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Original Article

Association of CYP3A polymorphisms with the pharmacokinetics of cyclosporine A in early post-renal transplant recipients in China

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Aim: To evaluate retrospectively the association of cytochrome P450 3A (CYP3A) and ATP-binding cassette sub-family B member 1 (ABCB1) gene polymorphisms with the pharmacokinetics of cyclosporine A (CsA) in Chinese renal transplant patients.
Methods: One hundred and twenty-six renal transplant patients were recruited. Blood samples were collected, and corresponding clinical indices were recorded on the seventh day after the procedure. The patients were genotyped for CYP3A4*1G, CYP3A5*3C, ABCB1 1236 C>T, ABCB1 2677 G>T/A, and ABCB1 3435 C>T polymorphisms. Whole blood trough concentrations of CsA at time zero (C_0) were measured before the drug administration. A multiple regression model was developed to analyze the effects of genetic factors on the CsA dose-adjusted C_0 (C_0/dose) based on several clinical indices.
Results: The CYP3A5*3C polymorphism influenced the C_0 and C_0/dose of CsA, which were significantly higher in patients with the GG genotype than in patients with the AA or GA genotypes. No significant differences were detected for other SNPs (CYP3A4*1G, ABCB1 1236 C>T, ABCB1 2677 G>T/A, and ABCB1 3435 C>T). In a univariate analysis using Pearson's correlation test, age, hemoglobin, blood urea nitrogen and blood creatinine levels were significantly correlated with the log-transformed CsA C_0/dose. In the multiple regression model, CYP3A5*3C, age, hemoglobin and blood creatinine level were associated with the log-transformed CsA C_0/dose.
Conclusion: CYP3A5*3C correlates with the C_0/dose of CsA on the seventh day after renal transplantation. The allele is a putative indicator for the optimal CsA dosage in the early phase of renal transplantation in the Chinese population.

Keywords: cyclosporine A; pharmacokinetics; CYP3A; ABCB1; gene polymorphism; pharmacogenetics; renal transplantation

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Introduction

Cyclosporine A (CsA) is a calcineurin inhibitor (CNI) that is efficient but costly in the clinic. Since 1978, it has been widely used to reduce delayed graft function (DGF) and the acute rejection (AR) response of patients receiving a renal transplant {Colombo, 2011 #4}[1]. Because of the narrow therapeutic window and large inter-individual variations in pharmacokinetics, therapeutic drugs are monitored in kidney transplant recipients to achieve target therapeutic trough blood levels[2]. Currently, the blood trough concentration at zero (C_0) is considered to be influenced by several clinical factors, including age, the hemoglobin level, and the blood creatinine (BCr) level[3]. However, variations in CsA pharmacokinetics are poorly explained by non-genetic factors. Therefore, genetic factors, particularly the cytochrome P450 3A (CYP3A) and ATP-binding cassette sub-family B member 1 (ABCB1) gene activity in the intestine and liver, could help predict CsA pharmacokinetics in early post-renal transplant recipients.

CYP3A, which participates in CsA metabolism, is primarily composed of CYP3A4 and CYP3A5 in adults[4]. Numerous CYP3A single nucleotide polymorphisms (SNPs) have been identified to date, and these can be obtained from several SNP

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databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and HapMap (http://www.hapmap.org). Some CYP3A polymorphisms have been shown to correlate with CsA pharmacokinetics. For example, CYP3A5*3C, a splicing mutation in intron 3, results in an mRNA splice defect with a premature stop codon, yielding truncated and non-functional proteins[5]. Several studies have reported that the CYP3A5*3C/*3C genotype correlates with a higher CsA C_0/dose in early renal transplant patients[6, 7], whereas other studies have demonstrated that the variant locus has no effect on the C_0/dose[8, 9]. Moreover, several studies on the high-frequency CYP3A4*1G allele in intron 10 revealed that the CYP3A4*1G/*1G genotype affects drug metabolism in healthy subjects, suggesting that CYP3A4*1G could affect CYP3A4 activity *in vivo*[10, 11]. Other independent research groups did not observe a correlation between CYP3A4*1G and CsA pharmacokinetics in patients on the seventh day after renal transplantation or in early bone marrow transplant patients[7, 12]. However, a linkage disequilibrium (LD) has been observed between CYP3A4*1G and CYP3A5*3C in Asian populations. Namely, CYP3A5*1 allele carriers display a greater probability of harboring the CYP3A4*1G allele than the CYP3A4*1 allele[13]. Taken together, these data highlight the importance of thoroughly validating the association between CYP3A polymorphisms and CsA pharmacokinetics.

