics of CK and its metabolite 20(S)-PPD in healthy Chinese individuals. Moreover, the interaction between CK and MRP4 was preliminarily validated using docking simulation.

2. Methods

2.1. Participant recruitment

The present analysis was performed using pooled data from two clinical trials. Both clinical trials were carried out at the phase I unit (Center of Clinical Pharmacology, The Third Xiangya Hospital, Central South University, China) and included healthy individuals selected using the same inclusion/exclusion criteria. All participants were required to be healthy and nonsmoking, aged from 18 to 45 years. The body weight of the male and female was not less than 50 and 45 kg, respectively, and the range of body mass index was within 19–24 kg/m². In addition, both trials were contraindicated for pregnant females and females of childbearing potential. Participants with any history of critical or infectious diseases, drug allergy, recent exposure to prescription or investigational medication, over-the-counter treatment, alcohol or drug abuse (within 6 months) were excluded. Participants were also excluded for the use of any medicine that induces or inhibits hepatic metabolism enzymes within 30 days. Before the random assignments, all participants were properly apprised of the risks of the trials, and read, understood, and signed the informed consent forms.

2.2. Clinical trials

All trials were approved by the ethics committee of the Third Xiangya Hospital affiliated to Central South University (No.14050 and No.14119) and were in accordance with the Declaration of Helsinki and the International Conference of Harmonization guidelines for Good Clinical Practice. For the duration of the trials, food was strictly controlled and standardized. In addition, smoking, caffeine, or alcohol consumption, the use of concomitant medications, and heavy exercise were not permitted.

Single-dose trial: This trial was double blinded, randomized, and placebo controlled, which included 76 healthy adults (male:female = 1:1). Volunteers took only one of seven doses (25, 50, 100, 200, 400, 600, and 800 mg) of CK (Ginsenoside Compound K Tab; Hisun Pharmaceutical Co., Ltd.) ($n = 8, 8, 10, 10, 10, 8,$ and 8 individuals in each dose group) or placebo (placebo Tab; Hisun Pharmaceutical Co., Ltd.) ($n = 2$ for each dose group), under fasting condition [12].

Food-effect trial: This trial was designed to be a randomized, two-period, two-treatment crossover trial, including 24 eligible participants (male:female = 1:1) who received a single 200-mg dose of CK after 10 hours of fasting or after a standard high-fat, high-calorie breakfast according to the Food and Drug Administration (150-calorie protein, 250-calorie carbohydrates, 500- to 600-calorie fat; total calories about 800–1,000). The alternate treatment was performed after a washout period that lasted for 14 days [11].

Blood samples (5 mL) for PK analysis were collected from each participant before dosing and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 36, 48, 72, and 96 hours after drug administration, which was designed based on preclinical animal PK data of CK. In the 25- and 50-mg dose groups of the single-dose trial, blood samples at 72 and 96 hours were not collected according to the research protocol. Samples were required to be processed within 1 hour after sampling as follows: Plasma was separated from whole blood by refrigerated centrifugation (3,000 rpm, 10 minutes) before transferred to labeled storage tubes and then stored at -70°C until chromatographic analysis. The plasma samples were stored and analyzed at the chromatography laboratory, Institute of Clinical Pharmacology, Central South University (Changsha, China).

We compared the main PK parameters of CK under the same administration condition (fast overnight) in the two trials. Accordingly, 30 participants from three dose groups (100, 200, and 400 mg) in the single-dose trial and 24 participants in the food-effect trial were enrolled in the present study to explore the impact of gene polymorphisms on the PK parameters of CK and 20(S)-PPD.

2.3. Measurement of plasma CK and 20(S)-PPD

Plasma concentrations of the target compounds were measured using mass spectrometry and liquid chromatography–tandem mass spectrometry (LC-MS/MS, API 4000; Applied Biosystems, Waltham, USA) in the trials. Concisely, internal standards (digoxin for CK and coumarin for 20(S)-PPD) were added to 0.5 mL of plasma sample and then deproteinized through several steps including addition of methyl tert-butyl ether (2 mL), mixing, and 10-minutes centrifugation at 4,000 r/min, 4°C . After that, the supernatant (1.4 mL) was transferred and evaporated by nitrogen blowing in a 40°C water bath. Finally, the residue was dissolved into 100 μL of the corresponding mobile phase, followed by mechanical shaking and centrifugation. After that, the supernatant was collected for flow injection analysis. A HypPURITY C18 (150 mm \times 2.1 mm, 5 μm ; Thermo Hypersil-Keystone, PA, USA) combined with mobile phases (for CK, acetonitrile:20 mM aqueous ammonium acetate = 60:40; for PPD, acetonitrile:10 mM aqueous ammonium acetate = 80:20) was used to separate the target compounds and internal standards from the matrix components. A satisfactory result of method validation indicated that the accuracy of quality control samples varied from 85 to 115%, with the intra and interassay precisions less than 15%. The lower limits of quantification and linear range were 1.002 ng/mL and 1.002 to 1002.0 ng/mL for CK, respectively. As for 20(S)-PPD, the aforementioned results were 0.151 ng/mL and 0.151 to 54.30 ng/mL.

2.4. Genotyping

For genetic analysis, a peripheral blood sample was drawn from every individual and kept in a -20°C freezer until DNA extraction. The Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) was used to extract DNA. A DNA sample from each participant was genotyped, with focus on 42 SNPs variants in seven genes (the *ABCC2* rs2756109, rs2273697, rs3740066, rs717620; *ABCC3* rs12051822, rs4148416, rs4793665; *ABCC4* rs4148546, rs1151471, rs1751034, rs1189437, rs2274406, rs2274407, rs11568658, rs1926657, rs869951; *CFTR* rs4148688, rs283054, rs213950, rs213976, rs4148711, rs213968, rs1042077, rs2106155, rs2237726; *ABCG2* rs2231148, rs2054576, rs12505410, rs2231142, rs6857600, rs3114018, rs2725248, rs17731799; *ABCB1* rs1128503, rs2032582, rs1045642; and *NR1I2* rs1464602, rs6785049, rs2276707, rs1523127, rs7643645, rs2472682) using Sequenom MassARRAY system. Direct sequencing confirmed the effectiveness of the method.

2.5. Pharmacokinetic analysis

WinNonlin version 6.1 (Pharsight Corporation, Mountain View, CA, USA) was used to assess all the PK data in this study. The maximum concentrations (C_{max}) and time to maximum plasma concentration (t_{max}) could be obtained from the plasma concentration–time data directly. The area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration (AUC_{last}) was calculated using linear trapezoidal rule. The elimination rate constant (K) was determined by linear regression analysis of the log-linear part of the plasma concentration–time curve. The half-life ($t_{1/2}$) was calculated based on the elimination rate constant, as equal to $(\ln 2)/K$. The apparent clearance (CL/F) and terminal volume of distribution (V_z/F) were also obtained. In addition, the dose-normalized (to 1 mg of CK) C_{max} (C_{max}/D) and AUC_{last} ($\text{AUC}_{\text{last}}/D$) were calculated by dividing each PK result with the homologous dosage of CK. The metabolite ratio was determined by the exposure of 20(S)-PPD divided by the corresponding exposure of CK.

2.6. Statistical analysis

The PK parameters for CK and 20(S)-PPD were summarized using descriptive statistics and compared in accordance with the genotype of gene polymorphisms in *NR1I2* and ABC transporter genes. Values of PK parameters were represented as mean (standard deviation), except for t_{max} which was expressed as median (range).

At first, one-way analysis of variance was applied on logarithmic transformed C_{max}/D , $\text{AUC}_{\text{last}}/D$, $t_{1/2}$, V_z/F , and CL/F , and nonparametric tests were performed on t_{max} to determine if there is a difference among the different groups. Based on what is mentioned previously, the criterion for the entry of this study was formulated. In the present study, the PK parameters for CK and 20(S)-PPD were compared in accordance with the genotype of gene polymorphisms in *NR1I2* and ABC transporter genes. The parameters used in these comparative analyses were $\text{AUC}_{\text{last}}/D$, C_{max}/D , CL/F , V_z/F , $t_{1/2}$, and t_{max} . The $\text{AUC}_{\text{last}}/D$ and C_{max}/D ratio of PPD/CK were also compared by genotype. All variables, except for t_{max} , were transformed logarithmically before statistical analysis. Analysis of variance was used to compare the differences of PK parameters, except for the t_{max} which was analyzed by nonparametric tests, among participants with different genotype. All p values in this study were considered statistically significant at less than 0.05.

2.7. Docking

2.7.1. Homology modeling

The first step in homology modeling was to identify template proteins for the target sequence. In this study, we used PSI-BLAST (<https://blast.ncbi.nlm.nih.gov/>) to reveal the templates of the human MRP4. Sequence alignment showed in Supplemental Fig. 1 was performed by Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Multiple templates were chosen to obtain maximum possible query coverage, which were submitted to Modeller 9.19 subsequently. Modeller is an automated approach for comparative modeling that depends on the satisfaction of spatial restraints. The distance, dihedral angle, and stereochemical restraints on the target sequence could be extracted by aligning them to those of the template. The homology model is constructed based on minimization of the restraints-based objective function of the target backbone as it is projected onto the template framework. During model refinement, conjugate gradient and simulated annealing molecular dynamics (MDs) were used to optimize the positions of heavy atoms. The simulated annealing combines the constraints in

the template structure to prevent the homologous model from expanding in the vacuum. **Supplemental Table 1** presents templates for homology modeling of the human MRP4.

2.7.2. Validation of 3-D homology models

The quality of the best model was assessed using SAVES (<http://services.mbi.ucla.edu/SAVES/>) server (**Supplemental Fig. 2**). Ramachandran plots of the model and template were generated by PROCHECK, which quantifies the residues in available zones of the Ramachandran plot and, thus, can be used to determine the stereochemical quality of the model.

