

## Original Article

# Associations of UDP-glucuronosyltransferases polymorphisms with mycophenolate mofetil pharmacokinetics in Chinese renal transplant patients

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**Aim:** To evaluate the effects of UDP-glucuronosyltransferases (UGTs) polymorphisms on the pharmacokinetics of the immunosuppressant mycophenolate mofetil (MMF) in Chinese renal transplant recipients.

**Methods:** A total of 127 renal transplant patients receiving MMF were genotyped for polymorphisms in *UGT1A9* -1818T>C, I399C>T, -118T9/10, -440C>T, -331T>C, *UGT2B7* IVS1+985A>G, 211G>T, -900A>G, *UGT1A8* 518C>G and *UGT1A7* 622T>C. The plasma concentrations of the MMF active moiety mycophenolic acid (MPA) and main metabolite 7-O-MPA-glucuronide (MPAG) were analyzed using HPLC. Univariate and multivariate analyses were used to assess the effects of UGT-related gene polymorphisms on MPA pharmacokinetics.

**Results:** The dose-adjusted MPA AUC<sub>0–12 h</sub> of the patients with the *UGT2B7* IVS1+985AG genotype was 48% higher than that of the patients with the IVS1+985AA genotype, which could explain 11.2% of the inter-individual variation in MPA pharmacokinetics. The dose-adjusted MPAG AUC<sub>0–12 h</sub> of the patients with the *UGT1A7* 622CC and *UGT1A9* -440CT/-331TC genotypes, respectively, was significantly higher than that of the patients with 622T homozygotes and -440C/-331T homozygotes. Furthermore, the genotypes *UGT1A9* -1818T>C and *UGT1A8* 518C>G were associated with a low dose-adjusted MPAG AUC<sub>0–12 h</sub>.

**Conclusion:** The *UGT2B7* 11+985A>G genotype is associated with the pharmacokinetics of MPA in Chinese renal transplant patients, which demonstrates the usefulness of this SNP for individualizing MMF dosing.

**Keywords:** renal transplantation; immunosuppressant; mycophenolate mofetil; mycophenolic acid; 7-O-MPA-glucuronide; pharmacokinetics; UDP-glucuronosyltransferases; genetic polymorphisms

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## Introduction

Mycophenolic acid (MPA) is the active moiety of mycophenolate mofetil (MMF), which is a potent immunosuppressive agent that is widely used in renal transplant patients<sup>[1]</sup>. MPA with a recommended AUC<sub>0–12 h</sub> of 30–60 mg·h·L<sup>-1</sup> has been shown to be effective in preventing allograft rejection after solid organ transplantation<sup>[1]</sup>. The pharmacokinetics of MPA

vary by approximately 7–10-fold among individuals, resulting in different states of immunosuppression<sup>[2]</sup>. Insufficient immunosuppression might result in the rejection of transplanted organs. Excessive immunosuppression might lead to side effects, such as diarrhea, anemia, leukopenia and infection<sup>[3]</sup>. It is important to understand the factors that cause this large pharmacokinetic variation to achieve the necessary drug concentration and avoid potential drug toxicity.

After administration, MMF is quickly hydrolyzed into MPA by esterase and then converted by UDP-glucuronosyltransferases (UGTs), predominantly in the liver, into phenolic glucuronide metabolite (7-O-MPA-glucuronide, MPAG, more than 90%) and acyl-MPAG (AcMPAG, less than 10%), which are

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excreted by the kidneys<sup>[1,2]</sup>. MPAG is characterized by enterohepatic circulation, which might affect MPA exposure, and MPA and MPAG exposure are important indicators of UGT activity *in vivo*. In addition, MPAG is reported to be related to the occurrence of diarrhea<sup>[4]</sup>. UGT isoforms that are involved in the metabolism of MPA include UGT1A9, 2B7, 1A8, and 1A7, among which UGT1A9 is the principal enzyme responsible for MPAG and 2B7 for AcMPAG<sup>[5,6]</sup>.