ABCB1, which encodes the drug transporter P-glycoprotein, plays an important role in pumping out exogenous substances (*eg*, CsA) from cells[14]. Numerous SNPs in ABCB1 have also been reported and can be obtained from the above-mentioned databases. To date, researchers have primarily focused on the associations between three polymorphisms (1236 C>T in exon 12, 2677 G>T/A in exon 21, and 3435 C>T in exon 26) and CsA pharmacokinetics, but the results have been inconsistent. Several studies have suggested that the non-synonymous variant 2677 G>T/A and synonymous variant 3435 C>T might contribute to the differences in CsA pharmacokinetics[6, 7], whereas others reported that neither polymorphism affected the CsA C_0[15, 16]. Furthermore, the three SNPs were in LD with one another. Additional studies have demonstrated that the TTT, CGC, and TGC haplotypes correlate significantly more with the CsA C_0 than with other ABCB1 1236-2677-3435 haplotypes[7, 17].

Apart from genetic factors, clinical indices, which are likely to yield confounding effects, may affect the inter-individual variability of CNI pharmacokinetics[18] and consequently lead to a reduction in or a complete blockade of genetic effects. Therefore, a combined analytical method (*eg*, regression model) may be advantageous for investigating the associations between genetic factors and CsA pharmacokinetics. A combined analytical method is commonly used to study differences in CsA pharmacokinetics in non-Chinese renal transplant patients, but it is rarely applied in studies involving Chinese patients. Therefore, we validated the effects of the CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms on CsA pharmacokinetics in early renal transplant patients by establishing a regression model.

In this study, we first examined the effects of CYP3A and ABCB1 polymorphisms on the CsA C_0 and C_0/dose in early renal transplant recipients. Next, we analyzed the impact of their haplotypes on CsA pharmacokinetics. Finally, we established a multiple linear regression model to further evaluate the contribution of polymorphism to CsA pharmacokinetics based on clinical indices.

Materials and methods

Patients

One hundred and twenty-six subjects were recruited from 634 Chinese patients who underwent *de novo* renal transplantation in Zhengzhou No 7 People's Hospital between May 2003 and July 2008. All demographic and clinical data were obtained from hospital records. All recipients accepted a triple immunosuppressive therapy regimen containing CsA (Neoral®; Novartis Pharma, Eberbach, Germany), mycophenolate mofetil (CellCept®; Roche, Shanghai, China), and a corticosteroid. A standard steroid regimen of 500 mg of methylprednisolone was given intravenously at the time of surgery and for each of the next two days, daily. On the third day after the procedure, oral prednisolone was administered at 100 mg and then progressively decreased to a daily dose of 20 mg by the end of the first month. Patients who met the following criteria were included in the study: (1) Han Chinese; (2) between 18 and 60 years of age; (3) an ABO blood type compatibility; (4) human leukocyte antigen matching to no less than two loci; (5) negative for panel reactive antibody; and (6) complement-dependent cytotoxicity less than 10%. Patients who had a history of renal transplantation or multiple organ transplantation; were taking other medications that influence CsA; had a history of hepatitis B, hepatitis C, or HIV; or could not procure medical records were excluded from the study.

