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Influence of *ABCB1*, *CYP3A4**18B and *CYP3A5**3 polymorphisms on cyclosporine A pharmacokinetics in bone marrow transplant recipients

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Abstract:
The aim of this study was to retrospectively evaluate the effect of polymorphisms in the *CYP3A4*, *CYP3A5* and *ABCB1* genes on the dose-adjusted concentration and dose requirement of cyclosporine A (CsA) in Chinese recipients during the early period after bone marrow or hematopoietic stem cell transplantation. Ninety-one bone marrow transplant recipients were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay or by direct sequencing for the C1236T, G2677T/A and C3435T polymorphisms in *CYP3A4**18B, *CYP3A5**3, and *ABCB1*, respectively. The concentration at zero before administration (C_0) and concentration at 2 h after administration (C_2) of whole blood CsA were measured by fluorescence polarization immunoassay. Dose-adjusted C_0 and C_2 were determined and compared among groups with different genotypes. Compared with *CYP3A5**3/*3 individuals, *CYP3A5**1/*1 subjects have a significantly lower dose-adjusted C_0 and C_2 at days 1–10 and a higher dose requirement for CsA at days 16–30 ($p < 0.05$). In addition, homozygotes for the *ABCB1* 3435T mutant have a significantly higher dose-adjusted C_0 and C_2 and a lower dose requirement compared with wildtype ($p < 0.05$). Similar results were also derived for carriers of the T-G-C haplotype in *CYP3A5* producers compared with non-carriers ($p < 0.05$ and $p < 0.01$, respectively). In summary, the *ABCB1* 3435T SNP, T-G-C haplotype in *CYP3A5* producers, and *CYP3A5**3 SNP are all associated with differences in CsA pharmacokinetics and dose requirements during the first month after bone marrow or hematopoietic stem cell transplantation. Genetic testing can therefore help to determine initial dosage and individualize immunosuppressive therapy.

Key words:
ABCB1, bone marrow transplantation, cyclosporine A, *CYP3A4**18B, *CYP3A5**3, hematopoietic stem cell transplantation

Introduction

With its unique ability to inhibit T cell activity, cyclosporine A (CsA) has become an important drug for use during human organ transplantation. CsA is effective in reducing the incidence of solid organ and bone marrow graft rejections. However, because CsA also has low oral bioavailability, a very narrow therapeutic index, and marked inter-individual differences in its pharmacokinetics, it is essential to monitor CsA concentrations to optimize the therapeutic dose for achieving the target concentration range after bone marrow or hematopoietic stem

cell transplantation [36]. As revealed by the daily practice of drug monitoring, there is substantial inter-individual variability in the required oral dose of CsA to achieve target blood concentrations. Such variability may be explained in part by polymorphisms in genes encoding biotransformation enzymes and drug transporters, which has received much attention.

As a substrate and an inhibitor of P-glycoprotein (P-gp), CsA is mainly metabolized by CYP3A4 and CYP3A5, both of which account for the variability in CsA pharmacokinetics [39]. Several recent studies have shown that CYP3A4, CYP3A5 and P-gp in human liver and intestine can contribute to inter- and intra-individual differences in the pharmacokinetics of CsA [15, 24]. P-gp is encoded by the ATP-binding cassette sub-family B member 1 gene (*ABCB1*) and acts as a trans-membrane efflux pump involved in energy-dependent export of xenobiotics, including a number of substrates, drugs, and physiological molecules, from the inside to the outside of the plasma membrane [1, 41]. Lown et al. showed that 30% and 17% of the variability in oral C_{max} and oral clearance of CsA, respectively, may be attributed to individual variation in P-gp levels [32]. The rate of intestinal absorption for certain drugs, including CsA, have been shown to correlate with several single nucleotide polymorphisms (SNPs) of the *ABCB1* gene [34]. Compared with polymorphisms, homozygotes for the mutation at position 3435 (TT) have significantly lower P-gp levels in the small intestine and the highest plasma concentrations of digoxin after oral administration [22]. Notably, this SNP was also found to be in linkage disequilibrium with two other SNPs, C1236T (in exon 12) and G2677T/A (in exon 21). It has been suggested that, compared with single SNPs, haplotype(s) formed by particular SNPs in the *ABCB1* gene may be more useful as a predictor of P-gp activity and therefore CsA dose requirements [6].