2.7.3. Molecular docking

The molecular docking experiment was performed using the AutoDock Vina 1.1.2 program. AutoDock Vina was used to prepare the protein before docking. Gasteiger partial charges were assigned to both the inhibitor and enzyme atoms. The docking sampled the ligands in a $40 \times 40 \times 40$ grid that was positioned to encompass the binding site. The ligand was set to have flexible torsion angles at all rotatable bonds, meanwhile the protein was prepared as a rigid structure.

3. Results

3.1. Demographic characteristics

Based on that the dose-normalized exposure of CK were not significantly different among the groups (**Supplemental Table 2**), a total of 54 healthy individuals were included in this analysis. The baseline demographics (age, height, weight, and body mass index) are provided in **Table 1**, and all values are presented as median (range).

3.2. Effects of gene polymorphisms on the pharmacokinetics of CK and 20(S)-PPD

The genotype frequencies of *NR1I2*, ABC transporter genes are presented in **Table 2**. The dose-normalized C_{max} and AUC_{last} values of CK in different genotypes are given in **Table 3** and **Fig. 1**, while the summary of other parameters are presented in **Supplemental Table 3**. *ABCC4* rs1751034 TT and rs1189437 TT and *NR1I2* rs1464602 AG were significantly associated with the higher dose-normalized C_{max} ($p = 0.002$, 0.028 , and 0.010 , respectively) and AUC_{last} values of CK ($p = 0.002$, 0.024 , and 0.035 , respectively). *CFTR* rs4148688 GG was associated with the higher dose-normalized C_{max} ($p = 0.027$), but not associated with the dose-normalized AUC_{last} ($p = 0.139$). Individuals who were carriers of CA for *NR1I2* rs2472682 had higher dose-normalized AUC_{last} ($p = 0.037$) and C_{max} (not statistically significant, $p = 0.050$) for CK. Besides, *ABCC2* rs717620, *ABCC4* rs2274407, and *CFTR* rs213976, rs2106155, and rs2237726 were significantly associated with the $t_{1/2}$ ($p = 0.021$, 0.010 , 0.045 , 0.012 , and 0.037 , respectively), and *ABCC4* rs1751034 and *NR1I2* rs1464602 and rs2472682 were related to CL/F

($p = 0.003$, 0.037 , and 0.037 , respectively). Obvious differences in Vz/F of CK between the different genotypes of *ABCC4* rs1751034 and rs1189437, *ABCC2* rs2231148, and *NR1I2* rs1464602, rs6785049, and rs2472682 were also observed ($p = 0.003$, 0.031 , 0.042 , 0.009 , 0.046 , and 0.016 , respectively). Plasma concentration–time profiles in participants with different genotypes that significantly affect the exposure of CK are presented in **Fig. 4**.

The dose-normalized C_{max} and AUC_{last} values of 20(S)-PPD in different genetic polymorphisms are also presented in **Table 3** and **Fig. 2**, and the gene effect on other PK parameters are summarized in **Supplemental Table 3**. Polymorphisms in *ABCC4* and *CFTR* were associated with the level of exposure to 20(S)-PPD in the plasma. Participants with TT genotype for rs1751034 and rs1189437 of *ABCC4* and GG genotype for rs2283054 of *CFTR* showed a significantly lower dose-normalized AUC_{last} ($p = 0.001$, 0.001 , and 0.009 , respectively) and C_{max} ($p < 0.001$, $p = 0.001$, and 0.002 , respectively) than other genotypes. The shorter $t_{1/2}$ of 20(S)-PPD was also associated with the *ABCC2* rs2273697 GG genotype and *ABCC4* rs1926557 CC genotype ($p = 0.006$ and 0.028 , respectively). Participants who were carriers of TT genotype for *ABCC4* rs1751034 and rs1189437 showed a higher CL/F of 20(S)-PPD ($p = 0.004$ and 0.004 , respectively). The shortest t_{max} of 20(S)-PPD was found in individuals who were GG genotype of *ABCC4* rs4148546 ($p = 0.048$). *ABCC2* rs3740066 and rs717620, *ABCC4* rs1751034 and rs1189437, and *CFTR* rs2283054 were significantly associated with the Vz/F of 20(S)-PPD ($p = 0.016$, 0.015 , 0.008 , 0.039 , and 0.034 , respectively). Plasma concentration–time profiles in participants with different genotypes that significantly affect the exposure of 20(S)-PPD are presented in **Fig. 5**.

The metabolite ratios were determined by the exposure of 20(S)-PPD divided by that of CK. The metabolic ratios in different genotypes are presented in **Table 3** and **Fig. 3**. A significantly lower AUC_{last}/D ratio was found for *ABCC3* rs12051822 GG ($p = 0.027$), *ABCC4* rs4148546 GG and rs1189437 TT ($p = 0.024$ and $p < 0.001$), and *CFTR* rs2283054 GG ($p = 0.024$) carriers. In addition, *ABCC4* rs1151471 CC and rs1751034 TT were significantly associated with a higher AUC_{last}/D ratio ($p = 0.002$, $p < 0.001$, respectively). The lower C_{max}/D ratio was also significantly associated with the *ABCC4* rs1151471 TT, rs1751034 TT, and rs1189437 TT genotypes ($p = 0.001$, $p < 0.001$, $p < 0.001$, respectively) and *CFTR* rs2283054 heterozygous ($p = 0.006$).

3.3. Docking results

The MRP4 protein homology model and docking grid generation, visualized in PyMOL (a molecular visualization system), are presented in **Supplemental Fig. 3**. The 2-D docking results between CK with the MRP4 protein homology model are shown in **Supplemental Fig. 4**. The amino acid residues Gln207, Val210, Phe211, Leu367, Phe368, Pro370, Arg375, Leu942, and Trp995 were in nonbonded contact with CK, whereas Ser371 and Asn320 were involved in hydrogen bonding interactions with CK.

4. Discussions

This work was performed to explore the associations between gene polymorphisms in *NR1I2*, ABC transporter genes and the pharmacokinetics of CK and 20(S)-PPD in healthy Chinese volunteers. The *ABCC4* rs1751034 and rs1189437 polymorphisms were associated with the AUC_{last} and C_{max} of CK and 20(S)-PPD, whereas the *ABCC4* rs1151471 variant was only associated with the AUC_{last} and C_{max} of 20(S)-PPD. Besides, *CFTR* rs2283054 was significantly correlated with the AUC_{last} and C_{max} of 20(S)-PPD, whereas rs4148688 was only associated with the C_{max} of CK. Apart from the polymorphisms in ABC transporter genes, rs1464602 and rs2472682 of *NR1I2* were also found to impact the exposure of CK.

Table 1
Demographics of the study participants

Sample	N ¹⁾	Age (years)	Height (m)	Weight (kg)	BMI (kg/m ²)
Sample 1	30	21 (18–26)	1.62 (1.51–1.80)	54.3 (46.5–77.0)	20.3 (19.1–23.9)
Sample 2	24	24 (18–29)	1.65 (1.55–1.78)	56.3 (49.0–76.0)	21.2 (19.0–24.0)
Total	54	21 (18–29)	1.64 (1.51–1.80)	55.5 (46.5–77.0)	20.8 (19.0–24.0)

BMI, body mass index.

Sample 1 was pooled from three doses (100, 200, and 400 mg of ginsenoside compound K) group of the single-dose trial. Sample 2 was pooled from the food-effect trial (200 mg of ginsenoside compound K under the fasting condition). All values are presented as median (range).

¹⁾ Number of participants.

Table 2
Candidate single nucleotide polymorphisms and genotype frequencies among participants

SNP	Localization	RefSNP alleles	N ¹⁾	Genotype frequency		
				wt/wt	wt/vt	vt/vt
Transporters						
ABCC2						
rs2756109	Intron	G/T	50	21 (42.0)	25 (50.0)	4 (8.0)
rs2273697 (Val 417 Ile)	Exon	G/A	54	39 (72.2)	15 (27.8)	0 (0)
rs3740066 (Ile 1324 Ile/Met)	Exon	C/G/T	54	CC: 29 (53.7)	CT: 24 (44.4)	TT: 1 (1.9)
rs717620	5'UTR	C/T	53	31 (58.5)	22 (41.5)	0 (0)
ABCC3						
rs12051822	Intron	G/A	53	41 (77.4)	12 (22.6)	0 (0)
rs4148416 (Gly 1013 Gly)	Exon	C/T	54	40 (74.1)	14 (25.9)	0 (0)
rs4793665	Upstream gene	C/T	54	0 (0)	12 (22.2)	42 (77.8)
ABCC4						
rs4148546	Intron	G/A	54	11 (20.4)	28 (51.9)	15 (27.8)
rs1151471	Intron	C/T	51	2 (3.9)	18 (35.3)	31 (60.8)
rs1751034 (Lys 1116 Lys)	Exon	T/C	53	39 (73.6)	14 (26.4)	0 (0)
rs1189437	Intron	T/G	51	35 (68.6)	15 (29.4)	1 (2.0)
rs2274406 (Arg 317 Arg)	Exon	T/C	54	9 (16.7)	31 (57.4)	14 (25.9)
rs2274407 (Lys 304 Asn)	Exon	C/A/G/T	54	CC: 41 (75.9)	CA: 13 (24.1)	AA: 0 (0)
rs11568658 (Gly 187 Trp)	Exon	C/A	51	35 (68.6)	15 (29.4)	1 (2.0)
rs1926657	Intron	C/T	53	25 (47.2)	22 (41.5)	6 (11.3)
rs869951	Upstream gene	G/C	53	23 (43.4)	21 (39.6)	9 (17.0)
CFTR						
rs4148688	Intron	G/C	53	19 (35.8)	25 (47.2)	9 (17.0)
rs2283054	Intron	G/A	54	14 (25.9)	25 (46.3)	15 (27.8)
rs213950 (Val 470 Met)	Exon	A/G	54	9 (16.7)	25 (46.3)	20 (37.0)
rs213976	Intron	G/T	54	7 (13.0)	24 (44.4)	23 (42.6)
rs4148711	Intron	T/A	53	17 (32.1)	26 (49.1)	10 (18.9)
rs213968	Intron	C/T	54	18 (33.3)	26 (48.1)	10 (18.5)
rs1042077 (Thr 854 Thr)	Exon	T/A/G	52	TT: 17 (32.7)	GT: 25 (48.1)	GG: 10 (19.2)
rs2106155	Intron	A/C	51	8 (15.7)	20 (39.2)	23 (45.1)
rs2237726	Noncoding transcript exon	C/T	52	24 (46.2)	23 (44.2)	5 (9.6)
ABCG2						
rs2231148	Intron	T/A	46	27 (58.7)	14 (30.4)	5 (10.9)
rs2054576	Intron	A/G	54	28 (51.9)	23 (42.6)	3 (5.6)
rs12505410	Intron	T/G	54	24 (44.4)	26 (48.1)	4 (7.4)
rs2231142 (Gln 141 Lys)	Exon	G/T	45	24 (53.3)	18 (40.0)	3 (6.7)
rs6857600	Intron	C/T	53	42 (79.2)	8 (15.1)	3 (5.7)
rs3114018	Intron	A/C	53	7 (13.2)	29 (54.7)	17 (32.1)
rs2725248	Intron	A/C	52	31 (59.6)	19 (36.5)	2 (3.8)
rs17731799	Intron	G/T	53	7 (13.2)	23 (43.4)	23 (43.4)
ABCB1						
rs1128503 (Gly 412 Gly)	Exon	G/A	54	4 (7.4)	26 (48.1)	24 (44.4)
rs2032582 (Ala 893 Ser/Thr)	Exon	C/A/T	53	CC: 13 (24.5)	CA: 30 (56.6)	AA: 10 (18.9)
rs1045642 (Ile 1145 Ile)	Exon	G/A/T	53	GG: 17 (32.1)	AG: 29 (54.7)	AA: 7 (13.2)
NR1I2						
rs1464602	Intron	G/A	54	11 (20.4)	19 (35.2)	24 (44.4)
rs6785049	Intron	G/A	54	14 (25.9)	30 (55.6)	10 (18.5)
rs2276707	Intron	C/G/T	54	CC: 15 (27.8)	CT: 31 (57.4)	TT: 8 (14.8)
rs1523127	5'UTR	C/A	54	1 (1.9)	14 (25.9)	39 (72.2)
rs7643645	Intron	A/G	53	21 (39.6)	25 (47.2)	7 (13.2)
rs2472682	Intron	A/C	54	17 (31.5)	28 (51.9)	9 (16.7)

