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UGT1A9, UGT2B7 and MRP2 genotypes can predict mycophenolic acid pharmacokinetic variability in pediatric kidney transplant recipients

Tsuyoshi Fukuda, PhD¹, Jens Goebel, MD², Shareen Cox, BS¹, Denise Maseck, RN¹, Kejian Zhang, MD, MBA³, Joseph R. Sherbotie, MD⁴, Eileen N. Ellis, MD⁵, Laura P. James, MD⁵, Robert M. Ward, MD⁶, and Alexander A. Vinks, PharmD, PhD¹

¹Division of Clinical Pharmacology, Cincinnati Children's Hospital Medical Center & Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

²Nephrology and Hypertension Division, Cincinnati Children's Hospital Medical Center & Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

³Division of Human Genetics, Cincinnati Children's Hospital Medical Center & Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

⁴Division of Pediatric Nephrology & Hypertension, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, USA

⁵Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, Arkansas, USA

⁶Division of Neonatology, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, USA

Abstract

Background—Mycophenolic acid (MPA) exposure in pediatric kidney transplant patients receiving body surface area (BSA)-based dosing exhibits large variability. Several genetic variants in glucuronosyl transferases (UGTs) and of multidrug resistance-associated protein 2 (MRP2) have independently been suggested to predict MPA exposure in adult patients with varying results. Here, the combined contribution of these genetic variants to MPA pharmacokinetic variability was investigated in pediatric renal transplant recipients who were on mycophenolic mofetil maintenance therapy.

Methods—MPA and MPA-Glucuronide (MPAG) concentrations from 32 patients were quantified by HPLC. MPA exposure (AUC) was estimated using a 4-point abbreviated sampling strategy (pre-dose/trough and 20min, 1h and 3h post-dose) using a validated pediatric Bayesian estimator. Genotyping was performed for all of the following single nucleotide polymorphisms (SNPs): UGT1A8 830G>A (*3), UGT1A9 98T>C (*3), UGT1A9-440C>T, UGT1A9-2152C>T, UGT1A9-275T>A, UGT2B7-900A>G and MRP2 -24T>C.

All correspondence to Tsuyoshi Fukuda, Ph.D., Division of Clinical Pharmacology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue MLC 6018, Cincinnati, OH 45229-3039, USA, Phone: (513) 803-0428, Fax: (513) 636-4402, tsuyoshi.fukuda@cchmc.org.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

Results—Recipients heterozygous for MRP2-24T>C who also had UGT1A9-440C>T and/or UGT2B7-900A>G (n=4), and MRP2-24T>C-negative recipients having both UGT1A9-440C>T and UGT2B7-900A>G (n=5) showed a 2.2 and 1.7-times higher dose- and BSA-normalized MPA-AUC compared to carriers of no or only one UGT-SNP ($P < 0.001$ and $P=0.01$, respectively) (n=7). Dose- and BSA-normalized pre-dose MPA concentrations were 3.0- and 2.4-times higher, respectively ($P < 0.001$). Inter-individual variability in peak concentrations could be explained by the presence of the UGT1A9-440C>T genotype ($P<0.05$).

Conclusion—Our preliminary study demonstrates that combined UGT1A9-440C>T, UGT2B7-900A>G and MRP2-24T>C polymorphisms can be important predictors of interindividual variability in MPA exposure in the pediatric population.

Keywords

Pediatric patient; Mycophenolic acid; Mycophenolate Mofetil; Pharmacogenetics; Pharmacokinetics; Kidney Transplantation; Uridine diphosphate glucuronosyltransferase; multidrug resistance-associated protein 2

INTRODUCTION

Mycophenolate mofetil (MMF) is an immunosuppressive pro-drug commonly used in organ transplantations [1–3]. After oral administration, MMF undergoes rapid and almost complete hydrolysis to its active form, mycophenolic acid (MPA). MPA exposure, measured as area under the concentration-time curve (AUC), is well associated with both drug efficacy as well as toxicity in kidney transplant recipients [4]. A therapeutic target range for the MPA AUC of 30–60 mg·h/L has been suggested, as individuals with AUCs > 60 mg·h/L are more likely to experience drug-related adverse events, while individuals with AUCs < 30 mg·h/L are at higher risk of kidney allograft rejection [5–7]. While these data were generated in adult patients, similar relationships have been suggested in children [8–10].