The intestinal and hepatic expression of the *CYP3A4* and *CYP3A5* genes can potentially influence the pharmacokinetics of CsA. Based on clinical studies, it was postulated that the *CYP3A4*18B* allele may be associated with increased *CYP3A4* expression or enzymatic activity [11, 38]. *CYP3A4**18B, a recently-discovered SNP in intron 10 of the *CYP3A4* gene, was initially identified in Japanese patients and is present at high frequency in Asian populations [5, 11, 38]. This SNP creates a G to A substitution at position 82266, which can increase the activity of CYP3A4. Several studies have indicated that this SNP is related to the pharmacokinetics of CsA [11, 19, 49]. *CYP3A5* is heterogeneously expressed in different populations and accounts for a significant amount of the total CYP3A enzyme levels in intestine and liver. A frequent SNP, 6896 A>G, has been found to be associated with CYP3A5 protein production and enzyme activity [29]. The G>A mutation in intron 3 results in a splice defect in the mRNA, which produces an unstable and non-functional protein. This mutant allele is named *CYP3A5**3, and the wild type is named *CYP3A5**1. Only individuals carrying at least one *CYP3A5**1 allele can express high levels of the CYP3A5 enzyme [20, 28].

For organ transplant recipients, achieving target blood concentrations of CsA as soon as possible after transplantation is a key to prevent rejection [4, 8]. However, it should also be noted that, in the early phase after organ transplantation, several external factors may significantly influence the pharmacokinetics of CsA. Yin et al. suggested that the pharmacokinetics of CsA are greatly variable, and the therapeutic window was from 50 to 500 ng/ml in patients with chronic myelocytic leukemia, acute non-lymphocytic leukemia, acute lymphoblastic leukemia, Mediterranean disease, or pancytopenia after bone marrow transplantation [48]. Recent studies have explored the association between polymorphisms in the *CYP3A4, CYP3A5* and *ABCB1* genes and the dose requirements and dose-adjusted concentration of CsA in kidney, liver and heart transplant recipients [6, 30, 42, 43]. However, no such study has been performed on bone marrow or hematopoietic stem cell transplant recipients.

The aim of this study was to investigate the genotypes and haplotypes of the *ABCB1*, *CYP3A4* and *CYP3A5* genes in bone marrow and hematopoietic stem cell transplant recipients and to assess whether such polymorphisms are associated with dose-adjusted concentrations of CsA and dose requirements to achieve the target therapeutic range following transplantation. Preoperative screening for such variants may facilitate individualized initial CsA dosing to ensure adequate drug exposure while minimizing its toxicity.

Materials and Methods

Patients

A total of 91 recipients (47 males and 44 females) who received bone marrow or hematopoietic stem cell transplantations for acute lymphocytic leukemia,

aplastic anemia, myelodysplastic syndrome, or B-cell lymphoma were recruited. From the time of transplantation, each patient was treated with CsA and maintained on a triple immunosuppressive regimen consisting of CsA capsules (Neoral, Sandoz, Basel, Switzerland), mycophenolate mofetil and steroids. CsA was given orally twice a day (BID) at a dose of 50 to 500 mg/day (median 150 mg) and then adjusted according to C_0 and C_2 levels, which were determined twice a week during the first month until they reached the target values of 200 and 800 ng/ml, respectively. Mycophenolate mofetil was given at a dose of 0.50–0.75 g BID during the first month after surgery. A commonly used steroid-tapering schedule was followed: (0.5–1.0 g of intravenous (*iv*) methylprednisolone was given at the time of surgery, 0.2–0.5 g was given on the following 3 days, and then 10–30 mg oral prednisolone was given daily, which was progressively tapered to 15–20 mg by the end of 1 month after transplantation. The dosages were subject to change in accordance with clinical and laboratory findings. Acute rejection was recorded, and when it occurred, steroid pulse therapy was started. Body weight, CsA dosage and whole blood CsA concentration were recorded at days 1–3, 8–10 and 16–30 after the transplantation. The dose requirement of CsA (mg/kg/d) and the dose-adjusted concentration of C_0 and C_2 (ng/ml per mg/kg/d) were calculated. Exclusion criteria included concurrent administration of drugs known to interact with CsA pharmacokinetics (except for prednisone as part of the immunosuppressive protocol), acute rejection episode within the previous 6 months, less than 2 years of a history of malignancies, drug and alcohol abuse, and transplantation of multiple organs.

The study was performed in accordance with the Declaration of Helsinki and its amendments. The protocols were approved by the Ethics Committee of Shengjing Hospital of China Medical University. Written informed consents were obtained from all participants. During routine visits, 2 ml of venous blood was drawn into an EDTA tube and stored at –80°C until use.