The location and RefSNP alleles' information of single nucleotide polymorphisms were obtained from Ensembl database. The wt indicates that the allele is an ancestral allele which is obtained from the Short Genetic Variations database of the NCBI. All values are expressed as number of individuals (%).

¹⁾ Number of participants; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism; wt, wild type; vt, variant type.

P-gp is one of the best characterized human efflux transporters expressed at the apical side, mediating an extrusion from inside of biomembranes for the majority of xenobiotic compounds. For an oral medicine, the first pass before entering the circulation is the brush border membranes of intestinal epithelial cells. The distribution and function of P-gp lead to the low bioavailability of its substrate compounds [24,25]. MDR1 has an unusually broad polyspecificity for numerous substrates [26], including many herbal phytochemicals [27]. Nowadays, there is more and more knowledge of its regulation, function, and effect of genetic variants, supported by the large number of *MDR1/ABCB1* gene polymorphisms that have been reported. The most widely studied variants are 1236C>T (rs1128503, p.G412G), 2677G>T/A (rs2032582, p.A893S/T), and 3435C>T (rs1045642, p.I1145I) for their high allele frequency [28].

Polymorphisms of *ABCB1* gene have been known to be associated with the pharmacokinetics and pharmacodynamics of substrate drugs. According to the results of Zhang et al, CK is absorbed by passive diffusion, accompanied with active efflux mediated by P-gp [17]. Another study confirmed that CK and 20(S)-PPD inhibit P-gp both *in vitro* and *in situ* [19]. However, to date, there has been no research on the relationship between the pharmacokinetics of CK and *ABCB1* gene polymorphisms. We first investigated the influence of *ABCB1* gene polymorphism on the exposure of CK and its metabolite 20(S)-PPD in healthy Chinese volunteers. The allele frequencies of *ABCB1* gene polymorphisms (rs1128503, rs2032582, and rs1045642) in the present study were very close to data in Asians according to the PharmGKB (<http://pharmacogenetics.ucsf.edu>) and literature [29,30]. Statistical analysis results of this study showed that none of the *ABCB1* mutations were markedly

Table 3
The impact of single nucleotide polymorphisms on ginsenoside compound K and 20(S)-PPD exposure

SNPs	Ginsenoside compound K		20(S)-PPD		Ratios (20(S)-PPD/ginsenoside compound K) %	
	C _{max} /D	AUC _{last} /D	C _{max} /D	AUC _{last} /D	C _{max} /D	AUC _{last} /D
ABCC2						
rs2756109						
GG	3.48 (1.34)	27.59 (12.16)	0.02 (0.02)	0.61 (0.41)	0.72 (0.57)	2.42 (1.84)
GT	3.58 (2.35)	27.83 (20.78)	0.03 (0.03)	0.89 (0.85)	1.45 (2.21)	5.15 (6.75)
TT	5.27 (3.56)	38.36 (23.40)	0.03 (0.02)	0.93 (0.62)	0.85 (1.08)	4.00 (4.67)
p	0.489	0.585	0.897	0.663	0.932	0.830
rs2273697 (Val 417 Ile)						
GG	3.35 (1.44)	25.98 (12.06)	0.03 (0.03)	0.83 (0.84)	1.22 (1.87)	4.19 (6.15)
GA	4.74 (3.23)	35.66 (26.59)	0.03 (0.02)	0.81 (0.63)	0.96 (1.36)	4.54 (6.68)
AA ¹⁾	—	—	—	—	—	—
p	0.184	0.322	0.803	0.873	0.429	0.802
rs3740066 (Ile 1324 Ile/Met)						
CC	4.02 (2.37)	32.09 (20.46)	0.02 (0.02)	0.64 (0.51)	0.78 (1.02)	3.03 (4.8)
CT	3.3 (1.87)	23.39 (11.335)	0.04 (0.03)	1.06 (0.99)	1.63 (2.29)	5.95 (7.51)
TT ²⁾	6.24 (—)	55.95 (—)	0.01 (—)	0.58 (—)	0.22 (—)	1.04 (—)
p	0.231	0.076	0.553	0.638	0.258	0.274
rs717620						
CC	3.98 (2.30)	31.60 (19.89)	0.02 (0.02)	0.64 (0.51)	0.77 (0.99)	3.02 (4.69)
CT	3.48 (2.01)	25.18 (13.50)	0.04 (0.04)	1.09 (1.02)	1.65 (2.39)	6.00 (7.83)
TT ¹⁾	—	—	—	—	—	—
p	0.381	0.211	0.207	0.233	0.126	0.105
ABCC3						
rs12051822						
GG	3.96 (2.39)	30.37 (18.95)	0.03 (0.03)	0.74 (0.72)	1.09 (1.81)	3.60 (5.48)
GA	3.09 (1.13)	23.66 (11.90)	0.04 (0.03)	1.17 (0.94)	1.44 (1.56)	6.84 (8.31)
AA ¹⁾	—	—	—	—	—	—
p	0.403	0.298	0.136	0.056	0.089	0.027*
rs4148416 (Gly 1013 Gly)						
CC	3.72 (2.07)	29.20 (18.77)	0.03 (0.02)	0.77 (0.69)	0.90 (1.07)	3.64 (5.23)
CT	3.78 (2.53)	27.15 (14.29)	0.04 (0.04)	0.99 (1.01)	1.87 (2.85)	6.12 (8.47)
TT ¹⁾	—	—	—	—	—	—
p	0.780	0.759	0.990	0.764	0.887	0.876
rs4793665						
CC ¹⁾	—	—	—	—	—	—
CT	4.04 (2.17)	29.14 (12.55)	0.02 (0.02)	0.61 (0.58)	0.54 (0.52)	2.27 (2.88)
TT	3.65 (2.19)	28.53 (18.94)	0.03 (0.03)	0.89 (0.82)	1.32 (1.92)	4.86 (6.83)
p	0.431	0.528	0.280	0.270	0.184	0.203
ABCC4						
rs4148546						
GG	4.87 (2.88)	39.82 (26.88)	0.02 (0.02)	0.47 (0.46)	0.41 (0.46)	1.34 (1.47)
GA	3.49 (1.91)	35.44 (12.28)	0.03 (0.03)	0.87 (0.75)	1.32 (1.94)	4.17 (4.77)
AA	3.43 (1.93)	26.51 (15.61)	0.03 (0.03)	1.01 (0.97)	1.37 (1.86)	6.66 (9.45)
p	0.159	0.101	0.235	0.139	0.060	0.024*
rs1151471						
CC ³⁾	2.08 (0.39)	13.68 (2.21)	0.09 (0.03)	2.65 (1.59)	4.33 (2.43)	20.61 (14.96)
TC	3.32 (2.06)	24.64 (13.71)	0.04 (0.03)	0.99 (0.81)	1.83 (2.49)	5.99 (7.30)
TT	4.16 (2.32)	32.65 (19.80)	0.02 (0.02)	0.58 (0.54)	0.52 (0.50)	2.12 (2.57)
p	0.114	0.056	0.014*	0.028*	0.001*	0.002*
rs1751034 (Lys 1116 Lys)						
TT	4.23 (2.32)	32.53 (18.96)	0.02 (0.02)	0.59 (0.51)	0.55 (0.50)	2.17 (2.45)
TC	2.45 (0.95)	18.58 (7.25)	0.05 (0.04)	1.51 (1.01)	2.88 (2.68)	1.39 (9.33)
CC ¹⁾	—	—	—	—	—	—
p	0.002*	0.002*	<0.001*	0.001*	<0.001*	<0.001*
rs1189437						
TT	4.06 (2.19)	31.84 (19.14)	0.02 (0.02)	0.54 (0.49)	0.50 (0.49)	2.03 (2.50)
GT	2.74 (1.29)	20.77 (9.40)	0.05 (0.03)	1.25 (0.80)	2.35 (2.55)	7.87 (7.32)
GG ²⁾	1.80 (—)	12.12 (—)	0.11 (—)	3.78 (—)	6.05 (—)	31.18 (—)
p	0.028*	0.024*	0.001*	0.001*	<0.001*	<0.001*
rs2274406 (Arg 317 Arg)						
TT	4.70 (2.25)	33.40 (14.80)	0.03 (0.03)	0.78 (0.55)	0.63 (0.46)	2.75 (2.87)
CT	3.68 (2.42)	28.24 (19.96)	0.03 (0.03)	0.77 (0.73)	1.15 (1.88)	3.81 (4.77)
TT	3.25 (1.32)	26.57 (13.77)	0.03 (0.03)	0.97 (1.01)	1.49 (1.93)	6.34 (9.72)
p	0.268	0.481	0.823	0.769	0.762	0.739
rs2274407 (Lys 304 Asn)						
CC	3.84 (2.38)	28.81 (18.82)	0.03 (0.03)	0.87 (0.83)	1.18 (1.84)	4.39 (6.18)
CA	3.43 (1.35)	28.22 (13.76)	0.03 (0.02)	0.70 (0.61)	1.04 (1.40)	3.96 (6.67)
AA ¹⁾	—	—	—	—	—	—
p	0.675	0.492	0.796	0.831	0.804	0.909
rs11568658 (Gly 187 Trp)						
CC	3.77 (2.48)	28.31 (19.56)	0.03 (0.02)	0.80 (0.74)	0.98 (1.14)	4.05 (5.68)
CA	3.72 (1.63)	30.79 (15.09)	0.04 (0.04)	1.01 (0.91)	1.73 (2.76)	5.46 (8.04)