Many UGT-related polymorphisms have been reported to affect UGT activity and thus affect drug metabolism<sup>[7,8]</sup>. UGT1A9 -440C>T, -331T>C, I399C>T, and -118T<sub>10</sub> were shown to be associated with higher activity *in vitro*<sup>[9-12]</sup>. UGT1A9 -1818T>C was reported to be related to slightly elevated UGT1A9 activity, without a significant difference<sup>[13]</sup>. UGT1A8\*2 (518C>G) led to the decreased activity of UGT1A8<sup>[14-16]</sup>. UGT2B7\*2 (802C>T) was reported to have a limited effect on UGT2B7 activity. However, UGT2B7 -900A>G, which was in complete linkage disequilibrium (LD) with UGT2B7\*2, has been shown to result in lower activity of the enzyme<sup>[15]</sup>. UGT2B7\*3 (211G>T) was associated with higher enzyme activity<sup>[10]</sup>. The UGT1A7 622T>C genotype was reported to abolish the activity of UGT1A7 *in vitro*<sup>[17]</sup>.

All of these single nucleotide polymorphism (SNPs) have been investigated in MPA pharmacokinetics *in vivo*, with conflicting results that might be due to the limitations of the sample size, the differences in the ethnicities and co-administered drugs, or the confounding effects of other genetic polymorphisms. Unlike other rare UGT1A9 SNPs, the UGT1A9 -1818T>C genotype is a common variation found in the Chinese population, with a mutant frequency of 52.4% and a distinct ethnic difference<sup>[13]</sup>. It is necessary to investigate the effect of the UGT1A9 -1818T>C genotype on MPA pharmacokinetics in Chinese patients. The influence of the UGT1A7 622T>C genotype on MPA metabolism in the Chinese population has not been studied. Innocenti *et al* recently discovered a novel SNP in the intron region, UGT2B7 IVS1+985G>A, which could affect UGT2B7 activity. No *in vivo* study has examined the influence of the IVS1+985G>A polymorphism on drug metabolism. We hypothesized that the UGT2B7 IVS1+985G>A polymorphism might affect the metabolism of MPA.

To evaluate the genetic factors influencing MPA pharmacokinetics, we systematically investigated the relationship between UGT-related polymorphisms and MPA pharmacokinetics in Chinese renal transplant patients, including well-understood SNPs that have been poorly examined in the Chinese population as well as SNPs that have not been studied in any population.

## Materials and methods

### Ethics statement

The study was performed according to the Declaration of Helsinki and with ethical approval from the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (No [2008]23). Written informed consent was obtained from all of the subjects.

## Patients and therapy

A total of 127 renal transplant recipients (82 males and 45 females) from the Kidney Transplant Department, Transplant Center, the First Affiliated Hospital, Sun Yat-sen University were enrolled in this study. The average age of the patients was 37.05±10.6 years, whereas the average weight was 58.53±11.0 kg.

All of the patients received a triple immunosuppressive regimen consisting of MMF (Cellcept<sup>®</sup>, Roche, Basel, Switzerland), tacrolimus (Prograf<sup>®</sup>, Fujisawa Ireland Ltd, Killorglin, Ireland) and steroids (prednisone, Guangdong Huanan Pharmacy, Ltd, Dongguan, China). Except for tacrolimus and prednisone, any medication that might affect the MPA concentration used during treatment, such as proton pump inhibitors, were prohibited. Patients with impaired hepatic function, combined organ transplantation or a smoking habit were excluded. All of the patients received maintenance therapy with a fixed dose of MMF for at least 2 weeks before blood sample collection. There should be no adverse reaction at least 1 week before or after blood sample collection. The MMF dose regimens ranged from 0.5 g q 12 h to 1 g q 12 h according to the clinical symptoms and side effects.