Therapeutic drug monitoring

CsA dosage was given daily in equal amounts at 7:00 a.m. and 7:00 p.m. C_0 and C_2 levels of CsA were assayed with a commercial CsA whole blood monoclonal antibody fluorescence polarization immunoassay, which was run on a TDxFLx analyzer according to the manufacturer's instructions (Abbott Laboratories, Chicago, IL, USA). The assay was calibrated daily with manufacturer-supplied reagents and verified for performance by assaying quality-control samples with expected concentrations in low (120–180 ng/ml), medium (340–460 ng/ml) or high (680–920 ng/ml) ranges. The between- and same-day variability was less than 4% in a concentration range between 150 and 800 ng/ml. The detection limit of the assay was 25 ng/ml. Data were recorded at days 1–3, 8–10, and 16–30 after bone marrow or hematopoietic stem cell transplantation. Dose-adjusted C_0 and C_2 were calculated by dividing the C_0 and C_2 with the corresponding 24-h dose on a mg/kg basis.

Genotype determination

Genomic DNA was extracted from EDTA-anti-coagulated whole blood using an EZ-10 Spin Column Genomic DNA Mini preps Kit (for blood) (Bio Basic Inc., Markham Ontario, Canada). The patients were genotyped for the *ABCB1*, *CYP3A4* and *CYP3A5* genes. PCR-RFLP was used to genotype the SNPs C1236T (exon 12, rs: 1128503) and C3435T (exon 26, rs: 1045642). Restrictive endonuclease ECO0109I and *Dpn*II (New England Biolabs, USA) were used to digest the PCR products accordingly. The genotypes of G2677T/A (rs: 2032582), *CYP3A4**18B (rs: 28371759) and *CYP3A5**3 (rs: 776746) were determined by direct sequencing [2, 42]. PCR conditions consisted of a denaturation step at 94°C for 3 min and then 35 cycles of denaturation at 94°C for 30 s, an annealing step at 50–56°C for 60 s, and elongation at 72°C for 1 min, followed by a final extension at 72°C for 7 min. The primer sequences are shown in Table 1.

To verify the results of the gel electrophoresis of the PCR-RFLP products, samples of each genotype (homozygous wildtype, heterozygous, and homozygous polymorphisms, a total of 30) were sequenced.

EMLD software was used for determining linkage disequilibrium (http://request.mdacc.tmc.edu/qhuang/software/pub.htm). Haplotypes used for analysis were based on those described by Kim et al., which are clearly in linkage disequilibrium [26]. For the *ABCB1* gene, the haplotypes were restricted to the C1236T, G2677T/A and C3435T SNPs. For homozygotes for all three variants or heterozygotes for only one variant, haplotypes were assigned unambiguously. Pa-

Tab. 1. Sequences of primers used for genotyping SNPs of the indicated genes

SNP	Primer
ABCB1 C1236T	Forward: 5' TCT TTG TCA CTT TAT CCA GC 3'
	Reverse: 5' TCT CAC CAT CCC CTC TGT 3'
ABCB1 G2677T/A	Forward: 5' AGT TTT CAG AAA ATA GAA GCA TGA GT 3'
	Reverse: 5' GGG AGT AAC AAA ATA ACA CTG ATT AGA 3'
ABCB1 C3435T	Forward: 5'TGT GCT GGT CCT GAA GTT 3'
	Reverse: 5' TAG GCA GTG ACT CGA TGA A 3'
*CYP3A4**18B	Forward: 5' GAG GGC TTC ACT TAG ATT 3'
	Reverse: 5' CTG CCA GTA GCA ACC ATT 3'
*CYP3A5**3	Forward: 5' ACC ACC CAG CTT AA CGA AT 3'
	Reverse: 5' AGC ACA GGG AGT TGA CCT T 3'

tients with uncertain haplotypes were classified as non-carriers of a particular haplotype.

Transplantation recipients carrying a *CYP3A4**18B or *CYP3A5**1 allele were defined as CYP3A4 or CYP3A5 producers, respectively. For CYP3A4, CYP3A5 producers or non-producers, carriers of particular haplotypes were analyzed independently.

Statistical analysis

The allelic frequencies for *ABCB1*, *CYP3A4* and *CYP3A5* were assessed using the Pearson χ^2 goodness-of-fit test supplemented by inference based on the Monte Carlo simulation when expected cell frequencies were smaller than 5 [40]. We also used the Pearson χ^2 test to assess the Hardy-Weinberg equilibrium. The estimation of haplotype frequencies for *ABCB1*, *CYP3A4* and *CYP3A5* were performed by maximum likelihood estimation based on the expectation-maximization (EM) algorithm. The likelihood ratio test was used to determine the significance of associations between loci, and the estimation was performed using Stata [33].