(continued on next page)

Table 3 (continued)

SNPs	Ginsenoside compound K		20(S)-PPD		Ratios (20(S)-PPD/ginsenoside compound K) %	
	C _{max} /D	AUC _{last} /D	C _{max} /D	AUC _{last} /D	C _{max} /D	AUC _{last} /D
AA ²⁾	2.47 (–)	19.11 (–)	0.01 (–)	0.30 (–)	0.28 (–)	1.59 (–)
<i>p</i>	0.858	0.676	0.598	0.713	0.793	0.931
rs1926657						
CC	4.02 (2.74)	30.01 (21.56)	0.03 (0.03)	0.80 (0.80)	0.97 (1.26)	3.92 (6.22)
TC	3.27 (1.48)	26.38 (13.50)	0.03 (0.03)	0.95 (0.83)	1.60 (2.30)	5.58 (7.02)
TT	3.90 (1.53)	28.85 (14.52)	0.01 (0.01)	0.46 (0.52)	0.33 (0.27)	1.43 (1.27)
<i>p</i>	0.539	0.797	0.274	0.336	0.177	0.279
rs869951						
GG	3.62 (1.92)	27.23 (15.19)	0.03 (0.02)	0.92 (0.82)	1.28 (1.55)	5.68 (7.87)
CG	4.21 (2.65)	32.82 (22.43)	0.02 (0.02)	0.59 (0.53)	0.49 (0.46)	2.10 (2.49)
CC	3.17 (1.46)	24.46 (7.59)	0.05 (0.05)	1.15 (1.09)	2.29 (3.18)	5.75 (3.18)
<i>p</i>	0.489	0.512	0.131	0.188	0.061	0.096
CFTR						
rs4148688						
GG	4.27 (1.88)	32.12 (14.86)	0.02 (0.02)	0.65 (0.55)	0.59 (0.64)	2.55 (3.22)
CG	3.04 (2.18)	24.59 (19.45)	0.03 (0.04)	0.87 (0.98)	1.49 (0.64)	5.04 (7.33)
CC	3.90 (1.72)	29.35 (15.08)	0.04 (0.02)	0.99 (0.52)	1.44 (1.59)	6.00 (7.81)
<i>p</i>	0.027*	0.139	0.332	0.427	0.203	0.332
rs2283054						
GG	3.97 (1.92)	27.39 (12.72)	0.02 (0.02)	0.58 (0.60)	0.58 (0.73)	2.77 (3.74)
GA	3.31 (1.41)	26.96 (13.21)	0.02 (0.02)	0.59 (0.46)	0.68 (0.58)	2.55 (2.37)
AA	4.24 (3.23)	32.71 (26.47)	0.05 (0.04)	1.45 (1.02)	2.47 (2.79)	8.60 (9.86)
<i>p</i>	0.620	0.963	0.002*	0.009*	0.006*	0.024*
rs213950 (Val 470 Met)						
AA	3.90 (1.72)	29.35 (15.08)	0.04 (0.02)	0.99 (0.52)	1.44 (1.59)	6.00 (7.81)
AG	3.36 (2.54)	26.46 (20.44)	0.03 (0.04)	0.91 (0.99)	1.45 (2.26)	4.96 (7.34)
GG	4.14 (1.85)	31.13 (15.12)	0.02 (0.02)	0.64 (0.54)	0.64 (0.66)	2.67 (3.18)
<i>p</i>	0.176	0.423	0.362	0.456	0.294	0.402
rs213976						
GG	3.62 (1.91)	27.63 (17.53)	0.04 (0.02)	1.12 (0.43)	1.68 (1.73)	7.39 (8.42)
GT	3.63 (2.58)	28.13 (20.65)	0.03 (0.03)	0.83 (0.78)	1.32 (2.08)	4.01 (4.96)
TT	3.89 (1.85)	29.55 (14.70)	0.02 (0.03)	0.74 (0.87)	0.81 (1.29)	3.63 (6.72)
<i>p</i>	0.662	0.756	0.177	0.167	0.156	0.136
rs4148711						
TT	3.89 (1.63)	29.34 (14.49)	0.02 (0.02)	0.63 (0.57)	0.66 (0.71)	2.87 (3.42)
AT	3.43 (2.53)	27.70 (20.72)	0.03 (0.04)	0.90 (0.98)	1.40 (2.23)	4.70 (7.26)
AA	3.81 (1.64)	28.15 (14.72)	0.04 (0.02)	0.97 (0.49)	1.40 (1.51)	5.87 (7.37)
<i>p</i>	0.366	0.799	0.263	0.378	0.274	0.382
rs213968						
CC	4.14 (1.90)	30.36 (14.71)	0.02 (0.02)	0.64 (0.55)	0.65 (0.69)	2.81 (3.33)
CT	3.43 (2.53)	27.70 (20.72)	0.03 (0.04)	0.90 (0.98)	1.40 (2.23)	4.70 (7.26)
TT	3.81 (1.64)	28.15 (14.72)	0.04 (0.02)	0.97 (0.49)	1.40 (1.51)	5.87 (7.37)
<i>p</i>	0.255	0.679	0.292	0.405	0.255	0.376
rs1042077 (Thr 854 Thr)						
TT	4.12 (1.95)	30.54 (15.14)	0.02 (0.02)	0.68 (0.55)	0.68 (0.70)	2.95 (3.37)
GT	3.46 (2.58)	27.96 (21.11)	0.03 (0.04)	0.92 (0.99)	1.44 (2.26)	4.85 (7.37)
GG	3.81 (1.64)	28.15 (14.72)	0.04 (0.02)	0.97 (0.49)	1.40 (1.51)	5.87 (7.37)
<i>p</i>	0.316	0.715	0.377	0.500	0.366	0.485
rs2106155						
AA	3.41 (1.86)	25.97 (16.89)	0.04 (0.02)	1.09 (0.41)	1.67 (1.60)	7.22 (7.81)
CA	4.02 (2.69)	31.04 (21.72)	0.04 (0.03)	0.88 (0.82)	1.39 (2.26)	4.03 (5.36)
CC	3.86 (1.85)	29.28 (14.69)	0.02 (0.02)	0.76 (0.87)	0.80 (1.29)	3.73 (6.73)
<i>p</i>	0.767	0.642	0.151	0.171	0.120	0.110
rs2237726						
CC	3.89 (1.81)	29.76 (14.41)	0.02 (0.02)	0.72 (0.85)	0.80 (1.27)	3.53 (6.59)
CT	3.35 (2.25)	26.16 (19.95)	0.03 (0.03)	0.84 (0.78)	1.42 (2.13)	4.43 (5.17)
TT	3.98 (2.20)	32.16 (19.24)	0.03 (0.02)	1.10 (0.47)	1.63 (2.04)	7.40 (10.14)
<i>p</i>	0.433	0.531	0.351	0.285	0.291	0.295
ABCG2						
rs2231148						
TT	3.09 (1.18)	23.05 (8.91)	0.03 (0.02)	0.80 (0.80)	1.11 (1.45)	4.75 (7.36)
AT	4.87 (3.24)	39.28 (26.36)	0.03 (0.04)	0.93 (0.97)	1.42 (2.72)	4.06 (6.40)
AA	4.43 (2.92)	35.54 (21.89)	0.03 (0.01)	0.68 (0.31)	0.84 (0.60)	3.01 (2.32)
<i>p</i>	0.175	0.060	0.889	0.887	0.670	0.681
rs2054576						
AA	3.31 (1.53)	25.61 (12.77)	0.03 (0.03)	0.78 (0.79)	1.26 (1.98)	4.17 (5.11)
GA	4.31 (2.78)	32.78 (22.38)	0.03 (0.02)	0.73 (0.53)	0.83 (1.11)	3.30 (5.27)
AA	3.29 (1.35)	25.70 (13.01)	0.06 (0.04)	2.05 (1.51)	2.56 (3.02)	12.95 (15.83)
<i>p</i>	0.422	0.539	0.203	0.154	0.304	0.197

Table 3 (continued)