## Collection of the blood samples

Venous blood samples were collected at 0.5, 1.5, 4, and 9 h after the morning dose of MMF. We used a validated abbreviated sampling strategy to estimate the AUC<sub>0-12 h</sub> of MPA and MPAG instead of a full time-concentration profile<sup>[18,19]</sup>. Briefly, blood samples before and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, and 12.0 h after the MMF morning dose were obtained from 42 adult renal transplant patients who used a fixed MMF dose combined with tacrolimus for at least 2 weeks. The best-fit description of the MPA AUC<sub>0-12 h</sub> was decided by multiple linear regression analysis with  $r^2=92.8\%$ , model  $P<1.0\times 10^{-6}$  and D-W=1.917. The MPAG AUC<sub>0-12 h</sub> was determined by the multiple linear regression analysis with  $r^2=95.6\%$ , model  $P<1.0\times 10^{-8}$  and D-W=2.245<sup>[20]</sup>, all of which were within the acceptable range. We calculated the dose-adjusted AUC<sub>0-12 h</sub> of both MPA and of MPAG.

## MPA and MPAG concentration detection

The plasma MPA and MPAG concentrations were simultaneously analyzed using a validated high performance liquid chromatography (HPLC) method<sup>[21-24]</sup>. Briefly, the plasma was extracted from whole blood after centrifugation at 4000 rpm/10 min. The plasma was stored at -80 °C until detection. A mixture of 5% ZnSO<sub>4</sub> and methanol (30:70, v/v) was used as the protein precipitator, and naproxen was used as the internal standard. A mixture of 100 μL of plasma, 10 μL of naproxen and 100 μL of the precipitator was fully blended by a 1-min vortex before centrifugation at 14000 rpm for 10 min to eliminate the denatured protein. The supernatant was separated on a solid reversed-column (4.6 mm×250 mm, 5 μm particle size; Xtimate<sup>™</sup> C18, Welch Materials, Inc, Shanghai, China) using a Waters 1525-717-2487 HPLC system with ultraviolet detection at 215 nm. The mobile phase consisted of 0.1%

trifluoroacetic acid and methanol (35:65, *v/v*), and the flow rate was 0.8 mL/min. All of the material used was of HPLC grade.

### DNA extraction and genotyping

Total genomic DNA extraction was conducted following a previously described method<sup>[25]</sup>. The *UGT1A8*\*2 polymorphism was detected using previously reported methods of polymerase chain reaction restriction-fragment length polymorphism (PCR-RFLP)<sup>[16]</sup>. The polymorphisms of *UGT1A9* -118dT<sub>9/10</sub> and I399C>T were genotyped by PCR-sequencing<sup>[10]</sup>. The other polymorphisms were genotyped by a MassArray<sup>®</sup> SNP genotyping system (Sequenom, Inc, San Diego, CA, USA)<sup>[26]</sup>.

### Statistical analysis

Nonparametric tests were used for the univariate analysis using SPSS (Statistical Package for the Social Sciences) statistical software (version 21.0). The Mann-Whitney *U*-test was used for the comparison of two groups, whereas the Kruskal-Wallis test was used for the comparison of more than two groups. The Hardy-Weinberg equilibrium was tested in the frequencies of all the genotypes using a chi-square test procedure. The LD between the SNPs was tested using SHESis<sup>[27]</sup>. A multivariate analysis by stepwise linear regression was applied to examine the association between MPA pharmacokinetics and various genotypes. The pharmacokinetic parameters were logarithmically transformed prior to the multivariate analysis. Only the variables with a *P* value of less than 0.1 in the univariate analysis were included in the multivariate analysis. The differences were considered to be statistically significant with *P*<0.05.

## Results

### Genotype frequencies

All of the 127 patients were genotyped for polymorphisms in *UGT1A9* -1818T>C, I399C>T, -118T<sub>9/10</sub>, -440C>T, -331T>C, *UGT2B7*\*3, IVS1+985A>G, -900A>G, *UGT1A8*\*2 and *UGT1A7* 622T>C. All of the allele and genotype frequencies complied with the Hardy-Weinberg equilibrium and previous reports in Chinese Han populations (Table 1). A complete LD was observed between *UGT1A9* -440C>T and 331T>C (*D'*=1). No significant link was found between the other individual combinations of SNPs.