To analyze the pharmacokinetic parameters, the Kolmogorov-Smirnov test was used to assess if the data were normally distributed. For the qualified data (presented as the mean ± standard deviation), one-way analysis of variance (ANOVA) was used to test for differences among three or more independent groups, followed by the least significant difference (LSD) *t*-test for multiple comparisons. An unpaired Student's *t*-test was used to evaluate the difference between two groups. Data from any abnormal distribution were expressed as the median and range and analyzed using non-parametric tests (i.e., the Kruskal-Wallis H test for comparison of three or more independent groups and the Mann-Whitney U test for comparing two groups). The incidence of acute rejection in different groups was compared by Fisher's exact test or Pearson's χ^2 as appropriate; $p < 0.05$ was considered to be statistically significant. Statistical analysis was conducted using the SPSS package (version 11.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 91 bone marrow or hematopoietic stem cell transplant recipients were recruited. The mean age of the patients was 21.7 ± 17.2 years, and the mean weight was 49.4 ± 23.3 kg. Table 2 lists the characteristics and frequencies of various alleles and genotypes for the *ABCB1*, *CYP3A4*, and *CYP3A5* genes. No significant difference was detected among the various genotype groups. The distribution of all alleles was consistent with the Hardy-Weinberg equilibrium ($p > 0.05$). A total of 11 haplotypes were identified from the recipients, with T-T-T being the most common (30.0%). Four additional major haplotypes, T-G-C, T-T-C, C-A-C, and C-G-C, were identified with frequencies of 16.7%, 15.0%, 13.3% and 11.7%, respectively. Together, they accounted for 86.7% of all haplotypes identified from the patients.

Tab. 2. Allelic and genotypic frequencies of the *ABCB1*, *CYP3A4* and *CYP3A5* genes among 91 bone marrow or hematopoietic stem cell transplant recipients

SNP	Allele frequencies			Genotype frequencies					
ABCB1 C1236T	C	T		CC	CT	TT			
	35%	65%		11%	41%	38%			
ABCB1 G2677T/A	G	T	A	GG	GT	GA	TT	AA	TA
	36%	46%	18%	10%	31%	13%	19%	4%	13%
ABCB1 C3435T	C	T		CC	CT	TT			
	60%	40%		35%	38%	17%			
*CYP3A4**18B	*1	*18B		*1/*1	*1/*18B	*18B/*18B			
	75%	25%		55%	26%	9%			
*CYP3A5**3	*1	*3		*1/*1	*1/*3	*3/*3			
	24%	76%		13%	17%	60%			

A strong linkage disequilibrium was detected between *CYP3A4**18B and *CYP3A5**3 (*D*' = 0.72). Carriers of the *CYP3A5**3 (G) allele were more likely to possess the *CYP3A4**1 (G) allele than *CYP3A4**18B (A). No significant linkage was found between other combinations of particular SNPs.

Table 3 summarizes the association between the SNPs of *CYP3A4*, *CYP3A5*, and *ABCB1* genes and the pharmacokinetic parameters of CsA based on assessments of C_0 and C_2 during the first month after transplantation. Significant differences in dose requirements and dose-adjusted concentrations were detected among the three genotypes of the *ABCB1* C3435T SNP. Compared with wildtype, the dose-adjusted C_0 and C_2 were 31.5% (p = 0.03) and 21.1% (p = 0.04) higher on days 1–3, 33.8% (p = 0.03) and 20.8% (p = 0.04) higher on days 8–10, and the dose requirement was 44.7% (p = 0.02) (days 16–30) lower in homozygote mutants. Similar results were also obtained for *CYP3A5**3: when compared to wildtype, dose-adjusted C_0 and C_2 for homozygotes of mutant alleles were 50.2% (p = 0.02) and 42.1% (p = 0.03) higher, respectively, on days 1–3; 21.1% (p = 0.04) and 20.7% (p = 0.04) higher, respectively, on days 8–10; and the dose requirement was 41.3% (p = 0.03) lower.

To eliminate potential confounding effects between *CYP3A4* and *CYP3A5* expression, data for *CYP3A4*, *CYP3A5* producers and non-producers were analyzed separately. Table 4 summarizes the effects of *ABCB1* SNPs on CsA dose-adjusted concentrations. For *CYP3A5* non-producers, a significant association between *ABCB1* C3435T and dose-adjusted C_0 and C_2 was detected. Compared with wildtype, the dose-adjusted C_0 and C_2 were 53.9% (p = 0.02) and 17.7% (p = 0.04) higher on days 1–3, 44.4% (p = 0.03) and 22.6% (p = 0.04) higher on days 8–10, and the dose requirement was 42.0% (p = 0.03) lower in homozygote mutants. *ABCB1* C1236T and G2677T/A SNPs were not related to differences in dose-adjusted CsA concentrations or dose requirements.