SNPs	Ginsenoside compound K		20(S)-PPD		Ratios (20(S)-PPD/ginsenoside compound K) %	
	C _{max} /D	AUC _{last} /D	C _{max} /D	AUC _{last} /D	C _{max} /D	AUC _{last} /D
rs12505410						
TT	3.00 (1.05)	22.29 (7.69)	0.02 (0.03)	0.63 (0.82)	0.97 (1.54)	4.03 (7.68)
GT	4.39 (2.76)	33.35 (21.92)	0.04 (0.003)	1.00 (0.74)	1.36 (2.02)	4.63 (5.21)
GG	3.96 (1.91)	36.50 (20.67)	0.03 (0.02)	0.92 (0.62)	0.90 (0.55)	3.61 (2.83)
p	0.187	0.112	0.100	0.059	0.520	0.421
rs2231142 (Gln 141 Lys)						
GG	3.51 (1.56)	27.45 (13.31)	0.02 (0.02)	0.62 (0.53)	0.78 (0.71)	2.95 (3.04)
GT	3.89 (2.60)	31.50 (23.56)	0.03 (0.04)	0.88 (0.83)	1.45 (2.39)	4.13 (5.64)
TT	3.29 (1.35)	25.70 (13.01)	0.06 (0.04)	2.05 (1.51)	2.56 (3.02)	12.95 (15.83)
p	0.945	0.877	0.111	0.071	0.177	0.119
rs6857600						
CC	3.72 (2.28)	29.60 (19.18)	0.03 (0.03)	0.89 (0.83)	1.26 (1.91)	4.61 (6.81)
TC	3.79 (1.18)	26.00 (7.52)	0.02 (0.02)	0.65 (0.64)	0.75 (0.80)	2.91 (3.24)
TT	2.37 (0.75)	16.47 (5.68)	0.02 (0.02)	0.48 (0.53)	0.95 (1.30)	4.27 (5.76)
p	0.428	0.350	0.441	0.479	0.520	0.683
rs3114018						
AA	3.40 (1.60)	25.71 (14.88)	0.03 (0.02)	0.91 (0.71)	0.96 (0.87)	4.78 (4.26)
CA	4.02 (2.12)	30.74 (15.11)	0.03 (0.03)	0.80 (0.73)	1.17 (1.94)	3.46 (4.62)
AA	3.36 (2.54)	26.43 (22.97)	0.03 (0.03)	0.88 (0.92)	1.25 (1.73)	5.73 (8.96)
p	0.405	0.310	0.996	0.943	0.877	0.779
rs2725248						
AA	3.68 (2.42)	26.94 (18.76)	0.02 (0.03)	0.69 (0.78)	0.92 (1.40)	3.94 (7.05)
CA	3.96 (1.89)	31.91 (15.94)	0.04 (0.03)	1.05 (0.79)	1.53 (2.29)	4.84 (5.49)
CC	4.46 (1.08)	40.01 (16.71)	0.03 (0.03)	0.96 (1.03)	0.74 (0.83)	3.24 (3.92)
p	0.601	0.252	0.198	0.144	0.397	0.429
rs17731799						
GG	3.40 (1.60)	25.71 (14.88)	0.03 (0.02)	0.91 (0.71)	0.96 (0.87)	4.78 (4.26)
GT	4.13 (1.88)	32.10 (14.45)	0.03 (0.03)	0.82 (0.77)	1.28 (2.16)	3.65 (5.14)
TT	3.47 (2.62)	26.40 (21.35)	0.03 (0.03)	0.80 (0.85)	1.08 (1.53)	4.76 (7.86)
p	0.280	0.173	0.967	0.882	0.893	0.733
ABCB1						
rs1128503 (Gly 412 Gly)						
GG	4.67 (3.44)	33.81 (17.43)	0.02 (0.02)	0.73 (0.70)	0.46 (0.25)	1.89 (0.94)
AG	3.66 (2.34)	28.43 (20.03)	0.03 (0.03)	0.77 (0.80)	1.12 (1.52)	4.67 (7.55)
AA	3.67 (1.80)	28.07 (15.30)	0.03 (0.03)	0.90 (0.79)	1.30 (2.07)	4.27 (5.15)
p	0.743	0.690	0.937	0.865	0.869	0.890
rs2032582 (Ser 893 Ala/Thr)						
CC	4.42 (3.14)	33.69 (25.60)	0.03 (0.02)	0.92 (0.62)	0.74 (0.49)	3.50 (2.84)
CA	3.46 (1.72)	27.47 (15.11)	0.03 (0.03)	0.82 (0.92)	1.34 (2.13)	4.60 (7.08)
AA	3.81 (2.02)	26.68 (12.71)	0.03 (0.02)	0.78 (0.53)	1.20 (1.55)	4.64 (7.42)
p	0.581	0.648	0.800	0.665	0.980	0.888
rs1045642 (Ile 1145 Ile)						
GG	4.13 (2.82)	31.19 (22.91)	0.03 (0.02)	0.82 (0.62)	0.80 (0.66)	3.23 (2.77)
AG	3.50 (1.91)	27.71 (15.58)	0.03 (0.03)	0.87 (0.93)	1.49 (2.26)	5.28 (8.05)
AA	4.03 (1.47)	28.81 (11.93)	0.02 (0.02)	0.59 (0.42)	0.54 (0.42)	1.99 (1.55)
p	0.526	0.798	0.769	0.649	0.637	0.637
NR1I2						
rs1464602						
GG	2.65 (1.27)	20.43 (9.71)	0.04 (0.04)	1.09 (1.05)	2.40 (3.04)	7.81 (8.88)
AG	4.62 (2.09)	33.46 (13.69)	0.03 (0.03)	0.85 (0.89)	0.80 (1.32)	3.45 (6.88)
AA	3.54 (2.35)	28.65 (21.81)	0.02 (0.02)	0.69 (0.50)	0.85 (0.79)	3.33 (3.32)
p	0.010*	0.035*	0.867	0.967	0.428	0.609
rs6785049						
GG	3.22 (1.69)	24.29 (12.99)	0.04 (0.04)	1.11 (1.21)	1.98 (2.90)	6.66 (9.43)
AG	4.27 (2.46)	32.86 (19.88)	0.03 (0.02)	0.72 (0.54)	0.69 (0.58)	2.64 (2.47)
AA	2.88 (1.46)	22.20 (13.19)	0.03 (0.02)	0.75 (0.59)	1.37 (1.63)	5.90 (7.79)
p	0.085	0.078	0.914	0.888	0.440	0.396
rs2276707						
CC	3.46 (1.86)	25.30 (13.16)	0.03 (0.02)	0.79 (0.54)	1.15 (1.37)	5.07 (6.48)
CT	3.88 (2.41)	30.38 (20.00)	0.03 (0.03)	0.79 (0.77)	1.12 (1.87)	3.49 (4.74)
TT	3.71 (1.90)	28.36 (15.79)	0.03 (0.03)	1.04 (1.19)	1.27 (1.99)	5.91 (10.37)
p	0.843	0.664	0.967	0.776	0.900	0.610
rs1523127						
CC ²⁾	3.49 (-) ²⁾	28.50 (-) ²⁾	0.01 (-) ²⁾	0.32 (-) ²⁾	0.22 (-) ²⁾	1.14 (-) ²⁾
CA	3.53 (1.71)	27.08 (14.85)	0.02 (0.02)	0.73 (0.52)	0.87 (0.80)	3.64 (3.52)
AA	3.82 (2.36)	29.24 (18.87)	0.03 (0.03)	0.87 (0.86)	1.27 (1.98)	4.60 (7.04)
p	0.984	0.941	0.723	0.946	0.747	0.909
rs7643645						
AA	3.76 (2.21)	26.64 (13.51)	0.03 (0.03)	0.95 (0.87)	1.16 (1.57)	5.31 (8.06)
AG	3.41 (1.51)	27.63 (13.83)	0.03 (0.03)	0.66 (0.76)	1.21 (2.09)	3.34 (5.02)
GG	5.06 (3.69)	39.83 (34.46)	0.04 (0.02)	1.14 (0.42)	1.03 (0.83)	4.98 (4.18)
p	0.499	0.610	0.326	0.139	0.699	0.300

(continued on next page)

Table 3 (continued)

SNPs	Ginsenoside compound K		20(S)-PPD		Ratios (20(S)-PPD/ginsenoside compound K) %	
	C_{max}/D	AUC_{last}/D	C_{max}/D	AUC_{last}/D	C_{max}/D	AUC_{last}/D
rs2472682						
AA	2.87 (1.20)	21.56 (9.07)	0.04 (0.04)	1.08 (1.14)	2.06 (2.76)	7.47 (9.73)
CA	4.45 (2.60)	34.34 (20.92)	0.02 (0.02)	0.66 (0.50)	0.59 (0.51)	2.12 (1.73)
CC	3.17 (1.39)	24.44 (13.14)	0.03 (0.02)	0.87 (0.59)	1.17 (0.97)	5.01 (4.45)
<i>p</i>	0.050	0.037*	0.716	0.629	0.227	0.221

AUC_{last}/D , the area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration normalized to doses of ginsenoside compound K; C_{max}/D , the maximum plasma concentration normalized to doses of ginsenoside compound K; PPD, protopanaxadiol; SD, standard deviation.

All values are presented as mean (SD). –, not applicable. * $p < 0.05$.

¹⁾ $n = 0$.

²⁾ $n = 1$.

³⁾ $n = 2$.