### The influence of the *UGT2B7* polymorphisms on MPA pharmacokinetics

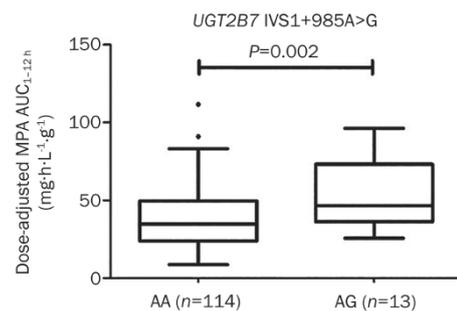
The MPA dose-adjusted AUC<sub>0-12h</sub> was significantly higher in the recipients with the *UGT2B7* IVS1+985AG genotype than that in the *UGT2B7* IVS1+985AA carriers (47.93±28.92 *vs* 32.42±24.35 mg·h·L<sup>-1</sup>·g<sup>-1</sup>, *P*=0.002) (Figure 1). We found that other *UGT2B7*-related polymorphisms did not affect the dose-adjusted MPA AUC<sub>0-12h</sub> (data not shown).

### The influence of the *UGT1A7*, *UGT1A8*, and *UGT1A9* polymorphisms on MPA/MPAG pharmacokinetics

The dose-adjusted AUC<sub>0-12h</sub> of MPAG was significantly greater

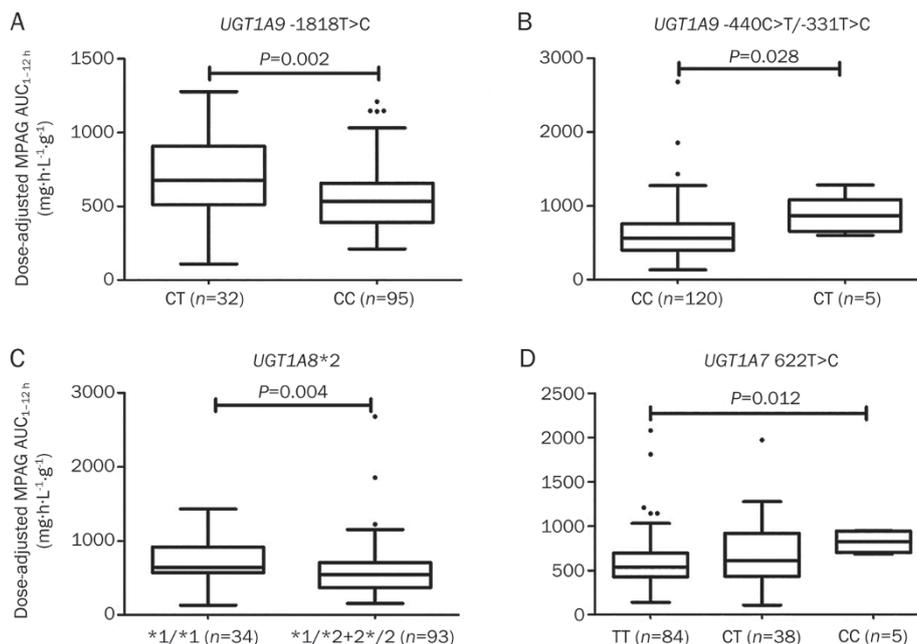
**Table 1.** The genotype frequencies of UGT-related polymorphisms.