We also assessed the effect of five main *ABCB1* haplotypes derived from SNPs C1236T, G2677T/A and C3435T on dose-adjusted concentrations and the dose requirement of CsA. For each haplotype, a comparison was performed between carriers and non-carriers. As shown in Table 5, the T-G-C haplotype was significantly correlated with dose requirements and dose-adjusted C_0 and C_2 in *CYP3A5* producers. Compared with non-producers of *CYP3A5*, the dose-adjusted C_0 and C_2 were 56.8% (p = 0.01) and 28.1% (p = 0.04) higher on days 1–3, 52.3% (p = 0.01) and 15.3% higher on days 8–10, and the dose requirement was 23.6% (p = 0.04) lower in carriers of the T-G-C haplotype. The other two *ABCB1* haplotypes were not associated with differences in the dose-adjusted CsA concentrations or dose requirements.

A total of 4 of the 91 patients experienced acute rejection. The times to the first rejection were 6, 8, 9 and 10 days after the transplantation. The number of patients with acute rejection in the different genotype groups is shown in Tables 3–5. No differences in the rates of acute rejection were detected among patients with different genotypes. However, considering that only a 1-month follow-up evaluation was conducted

Tab. 3. Association between *ABCB1*, *CYP3A4* and *CYP3A5* gene SNPs and dose-adjusted concentrations of CsA in 91 bone marrow or hematopoietic stem cell transplant recipients

	No.	Dose requirement (mg/kg)			C_0/D (ng/ml per mg/kg)			C_2/D (ng/ml per mg/kg)			No. of rejections (%)
		Days 1–3	Days 8–10	Days 16–30	Days 1–3	Days 8–10	Days 16–30	Days 1–3	Days 8–10	Days 16–30	
ABCB1 1236C/T											
CC	12	4.12 ± 1.80	4.09 ± 1.74	5.47 ± 1.78	29.91 ± 14.70	29.99 ± 13.16	44.67 ± 13.38	133.67 ± 21.49	139.02 ± 28.10	140.59 ± 28.99	0 (0.0)
CT	41	3.78 ± 1.81	3.85 ± 1.84	4.34 ± 1.92	28.86 ± 19.47	29.48 ± 20.79	34.39 ± 19.65	122.41 ± 34.83	119.35 ± 33.00	112.77 ± 27.54	3 (7.3)
TT	38	4.00 ± 2.81	4.07 ± 2.75	5.13 ± 2.83	28.14 ± 15.35	29.34 ± 15.03	36.94 ± 19.25	140.64 ± 59.71	127.53 ± 40.97	125.04 ± 33.57	1 (2.6)
ABCB1 2677G/T/A											
GG	10	3.52 ± 0.89	3.27 ± 0.84	4.72 ± 1.14	25.45 ± 15.78	25.77 ± 15.31	33.24 ± 19.29	131.77 ± 52.96	125.62 ± 43.55	123.72 ± 37.61	0 (0.0)
GT or GA	45	3.97 ± 2.81	4.24 ± 2.94	5.01 ± 2.75	29.83 ± 18.45	31.96 ± 19.68	39.03 ± 19.05	128.73 ± 43.06	120.45 ± 28.45	117.34 ± 25.71	2 (4.4)
TT,AA,TA	36	3.96 ± 1.72	4.03 ± 1.80	5.10 ± 1.84	35.32 ± 13.82	31.73 ± 12.84	39.71 ± 16.17	143.05 ± 34.46	146.53 ± 34.47	132.79 ± 30.61	2 (5.5)
ABCB1 3435C/T											
CC	33	4.21 ± 1.85	4.10 ± 1.76	5.14 ± 1.77	29.22 ± 18.56	28.05 ± 17.90	32.65 ± 19.77	125.43 ± 33.40	126.37 ± 35.58	119.11 ± 30.01	2 (6.1)
CT	39	3.69 ± 1.75	3.57 ± 1.68	3.85 ± 1.59	28.38 ± 15.58	31.60 ± 17.34	35.92 ± 16.70	126.76 ± 38.53	120.92 ± 29.11	120.38 ± 31.93	2 (5.1)
TT	19	3.88 ± 1.51	3.74 ± 1.45	3.55 ± 1.39*	38.44 ± 18.30*	37.64 ± 17.60*	37.18 ± 12.30	151.80 ± 40.75*	152.72 ± 49.27*	138.25 ± 33.82	0 (0.0)
*CYP3A4**18B											
*1/*1	55	3.86 ± 1.56	3.94 ± 1.61	4.37 ± 1.65	28.41 ± 16.97	29.44 ± 18.09	38.40 ± 19.88	123.46 ± 47.13	135.35 ± 34.24	123.41 ± 34.92	2 (3.6)
*1/*18B	27	3.87 ± 2.31	4.08 ± 2.24	4.51 ± 2.28	30.94 ± 18.88	32.40 ± 17.90	35.75 ± 17.69	113.17 ± 41.40	121.91 ± 38.84	117.65 ± 25.78	1 (3.7)
*18B/*18B	9	4.39 ± 2.27	4.24 ± 2.15	5.32 ± 2.43	23.71 ± 11.71	21.04 ± 9.23	34.32 ± 16.13	114.58 ± 54.76	115.81 ± 42.46	132.00 ± 26.26	1 (11.1)
*CYP3A5**3											
*1/*1	13	4.32 ± 2.75	4.83 ± 2.54	5.41 ± 2.09	22.53 ± 14.91	24.73 ± 13.78	37.69 ± 19.05	111.83 ± 32.07	120.63 ± 39.75	132.55 ± 20.92	1 (7.7)
*1/*3	17	3.81 ± 1.79	3.92 ± 1.84	4.94 ± 2.01	21.75 ± 15.22	23.41 ± 17.05	34.33 ± 18.66	127.39 ± 23.20	132.68 ± 29.65*	139.65 ± 26.98	2 (11.8)
*3/*3	61	3.77 ± 1.48	3.80 ± 1.53	3.83 ± 1.55*	33.82 ± 17.71*	35.13 ± 18.18*	37.32 ± 19.17	135.37 ± 32.74*	145.64 ± 33.84*	141.88 ± 34.71	1 (1.6)