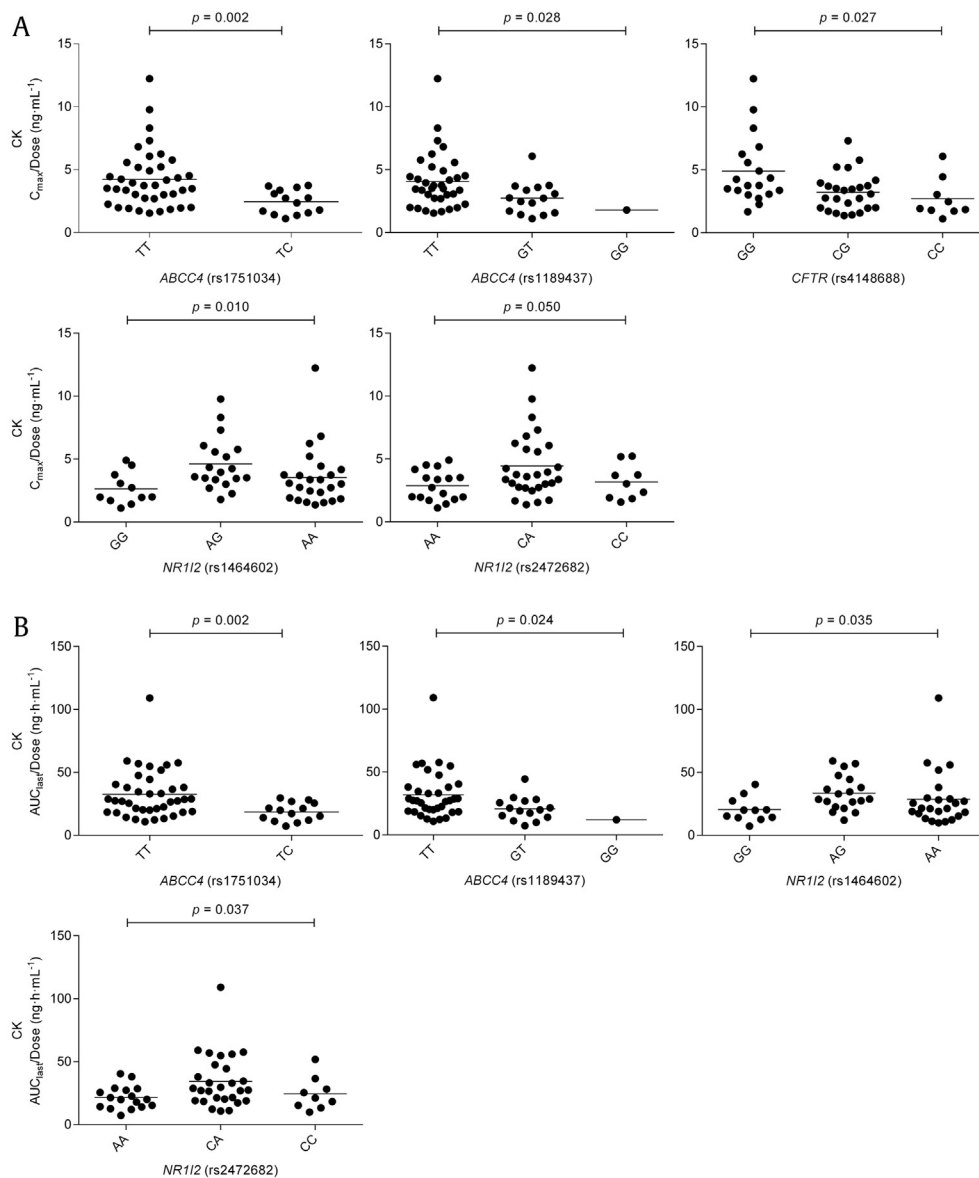


Fig. 1. Distribution of the CK pharmacokinetic parameters of the *ABCC4* (rs1751034 and rs1189437), and *NR112* (rs1464602 and rs2472682) gene variants. (A) CK $C_{max}/Dose$. (B) CK $AUC_{last}/Dose$. The mean is represented by a horizontal line through the given point. AUC_{last} , area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration;

CK, ginsenoside compound K; C_{max} , maximum concentrations.

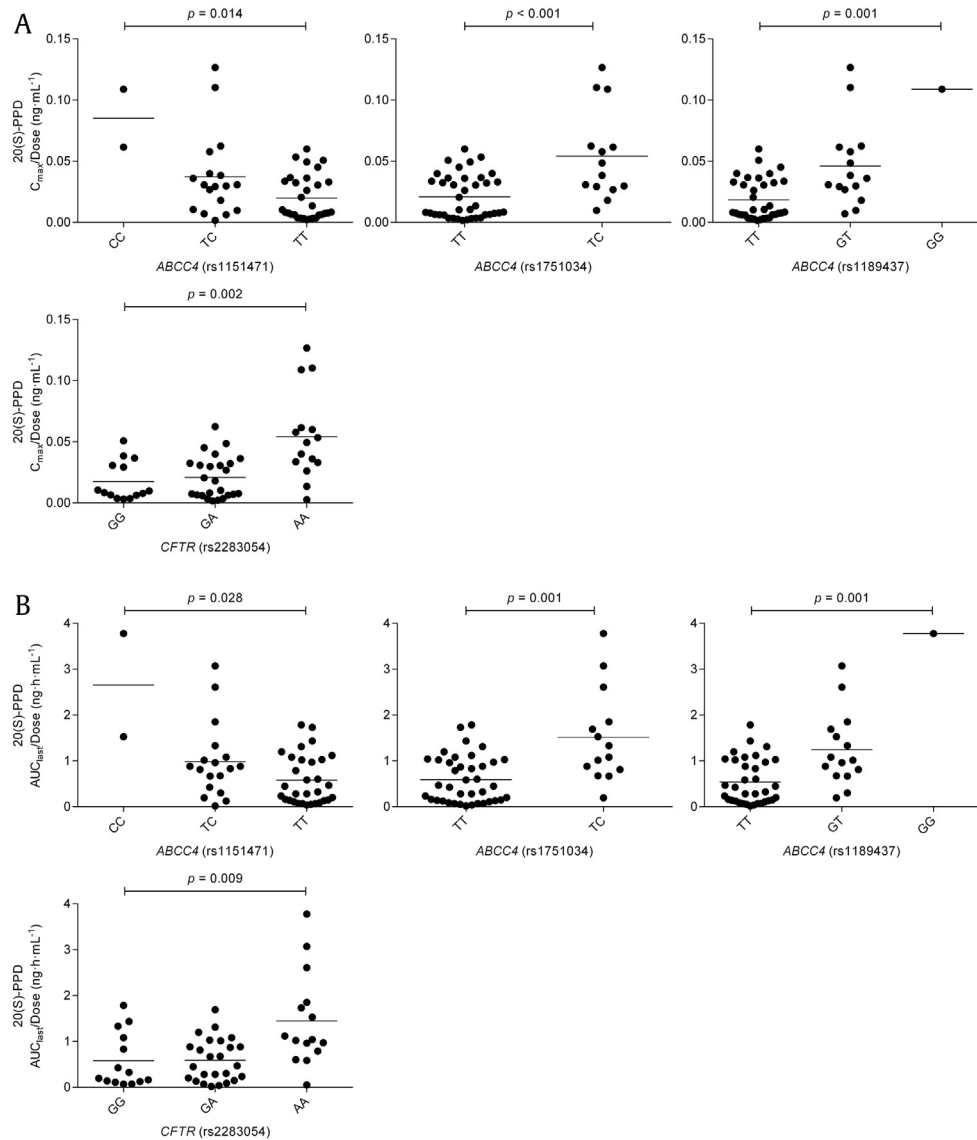


Fig. 2. Distribution of the 20(S)-PPD pharmacokinetic parameters of the *ABCC4* (rs1151471, rs1751034, and rs1189437) and *CFTR* (rs2283054) gene variants. (A) 20(S)-PPD C_{\max}/Dose . (B) 20(S)-PPD $AUC_{\text{last}}/\text{Dose}$. The mean is represented by a horizontal line through the given point. AUC_{last} , area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration; C_{\max} , maximum concentrations; PPD, protopanaxadiol.

correlated with the AUC_{last} and C_{\max} of CK or 20(S)-PPD or the ratio of the parent drug and metabolites, indicating no requirement of dose adjustment of CK according to the selected gene polymorphisms of *ABCB1*. Although the connection between absorption and transporters has been demonstrated in pre-clinical studies, both *in vitro* and *in vivo*, not all gene polymorphisms can affect the pharmacokinetics, treatment response, and drug-related toxicity. For example, it is widely known that P-gp plays a key role in the absorption and distribution of tacrolimus, an immunosuppressive agent, but most researches failed to find any impact of *ABCB1* gene polymorphisms on the pharmacokinetics of tacrolimus [31–33]. Therefore, we cannot deny the reported pre-clinical conclusions that the absorption of CK is mediated by P-gp. The reasons behind the failure to prove this connection in CK might include the following three points: First, the size of certain genotype groups analyzed in our study was relatively small, so these findings cannot rule out a potential influence that might be discerned in a larger population; Second, we screened the most frequently investigated variants and ignored a large number of relatively rare variants, and

the influence of rare variants in *ABCB1* on drug pharmacokinetics and/or pharmacodynamics could be larger than that of common missense variants; Finally, the dose range (100–400 mg) may be beyond the saturated dose of P-gp in the intestine, thus masking the transport function of P-gp.