Determination of the CsA C_0 and C_0/dose

The starting CsA dosage, which was initiated on the second day after the operation, was 4–6 mg/kg per day. Then, according to the CsA C_0, the daily dosage was adjusted to a full blood concentration of 200 ng/mL. Clinical indices, such as age, body weight, urinary volume, and full blood concentrations of hemoglobin, blood urea nitrogen (BUN), and BCr, were recorded on the seventh day after transplantation. CsA was administered in equal amounts at 8:00 AM and 8:00 PM daily. Blood samples were collected with ethylenediaminetetraacetic acid as the anticoagulant at 8:00 AM before drug administration. The CsA C_0 was determined using an Architect® i1000 analyzer (Abbott Laboratories, Abbott Park, IL, USA). The C_0/dose (ng/mL per mg·kg^{-1}·d^{-1}) was calculated using the following equation:

$$C_0/\text{dose}=C_0\ (\text{ng/mL})/\text{Dose}\ (\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1})$$

The initial C_0 was selected in this study based on its important role in rapidly attaining a target concentration for renal transplant patients compared with concentrations tested at other time points. The C_0/dose was chosen for analysis based on its consistency in patients. The study protocol was approved by the Ethics Committee of the Xiangya School of Medicine, Cen-

tral South University. Written informed consent was provided by all participants before the study began. We also obtained clinical permission from the Chinese Clinical Trial Register (Registration No ChiCTR-ONC-12002181) for this work.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using an SQ Blood DNA Kit (Omega, CO, USA) according to the manufacturer's protocol. CYP3A4*1G was genotyped using a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay, whereas CYP3A5*3C and ABCB1 3435 C>T were genotyped by pyrosequencing, and ABCB1 1236 C>T and ABCB1 2677 G>T/A were genotyped by direct sequencing. The primer sequences used are listed in Table 1. The following PCR conditions were employed: 94 °C for 7 min followed by 35 cycles of 94 °C for 30 s and 62 °C for 60 s for CYP3A4*1G and 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s and 50 °C for 30 s for CYP3A5*3C, ABCB1 1236 C>T, ABCB1 2677 G>T/A, and ABCB1 3435 C>T. PCR analysis was performed with an Eppendorf thermal cycler (Eppendorf AG, Hamburg, Germany), whereas pyrosequencing was performed using the Pyrosequencer 96MA (Biotage, Uppsala, Sweden). As a quality control, 5% of the samples were re-genotyped by direct sequencing.

Statistical analysis

SHEsis Online (http://analysis.bio-x.cn; access date: 2012-01-12) was used to calculate the frequencies of the CYP3A4, CYP3A5, and ABCB1 polymorphisms and their pairwise LDs. The haplotype analysis for CYP3A4-CYP3A5 and ABCB1 1236-2677-3435 was performed using PHASE 2.1 (downloaded from http://stephenslab.uchicago.edu/software.html; access date: 2012-01-02). Deviations from Hardy–Weinberg expectations were calculated using χ^2 analysis. The clinical characteristics of the renal transplant recipients were expressed as the mean±standard deviation, median (range).

The Shapiro-Wilk test was used to assess the normal distribution of the data from CsA pharmacokinetics. Data exhibiting an abnormal distribution were expressed as the median (range) and analyzed by non-parametric tests (*eg*, the Nemenyi test for comparing three groups and the Mann-Whitney U test for comparing two groups). The C_0/dose was log-transformed to attain normality. To select clinical candidate predictors for fitting a multiple regression model, we individually examined each one against the log-transformed C_0/dose using Pearson's correlation test. The variables associated with the log-transformed C_0/dose were retained (P<0.05) and further analyzed with the multiple regression model by the stepwise method. The dummy variables were used as category variables in the stepwise regression model.

All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA). For each analysis, P<0.05 was considered statistically significant.