SNP	Genotype	Frequency (n, %)
UGT1A7 622T>C rs11692021	TT	84 (66.1)
	CT	38 (30)
	CC	5 (3.9)
UGT1A8*2 (518C>G) rs1042597	CC	34 (26.8)
	CG	55 (43.3)
	GG	38 (29.9)
UGT1A9 -1818T>C rs13418420	CT	32 (25.2)
	CC	95 (74.8)
UGT1A9-440C>T/-331T>C rs2741045/rs2741046	CC/TT	120 (96)
	CT/TC	5 (4)
UGT1A9 I399C>T rs2741049	CC	23 (18.1)
	CT	34 (26.8)
	TT	70 (55.1)
UGT1A9 -118T9/10 rs35426722	9/9	43 (33.8)
	9/10	62 (48.8)
	10/10	22 (17.4)
UGT2B7 IVS1+985A>G rs62298861	AA	114 (89.8)
	AG	13 (10.2)
UGT2B7*3 211G>T rs12233719	GG	93 (73.2)
	GT	33 (26)
	TT	1 (0.8)
UGT2B7 -900G>A rs7438135	GG	14 (11)
	GA	45 (35.4)
	AA	68 (53.6)



**Figure 1.** Correlation of the *UGT2B7* IVS1+985A>G genotype with the dose-adjusted AUC<sub>0-12h</sub> of MPA in Chinese renal transplant patients.

in the patients with the *UGT1A9* -1818CT genotype than that in the carriers of the *UGT1A9* -1818CC genotype (671.39±338.62 *vs* 548.98±330.11 mg·h·L<sup>-1</sup>·g<sup>-1</sup>, *P*=0.002) (Figure 2A), and the dose-adjusted MPA AUC<sub>0-12h</sub> was greater in the patients with the *UGT1A9* -1818CT genotype than that in the carriers of the



**Figure 2.** Correlation of the *UGT1A9* -1818T>C genotype (A), the *UGT1A9* -440C>T/-331T>C genotype (B), the *UGT1A8*\*2 genotype (C) and the *UGT1A7* 622C>T genotype (D) with the dose-adjusted  $AUC_{0-12h}$  of MPAG in Chinese renal transplant patients.

*UGT1A9* -1818CC genotype, without a statistically significant difference ( $47.14 \pm 26.12$  vs  $38.18 \pm 18.14$   $\text{mg} \cdot \text{h} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ,  $P=0.061$ ). Although *UGT1A9* -440C>T/-331T>C mutant carriers were rare in this study group ( $n=5$ ), our data showed that these two variant alleles were related to an increase in the dose-adjusted  $AUC_{0-12h}$  of MPAG in comparison to that of the wild-type carriers ( $870.4 \pm 431.3$  vs  $622.1 \pm 363.5$   $\text{mg} \cdot \text{h} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ,  $P=0.028$ ) (Figure 2B). Compared to the carriers of *UGT1A8*\*1/\*1, the *UGT1A8*\*1/\*2 and \*2/\*2 subjects presented a 22% lower dose-adjusted  $AUC_{0-12h}$  of MPAG ( $597.6 \pm 337.6$  vs  $726.5 \pm 349.7$   $\text{mg} \cdot \text{h} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ,  $P=0.004$ ) (Figure 2C). The dose-adjusted  $AUC_{0-12h}$  of MPAG in the individuals with the *UGT1A7* 622CC genotype was significantly higher than that in the patients with the 622TT genotype ( $826.60 \pm 118.60$  vs  $539.20 \pm 156.40$   $\text{mg} \cdot \text{h} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ,  $P=0.012$ ) (Figure 2D). No association was observed between the polymorphisms of *UGT1A9* I399C>T and -118T<sub>9/10</sub> and MPA pharmacokinetics (data not shown).

#### Multivariate analysis of MPA pharmacokinetics

The results of the multivariate analysis are listed in Table 2. Two polymorphisms (*UGT2B7* IVS1+985A>G and *UGT1A9*

-1818T>C) as well as the age, weight, albumin level and gender were included in building the model. In the results, the *UGT2B7* IVS1+985A>G and *UGT1A9* -1818T>C polymorphisms were included in the final model (model  $P=0.001$ ). The *UGT2B7* IVS1+985AG genotype was independently predictive of a higher dose-adjusted MPA  $AUC_{0-12h}$ , which explained 11.2% of the inter-individual variations, whereas the *UGT1A9* -1818T>C polymorphism was independently predictive of a lower dose-adjusted MPA  $AUC_{0-12h}$ . In addition to the genetic factors, age and gender were included in the final model ( $P=0.004$  and  $P=0.019$ , respectively), the total of which led to a 23.1% explanation of the variation of MPA pharmacokinetics.