The C subset of the ABC transporter family has recently started attracting increasing attention. To date, 12 members of the human ABCC subfamily have been identified, including nine MRPs, CFTR (also named ABCC7), and two sulfonylurea receptors, namely SUR1 (ABCC8) and SUR2A/B (ABCC9) [34]. Their dominant expression in intestinal, liver, kidney, and blood-tissue barriers indicates an important role of ABCC transporters in absorption, distribution, metabolism, and excretion processes of oral drugs. So far, just MRPs 1–5 have been validated to have a conclusive role in the disposition of drugs [35,36], whereas CFTR was identified as a chloride channel responsible for the regulation of other ion channels, including the amiloride-sensitive epithelial sodium channel [37,38]. To our knowledge, no studies on the interactions of MRP2, 3, and 4 and CFTR with CK have been reported. Moreover, there have been no

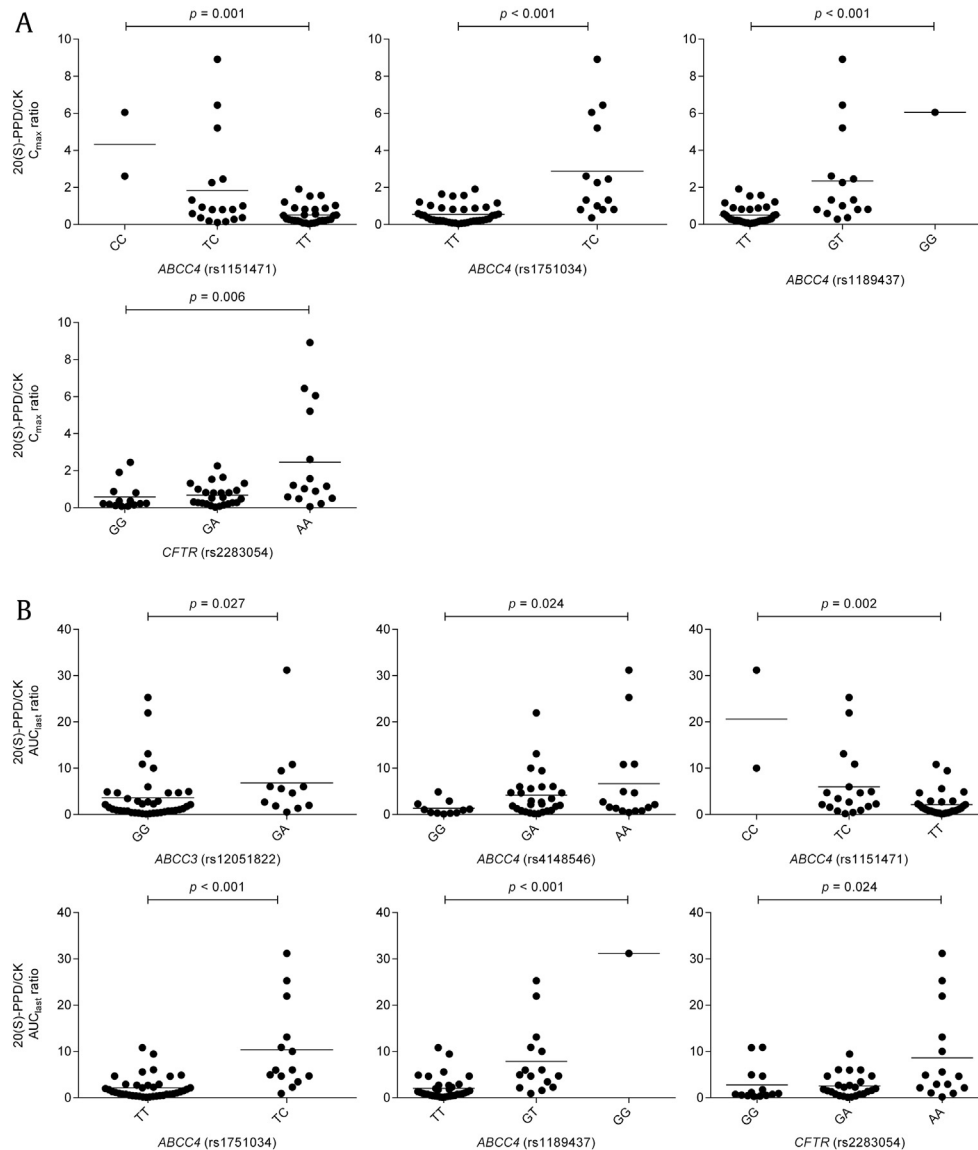


Fig. 3. Distribution of the pharmacokinetic parameters ratio of 20(S)-PPD to CK of the *ABCC3* (rs12051822), *ABCC4* (rs4148546, rs1151471, rs1751034, and rs1189437), and *CFTR* (rs2283054) gene variants. (A) $C_{max}/Dose$ ratio. (B) $AUC_{last}/Dose$ ratio. The mean is represented by a horizontal line through the given point. AUC_{last} , area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration; CK, ginsenoside compound K; C_{max} , maximum concentrations; PPD, protopanaxadiol.

reports on the impact of *CFTR* on drug disposition. In the present study, we did not detect any association between genetic variants of *ABCC2* and CK exposure in healthy Chinese individuals. Of the four SNPs of *ABCC3* screened, only the rs12051822 G>A polymorphism affected the AUC_{last} ratio of 20(S)-PPD to CK. Surprisingly, *ABCC4* gene mutations had a significant influence on the pharmacokinetics of CK, with four SNPs (rs1189437, rs1751034, rs1151471, and rs4148546) apparently associated with the PK parameters of CK or 20(S)-PPD. Among the variants in the *MRP4* gene, two SNPs (rs1751034 and rs1189437) had good correlations with the C_{max} and AUC_{last} of both CK and 20(S)-PPD, as well as their ratio. Although the rs1751034 T>C polymorphism is a synonymous variant, Sanchez-Martin et al showed a significant difference between the *ABCC4* rs1751034 T>C polymorphism and the pharmacokinetics of a nonnucleoside reverse transcriptase inhibitor, efavirenz [39]. The reason for this is that although a synonymous variant has no impact on the primary structure, there is a formal possibility that the polymorphism affects the secondary structure

of the mRNA by altering its stability and/or translatability, thus resulting to a diversity in protein expression levels [40]. Consistent with results reported in the literature, individuals with rs1751034 TC genotype in *ABCC4* had a lower plasma concentration and exposure of oral compounds than those with TT genotype [39]. The other three mutation sites (rs1189437, rs1151471, and rs4148546) belong to intron variants, and there have been no published studies on the association between drug pharmacokinetics and any of them, as far as we know. In general, the SNPs in regulatory regions and introns of the *MRP* genes are to a great extent unknown. Another unexpected result was that *CFTR/ABCC7* gene mutations could influence the pharmacokinetics of oral CK. The results indicated that *ABCC7* rs4148688 homozygous volunteers had an increased C_{max} of CK compared with heterozygous volunteers and the rs2283054 mutation was significantly associated with the C_{max} and AUC_{last} of 20(S)-PPD. In addition, three variants of *ABCC7* (rs213976, rs2106155, and rs2237726) impacted the $t_{1/2}$ of CK ($p < 0.05$). Mutations in the *CFTR* gene were found in patients with

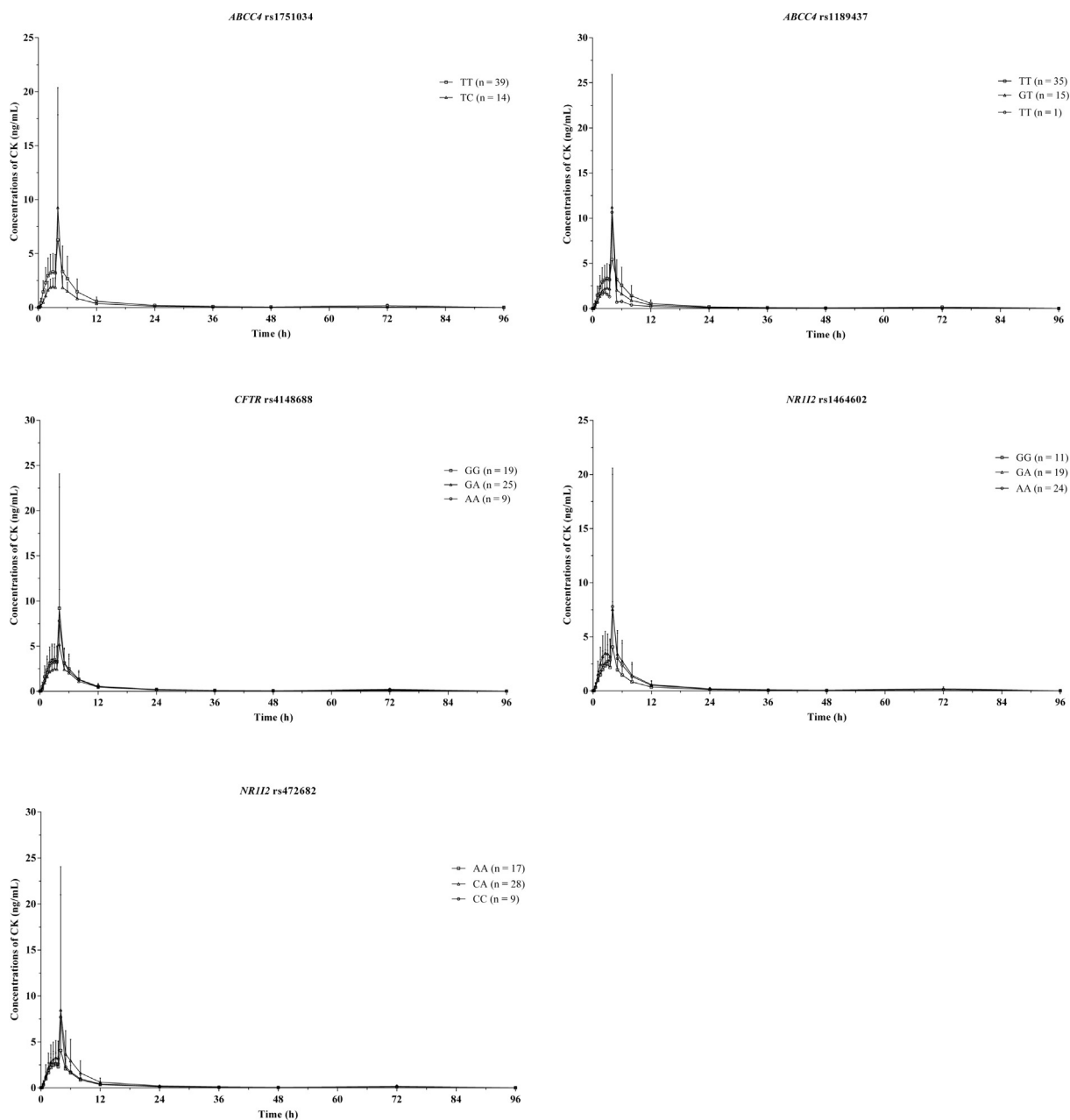


Fig. 4. Pharmacokinetic profiles of CK after a single oral dose of CK in 54 healthy Chinese volunteers with different *ABCG4*, *CFTR*, or *NR1I2* genotypes (mean \pm SD). CK, ginsenoside compound K; SD, standard deviation. All below error bars have been omitted for clarity.

cystic fibrosis and congenital bilateral absence of the vas deferens [41,42], and much attention has been paid on its role in disease susceptibility rather than in drug pharmacokinetics. Although *CFTR* has been associated with bile secretion and gallbladder emptying [43,44], we are unsure whether the PK differences of CK caused by *CFTR* mutations were due to the direct transport function, the increased solubility of CK by bile, or other physiological factors, which eventually affect its bioavailability. These results concerning the impact of *CFTR* mutations on the pharmacokinetics of CK and 20(S)-PPD provided a new sight into the function of *CFTR*, which should be validated in the future.