Results

Patient characteristics and polymorphism distribution

As shown in Table 2, the mean age of the 126 renal transplant recipients was 37.0±10.1 years; their mean body weight was 61.9±10.0 kg; and the ratio of male to female participants was 93:33. Table 3 lists the allele and genotype frequencies of CYP3A and ABCB1. The expected frequencies for all SNPs did not differ from the Hardy-Weinberg principle (P>0.05). As many studies have reported that an LD exists between CYP3A4*1G and CYP3A5*3C, and among ABCB1 1236, ABCB1 2677, and ABCB1 3435, we investigated these polymorphisms in our samples. CYP3A4*1G and CYP3A5*3C were in LD (D'=0.638), as were ABCB1 1236, ABCB1 2677, and ABCB1 3435 (D'=0.781, 0.831, and 0.554, respectively). We performed corresponding analyses according to the CYP3A4-CYP3A5 and ABCB1 1236-2677-3435 haplotypes and eventually obtained 4 and 11 haplotypes, respectively. Among the CYP3A4-CYP3A5 haplotypes, the main GG and AA haplotype

Table 1. Genotyping of CYP3A4, CYP3A5, and ABCB1 using the PCR-restriction fragment length polymorphism, pyrosequencing, and direct sequencing assays.

SNP	Primer sequence	Locus	Rs code	Restriction enzyme	Fragment length (bp)
CYP3A4 1026+12G>A	F, 5′-CACCCTGATGTCCAGCAGAAACT-3′ R, 5′-AATAGAAAGCAGATGAACCAGAGCC-3′	Intron 10	rs2242480	*Rsa* I	287
CYP3A5 6986 A>G	F, 5′-AGCTTAACGAATGCTCTACTGTCA-3′ R, 5′-GGTCCAAACAGGGAAGAAATA-3′* S, 5′-CAGGAAGCCAGACTTTGATCATT-3′	Intron 3	rs776746	NA	239
ABCB1 C1236T	F, 5′-ATGTGTCTGTGAATTGCCTTG-3′ R, 5′-CATCTCACCATCCCCTCTG-3′	Exon 12	rs1128503	NA	181
ABCB1 G2677T/A	F, 5′-TGTTGTCTGGACAAGCACTGA-3′ R, 5′-GCATAGTAAGCAGTAGGGAGTAACAA-3′	Exon 21	rs2032582	NA	141
ABCB1 C3435T	F, 5′-AGCTGAGAACATTGCCTATGGA-3′ R, 5′-CATGCTCCCAGGCTGTTTAT-3′* S, 5′-GGTGTCACAGGAAGAGAT-3′	Exon 26	rs1045642	NA	145

NA indicates not applicable. *Labeled by biotin.

Table 2. Patient characteristics.

Characteristic	*n*	Value/frequency (Mean±SD, Median, Range)
Age (years)	126	37.0±10.1, 36 (18–60)
Gender		
Male	93	73.8%
Female	33	26.2%
Body weight (kg)	126	61.9±10.0, 61.0 (40.0–91.0)
Native kidney disease		
Hypertension	44	34.9%
Chronic	33	26.2%
Glomerulonephritis		
Diabetes mellitus	20	15.9%
Other	11	8.7%
Nephrotic syndrome	7	5.6%
IgA nephropathy	5	4%
Polycystic kidney disease	3	2.4%
Chronic pyelonephritis	2	1.6%
Latent glomerulonephritis	1	0.8%
Donor		
Living related donor	109	86.5%
Deceased donor	17	13.5%
Cold ischemia time (h)	126	7.1±3.2, 8.0 (0.2–15.0)
CsA daily dose (mg/d)	126	302.2±49.4, 300.0 (200.0–450.0)
CsA C_0 (ng/mL)	126	209.5±98.8, 194.9 (33.8–547.6)
C_0/dose (ng/mL per mg/kg)	126	42.9±20.4, 39.1 (6.8–18.3)
Urinary volume (L)	126	3342.8±1326.9, 3240.0 (1200.0–8000.0)
Hemoglobin (g/L)	126	92.9±13.3, 92.0 (61.0–132.0)
BUN (mmol/L)	126	12.7±7.8, 10.7 (4.3–56.7)
BCr (μmol/L)	126	190.7±219.6, 127.1 (54.9–1640.2)

frequencies were 62.4% and 19.6%, respectively. Among the ABCB1 1236-2677-3435 haplotypes, the frequencies of the main TTT, TGC, and CGC haplotypes were 34.4%, 24.3%, and 17.2%, respectively.

Table 3. Genotyping of CYP3A and ABCB1.