* p-value < 0.05 (compared across genotypes using the least significant difference *t*-test)

Tab. 4. Association between *ABCB1* gene SNPs and dose-adjusted concentrations of CsA in *CYP3A5* non-producers

	No.	Dose requirement (mg/kg)			C_0/D (ng/ml per mg/kg)			C_2/D (ng/ml per mg/kg)			No. of rejections (%)
		Days 1-3	Days 8-10	Days 16-30	Days 1-3	Days 8-10	Days 16-30	Days 1-3	Days 8-10	Days 16-30	
ABCB1 1236C/T											
CC	8	4.56 ± 2.06	4.62 ± 1.83	5.24 ± 1.92	27.87 ± 13.62	28.18 ± 12.56	43.41 ± 10.21	133.10 ± 20.25	137.55 ± 26.13	138.70 ± 29.30	0 (0.0)
CT	28	3.76 ± 2.01	3.92 ± 2.10	4.35 ± 1.94	30.72 ± 21.21	31.93 ± 22.11	33.71 ± 19.14	120.91 ± 37.76	118.48 ± 31.71	108.88 ± 28.39	1 (3.6)
TT	25	3.61 ± 1.42	3.94 ± 1.55	4.90 ± 1.72	29.43 ± 14.89	31.17 ± 15.06	39.40 ± 21.08	122.30 ± 28.45	129.85 ± 37.52	131.06 ± 38.41	0 (0.0)
ABCB1 2677G/T/A											
GG	7	3.76 ± 0.87	4.02 ± 1.15	4.74 ± 1.23	24.88 ± 15.25	25.60 ± 16.49	33.84 ± 21.02	137.18 ± 59.67	127.57 ± 39.66	128.94 ± 42.48	0 (0.0)
GT or GA	28	3.55 ± 1.91	4.10 ± 1.74	4.57 ± 1.80	34.10 ± 20.09	37.13 ± 19.58	40.51 ± 18.35	134.41 ± 51.05	121.79 ± 29.11	113.54 ± 26.16	0 (0.0)
TT,AA,TA	26	4.10 ± 1.85	4.33 ± 1.94	5.46 ± 2.11	31.03 ± 12.57	27.68 ± 11.53	37.46 ± 14.80	132.54 ± 34.98	133.91 ± 30.31	129.05 ± 29.09	1 (3.8)
ABCB1 3435C/T											
CC	20	3.58 ± 1.80	4.11 ± 1.82	5.75 ± 1.74	21.93 ± 19.54	24.96 ± 18.25	32.00 ± 19.21	123.43 ± 34.02	125.25 ± 32.16	116.24 ± 29.72	0 (0.0)
CT	28	3.90 ± 1.86	4.34 ± 1.97	5.11 ± 1.92	28.36 ± 16.48	31.00 ± 18.29	35.97 ± 17.49	130.06 ± 44.38	122.24 ± 33.35	119.69 ± 36.62	1 (3.6)
TT	13	3.97 ± 1.74	4.03 ± 1.80	4.05 ± 1.76*	33.70 ± 18.44*	36.11 ± 19.23*	39.16 ± 23.59	145.18 ± 39.85*	153.57 ± 38.61*	135.29 ± 36.71	0 (0.0)