Not only *ABCB1* and the *CFTR*/*MRP* (*ABCC*) subfamily but also *BCRP* is an important transporter which has elicited increased research attention recently. Numerous functional assessment experiments performed on animal models and multiple

pharmacogenetics studies in humans have concluded that *ABCG2* contributes to poor drug bioavailability [45–48]. *ABCG2* efficiently extrudes various compounds from cells, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, antibacterials, antineoplastic agents, phytoestrogens etc. [49,50]. To date, investigations in different races or subpopulations have revealed about 200 genetic polymorphisms of *ABCG2*, and many of these have been analyzed to evaluate their impact on *ABCG2* mRNA stability, expression, function, or clinical outcome. Unfortunately, no significant differences between the PK parameters of CK or 20(S)-PPD and selected *ABCG2* gene polymorphisms were found in our study.

PXR is an “adopted” orphan nuclear receptor encoded by the *NR1I2* gene. It was involved in the present study as a main regulator of transporters, acting as a promiscuous receptor that binds to chemically diverse compounds. Generally, PXR is activated by a

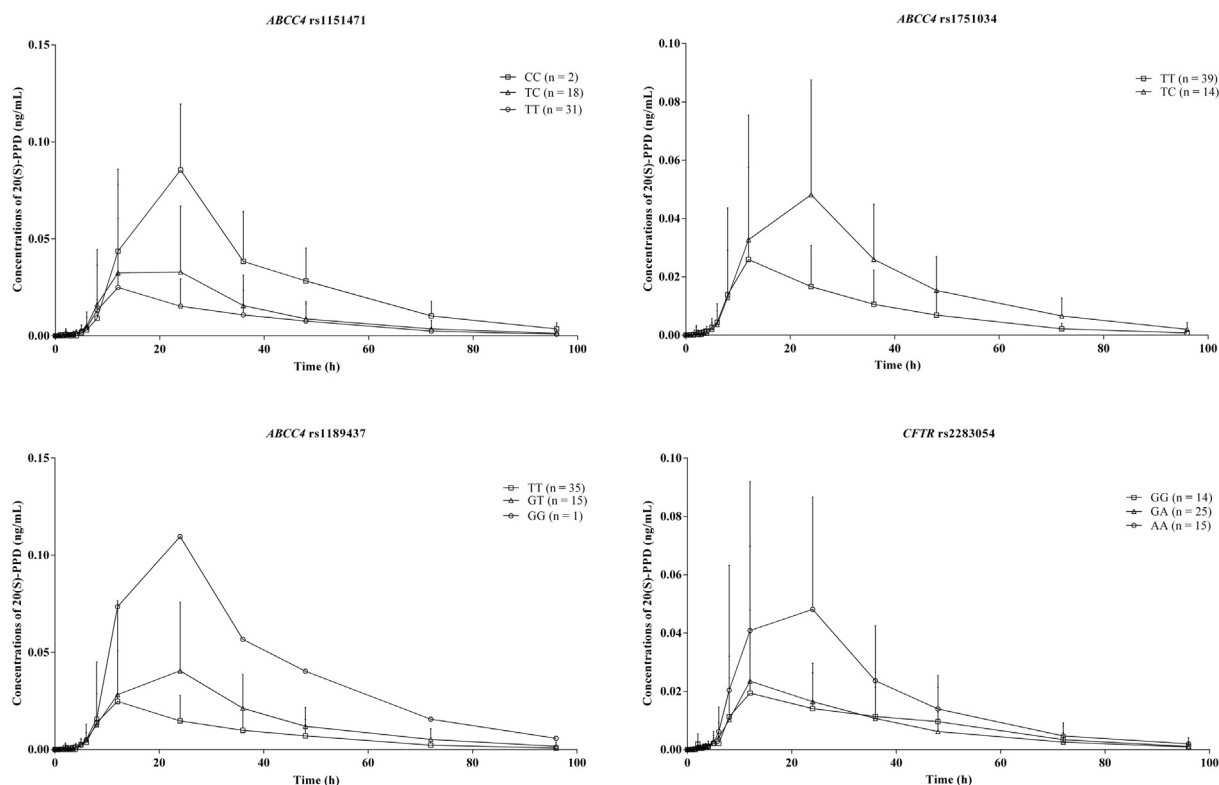


Fig. 5. Pharmacokinetic profiles of 20(S)-PPD after a single oral dose of CK in 54 healthy Chinese volunteers with different *ABCC4* or *CFTR* genotypes (mean \pm SD). CK, ginsenoside compound K; PPD, protopanaxadiol; SD, standard deviation. All below error bars have been omitted for clarity.

large and flexible ligand-binding cavity [51,52]. Studies on the regulatory role of PXR in transporters have reported that PXR increases MDR1 in LS174T cells [53], MRP2 in human liver slices [54] and intestine [55], MRP3 in human hepatocytes [56], BCRP in MDA-MB-231 and MCF-7 cells [57] etc. Knowledge concerning the polymorphisms of *NR112* may help with understanding the variations in drug pharmacokinetics and pharmacodynamics. According to our results, rs1464602 and rs2472682 were significantly associated with the oral bioavailability of CK. One previous study also reported the impact of rs2472682 (A>C) mutation on the pharmacokinetics of a drug as its results confirmed that the rs2472682 (A>C) mutation of PXR exhibited a significant correlation with stable warfarin doses [58]. Although PXR rs2472682 is detected in the intron region, on one hand, this noncoding region SNP could result in the production of different protein isoforms by altering the stability and degradation of messenger RNA (mRNA), gene expression, and alternative splicing [59]. On the other hand, this SNP might also alleviate interaction with its partner microRNA (miRNA) and result in the regulation of target genes through combining with the complementary regions of transcripts, eventually leading to repression of translation or mRNA degradation [60].

The results of this study point to a crucial role of MRP4 in the pharmacokinetics of CK *in vivo*. However, owing to the lack of data on the interaction between MRP4 and CK, we first aimed to prove that CK is a substrate for MRP4. Classic methods include intestinal perfusion, the Caco-2 monolayer model, and so on [61]. However, these classic methods have obvious limitations. On the one hand, *in vitro* experiments are generally time-consuming. On the other hand, probe drugs of common ABC transporters are usually not specific. By comparison, the molecular docking technique can overcome the aforementioned defects to some extent. In addition, a more advanced technology is MDs—a computer simulation method

for investigating the physical movements of molecules and atoms [62]. Regrettably, it is costly to allow the flexibility of the protein to be calculated, and it is still unrealistic compared with the current most advanced technology, especially for proteins with unknown 3-D structures. Consequently, we performed a preliminary docking study to investigate whether CK is a substrate of MRP4. Wittgen et al suggested that Phe368 of MRP4 plays an essential role in its substrate-specific excretion [63]. Our docking results showed that the amino acid residue Phe368 was in nonbonded contact with CK, which could be served as a theoretical basis for the transport of CK through MRP4 *in vivo*. Furthermore, it helps explain the significant correlation between *ABCC4* polymorphisms and the PK parameters of CK. Work in next stage will include the validation of this result using the classic approaches.

Integrating all aforementioned information, we did not find strongly significant correlations between the pharmacokinetics of CK and the gene polymorphisms of transporters including P-gp, which has been demonstrated to mediate the transport of CK, as well as *MRP2*, *MRP3*, and *BCRP*, which have been studied extensively. Nevertheless, our results revealed that *MRP4* gene polymorphisms could impact the disposition of CK, while *CFTR* and *NR112* mutations play indispensable roles in the pharmacokinetics of CK. To our knowledge, this was the first investigation on the impact of gene polymorphisms in *CFTR* on drug pharmacokinetics in clinical trials. Without doubt, further researches are required to study the mechanisms underlying the phenomenon observed in this work. In addition, we cannot conclude that the transporters whose polymorphisms displayed no significant associations with the pharmacokinetics of CK do not mediate the transport of CK *in vivo*. First, the dose range of CK in this study may lead to the over saturation of its transport proteins; thus, the variations of transporter expression or activity caused by gene mutations did not impact the oral bioavailability of CK. Another unneglectable

issue is the counteractive effect among transporters, caused by the differences in tissue distribution and function, and the fact that CK may behave as a substrate for several transporters at the same time. In addition, there are some unavoidable limitations in our experiments. Considering the sample size of the present study, the selection of mutation sites to be assessed was based on a higher mutation rate in the Han population of South China according to a database (1000 Genomes Browser <https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>), which inevitably ignores some important mutation sites. Moreover, the differences in study population and sample size may also cause inconsistent conclusions. In this sense, further studies in different and larger populations are essential. Moreover, the variability in pharmacokinetics and pharmacodynamics induced by transporters may be attributed not only to genetic polymorphisms but also to transcriptional regulations or posttranscriptional modifications, suggesting that we should perform a multilevel study to gain a more comprehensive understanding of individual differences in the pharmacokinetics of CK.

5. Conclusions

This was the first study to investigate the impact of genetic polymorphisms on the pharmacokinetics of CK and 20(S)-PPD. The research results showed that the *ABCC4* rs1751034 and rs1189437 were associated with the disposition of both CK and its metabolite 20(S)-PPD. In contrast, *NR1I2* rs1464602 and rs2472682 were solely correlated with the pharmacokinetics of CK. Thus, these hereditary variances could partly elucidate the interindividual differences in the pharmacokinetics of CK.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jgr.2018.04.003>.

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