SNP	Allele frequency (*n*, %)		Genotype frequency (*n*, %)	
ABCB1 C1236T	C	87 (34.5)	CC	19 (15.1)
	T	165 (65.5)	CT	49 (38.9)
			TT	58 (46)
ABCB1 G2677T/A	G	109 (43.3)	GG	26 (20.6)
	T	107 (42.5)	GT	39 (31)
	A	36 (14.3)	GA	18 (14.3)
			TT	29 (23)
			AA	4 (3.2)
			TA	10 (7.9)
ABCB1 C3435T	C	154 (61.1)	CC	51 (40.5)
	T	98 (38.9)	CT	52 (41.3)
			TT	23 (18.3)
CYP3A4*1G	G	186 (73.8)	GG	72 (57.1)
	A	66 (26.2)	GA	42 (33.3)
			AA	12 (9.5)
CYP3A5*3C	A	78 (31)	AA	16 (12.7)
	G	174 (69)	AG	46 (36.5)
			GG	64 (50.8)

Impact of genetic factors on CsA pharmacokinetics

We examined CYP3A4, CYP3A5, and ABCB1 polymorphism associations with the CsA C_0 and C_0/dose. All results are shown in Figure 1 and Table 4. The CYP3A5*3C polymorphism exhibited significant differences between the AG and GG genotypes, and between AA and GG (P<0.05), in the C_0 and C_0/dose groups. No significant differences were detected for other SNPs (CYP3A4*1G, ABCB1 1236 C>T, ABCB1 2677 G>T/A, and ABCB1 3435 C>T). We selected GG and AA

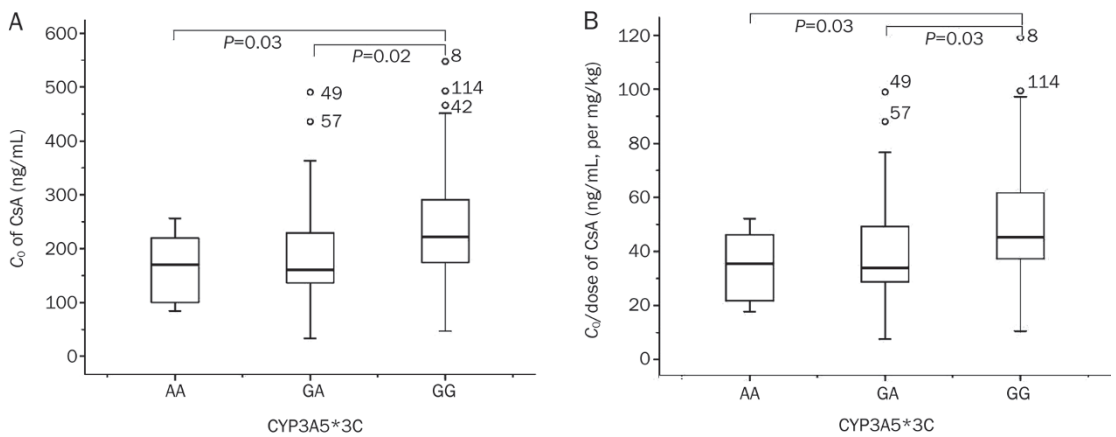

Figure 1. Differences in the CsA (A) C_0 and (B) C_0/dose among CYP3A5*3C genotypes on the seventh day after renal transplantation in the study group (n=126).

Table 4. Associations between the CYP3A and ABCB1 polymorphisms and CsA pharmacokinetics.