* p-value < 0.05 (compared across genotypes using the least significant difference *t*-test)

Tab. 5. Effect of major *ABCB1* gene haplotypes on dose-adjusted concentrations of CsA in *CYP3A5* producers

	No.	Dose requirement (mg/kg)			C_0/D (ng/ml per mg/kg)			C_2/D (ng/ml per mg/kg)			No. of rejections (%)
		Days 1–3	Days 8–10	Days 16–30	Days 1–3	Days 8–10	Days 16–30	Days 1–3	Days 8–10	Days 16–30	
Haplotype TTT											
Carrier	5	3.56 ±1.25	4.24 ±1.36	4.79 ±1.45	24.01 ±19.50	23.94 ±15.23	27.30 ± 17.45	119.27 ± 38.80	131.23 ± 78.93	105.12 ± 16.56	0 (0.0)
Non-carrier	9	4.21 ± 1.74	4.53 ± 1.62	5.13 ± 1.87	29.49 ± 17.31	28.34 ± 15.64	39.42 ± 18.44	131.18 ± 34.75	137.15 ± 37.85	126.83 ± 29.34	0 (0.0)
Haplotype TGC											
Carrier	5	4.39 ± 1.87	4.55 ± 1.79	4.44 ± 1.74	35.11 ± 5.77	37.10 ± 2.89	38.94 ± 16.73	135.12 ± 33.86	139.96 ± 36.87	137.36 ± 10.22	0 (0.0)
Non-carrier	9	4.46 ± 1.26	4.81 ± 1.33	5.49 ± 1.65*	22.38 ± 7.32*	24.36 ± 11.22*	34.05 ± 23.13	105.53 ± 30.69*	121.59 ± 46.59	133.72 ± 46.77	0 (0.0)
Haplotype TTC											
Carrier	4	4.46 ± 2.07	4.53 ± 1.93	4.95 ± 2.11	23.31 ± 20.41	19.69 ± 15.60	28.26 ± 18.38	100.75 ± 20.20	104.57 ± 25.13	100.37 ± 19.03	0 (0.0)
Non-carrier	10	3.51 ± 1.12	4.29 ± 1.26	4.78 ± 1.54	28.29 ± 14.29	30.71 ± 14.98	40.11 ± 20.32	164.30 ± 74.53	130.87 ± 30.24	130.68 ± 21.40	1 (10.0)

* p-value < 0.05 (compared across haplotypes using unpaired Student's *t*-test)

and given the small number of patients with acute rejection, our results may not illustrate the association between *ABCB1* and *CYP3A4/5* polymorphisms and the rate of acute rejection.

Discussion

As a major component of an immunosuppressant regimen for organ transplantation, CsA has shown substantial pharmacokinetic variability among patients, particularly in the early period after organ transplantation [21, 23]. Attaining therapeutic levels of CsA immediately after transplantation can significantly reduce the rate of acute organ rejection [44]. Many factors, including time after transplantation, gastrointestinal tract status, activity of metabolic enzymes, age, and concomitant medication, can influence the oral bioavailability and inter-individual variability of CsA [25, 31]. Among these, the contributions of the P-gp, *CYP3A4* and *CYP3A5* genes have been recognized as being clinically important [12, 45].

Intestinal P-gp levels and hepatic CYP3A4 and CYP3A5 activities are determinants for up to 75% of the CsA variation after oral administration [13]. The most extensively studied SNP of *ABCB1* has been C3435T [2]. With an estimated allele frequency of 40%, this mutation appears to be fairly common in our patients, which was similar to that reported by Hoffmeyer and Kim et al. [17, 26]. Another factor contributing to CsA pharmacokinetics may be the variable expression of functional CYP3A4 and CYP3A5 enzymes. Genetic factors are considered as the most important cause of the considerable inter-individual differences in *CYP3A4* and *CYP3A5* expression and activity [10, 37]. Therefore, variations in such genes may contribute to inter-individual differences in the pharmacokinetics of orally administered CsA.

Notably, most studies have used C_0 as the only measure of drug exposure. In this study, we have added C_2, which may better reflect intestinal absorption because it decreases the role of hepatic metabolism and renal excretion. A report by Levy et al. showed that C_2 target values should be achieved by days 3 to 5 post transplantation to maximize clinical benefit [30].