#### Discussion

The large inter-subject variations in MPA pharmacokinetics are characterized by large differences in the MPA/MPAG plasma concentrations. In this work, we found that the *UGT2B7* IVS1+985A>G, *UGT1A9* -440C>T/-331T>C, -1818C>T, *UGT1A8*\*2 and *UGT1A7* -622T>C polymorphisms were associated with MPA pharmacokinetics in Chinese renal transplant patients.

**Table 2.** The stepwise linear regression analysis for prediction of dose-adjusted  $AUC_{0-12h}$  of MPA.

Dependent factor	Independent factor	Coefficient	Partial $r^2$	$P$	Model $P$
Dose-adjusted MPA $AUC_{0-12h}$	<i>UGT2B7</i> IVS1+985AA vs AG	0.541	0.112	<0.001	<0.001
	Age	-0.011	0.0713	0.004	
	Gender (male=1, female=2)	0.219	0.0462	0.019	
	<i>UGT1A9</i> -1818 TC vs CC	-0.218	0.0372	0.036	

In our research, the IVS1+985AG genotype was found to be associated with an elevated dose-adjusted MPA AUC<sub>0-12 h</sub> ( $P=0.002$ ), which could explain 11.2% of the inter-individual variations in MPA pharmacokinetics ( $P<0.001$ ). Innocenti *et al* discovered a haplotype consisting of the UGT2B7 IVS1+985A>G polymorphism and two other SNPs, which were in high LD with each other, to be associated with an increasing enzyme activity by regulating mRNA expression of UGT2B7 *in vitro*<sup>[28]</sup>. However, no *in vivo* data focusing on the effect of the IVS1+985A>G polymorphism on any drug metabolism has been reported. Despite a tendency for the up-regulation of the UGT2B7 activity by the IVS1+985A>G polymorphism *in vitro*, we found a negative effect of this polymorphism on transforming the MPA. This discordancy between *in vivo* and *in vitro* studies might have many causes. First, alternative splicing mechanisms are very complex, and the *in vitro* data could not exactly predict the actual situation *in vivo*. The UGT2B7 IVS1+985A>G polymorphism was reported to favor the formation of splicing variant 1, which is expressed most frequently in the liver and intestinal tract<sup>[29, 30]</sup>. Additionally, other splicing variants naturally occur in other tissues, including the kidneys, in which the effects of the IVS1+985A>G polymorphism on these splicing variants were unknown. Moreover, it was reported that different splicing variants could interact with each other by competitive binding, which further complicates the process. It is possible that this polymorphism not only influences splicing variant 1 formation but also affects the formation of other splicing variants, which in turn could affect the formation of splicing variant 1. Second, the effect of the Y483D polymorphism on UGT1A9 activity was substrate dependent<sup>[31, 32]</sup>. The IVS1+985A>G mutation might affect the substrate specificity of UGT2B7 in the similar way, which might lead to unexpected responses in MPA pharmacokinetics. Third, all of the patients in this study were co-administered with tacrolimus, which might inhibit UGT enzyme activity, counteracting the influence of this polymorphism on MPA pharmacokinetics. However, the clear mechanisms by which this SNP leads to an increased exposure to MPA remain to be explained.

We found that individuals with the UGT1A9 -440CT/-331TC genotypes had higher dose-adjusted AUC<sub>0-12 h</sub> of MPAG than those of the individuals with the wild type ( $P=0.028$ ), whereas the UGT1A9 -1818CC genotype was associated with a lower dose-adjusted AUC<sub>0-12 h</sub> of MPAG ( $P=0.002$ ). The most well-studied gene involved in the metabolism of MPA is UGT1A9, which accounts for more than 50% of MPAG production. The UGT1A9 -440C>T/-331T>C polymorphism is associated with enhanced UGT activity in human liver microsomes<sup>[6, 33]</sup>, a finding that was consistent with our result. Very few *in vivo* studies demonstrated the effect of the UGT1A9 -1818T>C polymorphism on MPA pharmacokinetics. An *in vitro* study showed that the -1818C allele might lead to slightly evaluated enzyme activity; however, the result was inconclusive and without significant differences. We showed, for the first time, that the UGT1A9 -1818T>C polymorphism was associated with a lower dose-adjusted AUC<sub>0-12 h</sub> of MPAG in the Chinese population.