	Number	CsA C_0 (ng/mL) Median (Range)	C_0/dose (ng/mL, per mg/kg) Median (Range)
ABCB1 1236 C>T			
CC	19	183.6 (71.5–363.2)	38.0 (14.3–75.7)
CT	49	200.9 (33.8–435.9)	41.5 (6.8–87.2)
TT	58	194.9 (47.1–547.6)	39.1 (9.7–118.3)
ABCB1 2677 G>T/A			
GG	26	176.8 (52.6–390.3)	36.7 (11.0–79.5)
GT/GA	57	203.5 (33.8–547.6)	41.5 (6.8–118.3)
TT/AA/TA	43	194.5 (82.2–490.4)	39.2 (17.5–98.1)
ABCB1 3435 C>T			
CC	51	177.8 (33.8–451.3)	37.0 (6.8–93.0)
CT	52	208.4 (47.1–547.6)	42.7 (9.7–118.3)
TT	23	201.2 (82.2–490.4)	41.6 (17.5–98.1)
CYP3A4*1G			
GG	72	205.4 (33.8–547.6)	42.4 (6.8–118.3)
AG	42	181.4 (52.6–490.4)	37.8 (11.0–98.1)
AA	12	170.7 (84.5–288.5)	34.6 (16.9–61.5)
CYP3A5*3C			
AA	16	170.7 (84.5–256.3)[b]	34.6 (16.9–51.3)[b]
AG	46	160.5 (33.8–490.4)[b]	33.1 (6.8–98.1)[b]
GG	64	221.7 (47.1–547.6)	44.3 (9.7–118.3)

[b]P<0.05 compared with GG (Nemenyi test for comparing three groups; Mann-Whitney U test for comparing two groups).

among the 4 CYP3A4-CYP3A5 haplotypes for further analysis because they were more frequent than AG and GA. Patients with the AA haplotype exhibited lower CsA pharmacokinetics than those with non-AA haplotypes (P<0.05). GG haplotype patients did not differ from non-GG haplotype patients. Moreover, among the 11 ABCB1 1234-2677-3435 haplotypes, we selected the TTT, TGC, and CGC haplotypes for further analysis because they were more frequent than the 9 other haplotypes. None of them correlated with CsA pharmacokinetics (Table 5).

Combined impact of genetic factors and clinical indices on CsA pharmacokinetics

Because our analysis revealed that only CYP3A5*3C correlated with CsA pharmacokinetics, we established a multiple regression model to assess the association between the SNP and CsA pharmacokinetics more efficiently by considering clinical factors. First, we individually examined age, sex, cold ischemia time, as well as hemoglobin, BUN, and BCr levels, against the log-transformed C_0/dose using Pearson's correlation test to select candidate predictors for further multiple regression analysis. Age and hemoglobin, BUN, and BCr levels were retained in the model (P<0.05). CYP3A5*3C maintained a significant correlation with the log-transformed C_0/dose and accounted for 44.2% of the stepwise regression model (r^2=0.31) (Table 6).

Table 5. Associations between the CYP3A and ABCB1 haplotypes and CsA pharmacokinetics.

	Number	CsA C_0 (ng/mL) Median (Range)	C_0/dose (ng/mL, per mg/kg) Median (Range)
ABCB1 1236-2677-3435			
TTT	86	198.6 (33.8–547.6)	40.6 (6.8–118.3)
non-TTT	40	180.7 (52.6–451.3)	37.5 (11.0–93.0)
TGC	58	187.4 (33.8–547.6)	38.4 (6.8–118.3)
non-TGC	68	198.6 (65.1–490.4)	40.4 (13.0–98.0)
CGC	36	208.4 (52.6–395.0)	43.2 (11.0–79.0)
non-CGC	90	185.9 (33.8–547.6)	38.4 (6.8–118.3)
CYP3A4-CYP3A5			
GG	109	200.9 (33.8–547.6)	41.3 (6.8–118.3)
non-GG	17	181.5 (84.5–288.5)	37.2 (16.9–61.5)
AA	40	155.1 (82.2–490.4)[b]	31.6 (16.9–98.1)[b]
non-AA	86	208.4 (33.8–547.6)	43.2 (6.8–118.3)

[b]P<0.05 compared with non-AA (Mann–Whitney U test).

Table 6. Multiple linear regression analyses of variables influencing the log-transformed C_0/dose.