Our results demonstrate for the first time a clear relationship between *ABCB1* 3435T, the T-G-C haplotype in *CYP3A5* producers and *CYP3A5**3 and the dose requirement, dose-adjusted C_0 and C_2 of CsA. When a standard dose of CsA was administered, the dose-adjusted C_0 and C_2 concentrations showed a significant correlation with *ABCB1* 3435T and *CYP3A5**3, with higher concentrations detected in patients carrying the *ABCB1* 3435TT and *CYP3A5**3/*3 mutations at days 1–10. After 2 weeks of adjusted doses, the mean C_0 and C_2 levels of CsA both reached the target therapeutic range, with no significant differences detectable among different groups. In our previous study, significant inter-individual variability was found for the dose required for achieving the therapeutic concentration range. The CsA doses required to maintain therapeutic C_0 and C_2 levels were significantly lower in homozygous mutants (*ABCB1* 3435TT and *CYP3A5**3/*3) compared with wildtype (*ABCB1* 3435CC and *CYP3A5**1/*1). Similar results were also derived from patients with the *ABCB1* T-G-G haplotype in *CYP3A5* producers.

Several studies have been conducted on the pharmacokinetics of CsA in the setting of renal transplantation. Notably, conflicts seem to exist with regard to the *ABCB1*, *CYP3A4* and *CYP3A5* polymorphisms and their relationships with CsA concentration and/or outcome of treatment [3, 6, 14, 16, 35, 47]. In 124 stable Caucasian renal-transplant recipients with various *ABCB1* C3435T genotypes, von Ahsen et al. [44] failed to detect any significant difference in the doses required to maintain a similar C_0 concentration of CsA for 6 months or more after the transplantation. Wang et al. [46] also investigated the effect of *ABCB1* gene polymorphisms on blood concentration of CsA in renal transplant recipients and found that blood CsA concentrations per unit dose in 3435CC homozygotes were lower than those of either 3435CT or 3435TT. They concluded that the C3435T genotype is the best predictor for systemic CsA exposure, which is in agreement with our results.

Previous studies on Chinese renal transplant recipients showed that the dose-adjusted concentrations of both C_0 and C_2 were significantly lower in the *CYP3A4**18B/*18B group compared with *CYP3A4**1/*1. In the present study, the change of dose-adjusted concentrations of C_0 and C_2 were consistent with that reported by Qiu et al. [38], but the difference among the groups was not significant, which is probably due to the small sample size and difference in disease status. Although some studies have found no influence of the *CYP3A5**3 genotype on CsA concentration [3, 9, 16, 27], our results indicate that dose-adjusted C_0 and C_2 values of

CsA were significantly higher and dose requirement was lower in homozygotes for *CYP3A5**3 compared with *CYP3A5**1 allele carriers, which is similar to previous findings with Chinese renal transplant recipients [7, 18, 38]. This result may be explained by the fact that CYP3A5 accounts for up to 50% of the total hepatic CYP3A in *CYP3A5* expressers with a large variability and may therefore enhance the metabolism of CYP3A substrates [29]. Qiu et al. [38] also found that the frequency of the *ABCB1* C-A-C haplotype was 14.8%, and when compared to other haplotypes, the dose-adjusted C_0 was higher in the first month after renal transplantation in individuals with *ABCB1* C-A-C haplotype. In the patients in the current study, the frequencies of the *ABCB1* C-A-C haplotype was 13.3%, which was similar to that of Chinese renal transplant recipients. Notably, we have found a correlation between the *ABCB1* T-G-C haplotype in *CYP3A5* producers and CsA pharmacokinetics. This discrepancy may in part be explained by different disease status, small sample size and other unknown factors.

In the present study, linkage disequilibrium was found between *CYP3A4*, *CYP3A5* and/or *ABCB1* genes. This result may in part explain the discrepancies between the effect of such genes on CsA pharmacokinetics and dose requirement. Another reason may be the delay after transplantation. Several months after transplantation, side effects of the immunosuppressive treatment usually require additional concomitant medications, which may interact with the absorption, metabolism and/or excretion of CsA. As shown by our results, significant differences may be detected among various *ABCB1* 3435 genotypes in CYP3A5 non-producers and between carriers and non-carriers of T-G-C in CYP3A5 producers. Similar results were also derived by Qiu et al. [38]. The differences between CYP3A4, CYP3A5 producers and non-producers combined with P-gp are unknown. Due to the small sample size, we cannot clarify this question, and further studies are needed.

To our knowledge, the present study has been the first to suggest an association between *ABCB1*, *CYP3A*4 and *CYP3A5* polymorphisms and CsA pharmacokinetics in bone marrow transplant recipients, though a duplicate study with a larger patient collection may still be necessary. Analysis of *ABCB1* or *CYP3A5* genotypes or haplotypes may provide useful guidance for individualizing CsA dosages in such patients at the beginning of treatment, which may improve the efficacy while reducing side effects.

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