In addition, the UGT1A9, UGT1A8, and UGT1A7 polymorphisms play important roles in regulating the metabolism of MPA. We found a positive relationship between the dose-adjusted AUC<sub>0-12 h</sub> of MPAG and the UGT1A7 622C allele in Chinese renal transplant patients ( $P=0.030$ ), and UGT1A8\*2 was found to be associated with a lower dose-adjusted AUC<sub>0-12 h</sub> of MPAG ( $P=0.004$ ). The influence of the UGT1A7 -622T>C polymorphism on MPA metabolism *in vivo* had only been investigated in populations other than Chinese populations, and conflicting results have been observed. Patients with the UGT1A7 622TC genotype demonstrated increased oral clearance and a decreased C<sub>max</sub> of MPA<sup>[8]</sup>, which resulted in an accumulation of MPAG. These findings were in accordance with our results. Compared to the wild type, the UGT1A8\*2 polymorphism was found to have a limited effect on MPAG production *in vitro*<sup>[5, 6]</sup>. The MPA dose-adjusted trough concentrations were 60% higher in the \*1/\*2 and \*2/\*2 carriers than those in the patients with the wild type *in vivo*<sup>[34]</sup>, from which we could assume an inhibitive effect of the UGT1A8\*2 polymorphism on UGT1A8 activity. Correspondingly, our result that the UGT1A8\*2 polymorphism had a negative effect on MPAG formation *in vivo* was consistent with this assumption.

No association was observed between the UGT1A9 I399C>T, -118T<sub>9/10</sub>, UGT2B7\*3, -900A>G polymorphisms and MPA pharmacokinetics in our research. Conflicting results concerning these polymorphisms have been observed in other studies<sup>[15, 35, 36]</sup>, which might have resulted from the specific binding of different substrates to UGT1A9 or UGT2B7 affected by mutant alleles, different co-administered drugs and limitation of the sample size. Thus, a larger sample size is needed to evaluate the effect of these polymorphisms on MPA pharmacokinetics, whereas co-medication should be taken into consideration as well.

In this study, we systematically evaluated the potential influence of the UGTs-related polymorphisms on MPA pharmacokinetics in Chinese renal transplant patients. We found that the UGT2B7 IVS1+985A>G polymorphism was the most profound factor in the large variation in MPA pharmacokinetics. Patients who carry the UGT2B7 IVS1+985AG genotype might be at a greater risk of a higher dose-adjusted MPA AUC<sub>0-12 h</sub>, which would lead to infection and other side effects that might require reducing the MMF dosage. In addition, the UGT1A9 -440C>T/-331T>C, -1818C>T, and UGT1A7 -622T>C and UGT1A8\*2 polymorphisms were related to the dose-adjusted AUC<sub>0-12 h</sub> of MPAG, which might influence MPA exposure and thus clinical outcomes. The UGTs-related polymorphisms might be useful for the individualization of MMF dosing.

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### Author contribution

Xiao-chun XIE, Jia-li LI, Min HUANG, and Chang-xi WANG designed the research. Xiao-chun XIE, Jun LI, Qian FU, Hong-yang WANG, and Ping-ping SUN performed the research. Xiao-chun XIE, Xue-ding WANG, Jing LIU, Jia-wen HUANG, and Hong-liang LI contributed new reagents and analytic tools. Xiao-chun XIE, Guo-ping ZHONG, and Chen ZHU analyzed the data. Xiao-chun XIE and Jia-li LI wrote the paper.

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