Dependent variable	Independent variables	Standardized coefficients beta	t	Sig
CsA/Dose log-transformed	(Constant)		7.668	0.000[b]
	CYP3A5_D1	-0.189	-2.360	0.020[b]
	CYP3A5_D2	-0.253	-2.360	0.002[b]
	Hemoglobin	0.285	-3.171	0.000[b]
	BCr	-0.253	-3.248	0.002[b]
	Age	0.228	2.952	0.004[b]

CYP3A5_D1 represents a dummy variable defined as AG relative to the GG genotype for CYP3A5*3C, and CYP3A5_D2 represents a dummy variable defined as AA relative to the GG genotype for CYP3A5*3C. [b]P<0.05.

Discussion

In this study, we observed that neither the CYP3A4*1G, ABCB1 1236C>T, ABCB1 2677G>T/A, and ABCB1 3435C>T alleles nor the CYP3A4-CYP3A5 GG, ABCB1 1236-2677-3435 TTT, CGC, and TGC haplotypes was associated with the CsA C_0/dose in early renal transplant recipients. In contrast, carriers of the CYP3A5*3C allele and CYP3A AA haplotype exhibited a higher CsA C_0/dose than CYP3A5*1 allele and CYP3A4-CYP3A5 non-AA haplotype carriers. We concluded that CYP3A5*3C allele carriers retained a high CsA C_0/dose in the multiple linear regression model.

Previous studies have shown that CYP3A4*1G allele frequency is characterized by extensive ethnic variation. This allele is infrequently observed in Caucasians but common in Asians. CYP3A4*1G is observed in approximately 25%–49% of Asian populations[19], and our data confirmed this result (26.2%). We investigated the role of CYP3A4*1G in CsA pharmacokinetics in early renal transplant recipients and revealed that the wild type and mutant patients did not differ in CsA pharmacokinetics, including in the C_0 and C_0/dose. However, one study demonstrated that the CYP3A4*1G allele correlated with the CsA C_0 during testing days 16–30[7]. We speculate that the selection of different time points for CsA pharmacokinetics could contribute to such disparate results.

Our findings also revealed that the CYP3A5*3C frequency was 69%, similar to data from other studies in Chinese patients[20, 21], whereas the frequencies in Caucasian and African American populations have been reported as 85% and 45%, respectively[22]. These results indicate that significant ethnic variations for the allele do exist. Although studies have consistently shown an association between CYP3A5*3C and the pharmacokinetics of tacrolimus, another CNI, reports on the association between the SNP and CsA pharmacokinetics are contradictory. We compared several studies that analyzed CsA pharmacokinetics on the seventh day after renal transplantation in Chinese patients, and interestingly, we did not observe conflicting results. Thus, CsA pharmacokinetic time points and patient ethnicity affected the association between CYP3A5*3C and CsA pharmacokinetics. Furthermore, a meta-analysis suggested that CYP3A5*3 correlates with CsA pharmacokinetics in Asians[23].

An LD has been observed between CYP3A4*1G and CYPE3A5*3C carriers in Asian populations[24]. Our data confirmed this correlation (D'=0.64) and revealed that GG and AA frequencies jointly accounted for 84% of CYP3A4-CYP3A5 haplotypes. We then performed a CYP3A4-CYP3A5 haplotype analysis to assess their combined effect, which indicated that CsA pharmacokinetics were lower in patients with the AA haplotype than in patients with non-AA haplotypes (P<0.05), thereby validating the effect of CYP3A5*3C on CsA pharmacokinetics. However, CsA pharmacokinetics did not differ between patients with the GG haplotype and patients with non-GG haplotypes. This result could be attributed to the low statistical power limited by the small sample size.

ABCB1 polymorphisms were distributed differently between ethnic groups. For example, ABCB1 2677 G>T/A is one of the most common and extensively reported SNPs. Previous research has shown that the A allele is observed in 13% of Chinese, 2% of Caucasians, and 0% of Africans[25–27]. We detected a rate of 14.3% in our Chinese patients, consistent with the above data for the Chinese population. In addition, a strong LD among ABCB1 1236, ABCB1 2677, and ABCB1 3435 has been observed in previous studies[28]. Our results showed that the TGC frequency accounted for 24.3% of ABCB1 1236-2677-3435 haplotypes, similar to the value of 20.5% reported by Qiu *et al*[7]. However, the frequency of this haplotype only accounted for 4.2% in Czech nationals[29]. Thus, our data confirmed the diversity of ABCB1 mutations in several populations. We also assessed the impact of ABCB1 polymorphisms and their haplotypes on CsA pharmacokinetics in renal transplant patients, demonstrating that none of the three classic polymorphisms (1236 C>T, 2677 G>T/A, and 3435 C>T) or the three main haplotypes (TTT, TGC, and CGC) were linked to CsA pharmacokinetics, in agreement with several studies[8, 29] but in contrast to others[6, 7]. We postulate that the different experimental methods and conditions used in the present and previous studies could partly account for the conflicting results. Moreover, different studies on the associations between ABCB1 polymorphisms and CsA pharmacokinetics have selected different pharmacokinetic parameters: some used C_0, whereas others used C_2, which is the concentration at 2 h post-dose. C_2 has been proposed as more efficient than C_0 by some studies and not by others[30, 31]. Therefore, identification of appropriate selection parameters requires further independent validation.

Finally, to elucidate the impact of CYP3A5*3C on CsA pharmacokinetics, we screened several variables correlated with the CsA C_0/dose using Pearson's correlation test. For example, age was positively associated with the C_0/dose, which might be due to CYP3A's decreasing ability to metabolize CsA with increased age[32]. However, the positive correlation of hemoglobin with C_0/dose is likely a result of low hemoglobin values obtained from low hematocrit values, potentially resulting in a reduced proportion of CsA bound to red blood cells and an increased plasma portion more readily metabolized by the liver[21]. Moreover, specific indices for identifying DGF and AR in clinical practice, BUN and BCr were identified as negative correlates of the C_0/dose. We speculated that their high values were attributable to the low CsA C_0, which readily led to DGF or AR. Therefore, we established a stepwise regression model to further observe the effects of CYP3A5*3C on CsA pharmacokinetics while considering clinical factors. We selected clinical variables, such as age and hemoglobin, BUN, and BCr levels, that, except BUN, were in the stepwise regression model. Bun was excluded possibly because it was influenced by many factors, such as food, drugs, and sports activities, yielding a diminished effect in the complicated multiple-factor environment. Our model demonstrated that CYP3A5*3C exhibited an independent, significant correlation with CsA pharmacokinetics and accounted for 44.2% of the model.

This study was not without limitations, partly reflected by the low r^2 value in the regression model. We speculate that other factors may affect CsA pharmacokinetics, including methylation, microRNA regulation, and the copy number variations of relevant genes[33], as well as serum lipid profiles, hematocrit levels, and comorbidities (*eg*, diabetes and hypertension)[3, 18]. Concurrently, the medication prednisone was routinely used in renal transplant patients because of its efficacy in reducing AR. Thus, although studies have shown that the administration of this drug induced CYP3A[34], we could not have excluded those patients who were taking prednisone. Moreover, we did not consider the genetic factors of donors

because their organ samples did not remain intact, whereas several studies have demonstrated that the ABCB1 3435 C>T genotype of donors correlated with CsA pharmacokinetics[35].

In summary, our study assessed the impact of CYP3A5*3C on CsA pharmacokinetics on the seventh day after renal transplantation in Chinese patients. This allele serves as a putative indicator for predicting the optimal CsA dose for early renal transplantation.

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Author contribution

Hong-hao ZHOU, Xiang-guang MENG, and Wei ZHANG designed the study; Xiang-guang MENG, Cheng-xian GUO, Xiang-dong PENG, Qi PEI conducted the research; Guo WANG, Meng HE, Min LIU, and Jing-ke YANG contributed new analytical tools and reagents; Xiang-guang Meng collected the medical records of the patients and analyzed the data; Jian-le HAN, Xin CHEN, Yong SHI, and Hong-yao SHI collected samples from the patients; and Xiang-guang MENG, Ji-ye YIN, Guo-qing FENG, Ying-chun ZHAO, and Bo-ting ZHOU wrote the manuscript.

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