The large inter-individual variability in MPA exposure as documented in many studies has prompted specific focus on pharmacogenetic factors including genetic polymorphisms in the uridine diphosphate-glucuronosyltransferases (UGTs), the enzyme family responsible for MPA's main metabolic pathways [11–16]. MPA is glucuronidated to mycophenolic acid glucuronide (MPAG) and the acyl glucuronide of mycophenolic acid (AcMPAG) [17]. UGT1A family members, particularly UGT1A9 and UGT1A8, are involved in the formation of MPAG, while UGT2B7 contributes to the acyl-glucuronidation [18]. It has been reported that these three UGT enzymes have functional genetic variants [19–21].

UGT1A9 is mainly expressed in the liver but also in the gastrointestinal tract and kidney [22]. Two single nucleotide polymorphisms (SNPs) in the promoter region of the UGT1A9 gene, UGT1A9-275T>A and UGT1A9-2152C>T, are reported to enhance the glucuronidation of MPA to MPAG [19] and, in turn, result in a lower MPA exposure [11]. Substantial interindividual variability in MPA exposure was explained in part with the presence of the UGT1A9-440C>T/-331T>C genotype in the promoter region of the UGT1A9 gene ($p=0.005$) in a study of 40 adult kidney transplant recipients [12]. UGT1A9 also has a rare coding SNP T>C at 98 (*UGT1A9*3*) resulting in dramatically decreased glucuronidation and increased MPA exposure [23]. UGT1A8 expressed in the kidney and throughout the gastrointestinal tract is also associated with the glucuronidation of MPA to MPAG [24]. *In vitro* studies have shown that *UGT1A8*3* confers decreased glucuronidation activity which may result in increased MPA exposure [18, 20, 24]. However, the clinical relevance of this SNP has not been clearly addressed, probably due to the presence of confounders including functional SNPs in the UGT1A9, UGT2B7 and transporter genes and

also to the complicated gene structure of the UGT1A family, e.g. a strong linkage between *UGT1A8**3 and *UGT1A9*-275T>A [25]. Another important UGT enzyme for MPA metabolism is UGT2B7 expressed in the liver and intestine [18]. A SNP in the promoter region of UGT2B7, *UGT2B7*-900A>G (formerly -842 or -840), was associated with lower AcMPAG concentrations [21]. Subjects with *UGT2B7**2 (802T), which is almost completely linked with *UGT2B7*-900G showed clearly higher AUCs of even MPA itself than non-carriers [13]. In summary, the presences of UGT alleles associated with decreased metabolic activity may increase the likelihood of MPA-related adverse events. Along these lines, we reported that the *UGT1A9*-440C>T/-331T>C genotype along with *UGT2B7*-900A>G indicates a risk factor for adverse events in pediatric kidney transplant recipients [26].

On the other hand, enterohepatic recirculation of MPA causes a considerable increase in total MPA exposure [27]. Several reports describe that excretion of the MPA metabolite MPAG in the bile mainly occurs through multidrug resistance-associated protein 2 (MRP2) [28, 29]. This MRP2 transporter encoded by a polymorphic gene *ABCC2* is expressed at the canalicular membrane of hepatocytes, the luminal/apical surface of epithelial cells of the small intestine, proximal tubular cells of the kidneys and endothelial cells of the brain [30]. It has been reported that the *MRP2*-24C>T in the promoter region of the *ABCC2* gene was associated with higher MPA trough levels and exposure (AUC) in kidney transplant recipients who received MMF in combination with tacrolimus [31], but not with cyclosporine [12].

The objective of this preliminary study was to explore the potential contribution of genetic variants in the *UGT1A9*, *UGT2B7* and *ABCC2* genes to MPA pharmacokinetics in pediatric kidney transplant recipients.

METHODS

Patients

This was a prospective multi-center open-label, Pharmacokinetic-Pharmacogenetic study of mycophenolate-mofetil (MMF) in *de novo* pediatric kidney transplant recipients aged 2 to 19 years during stable ambulatory treatment. The study was approved by the Institutional Review Boards of the participating institutions and parents/guardians and patients provided written consent and assent as required. Cincinnati Children's Hospital Medical Center (CCHMC) was the lead site with two additional sites from the National Institute of Child Health and Human Development (NICHD) Pediatric Pharmacology Research Units (PPRU) network participating: Arkansas Children's Hospital and University of Utah.

All patients were treated as per institutional protocols with an initial MMF dose of 450–600mg/m² (as CellCept, 250 mg capsule or 500 mg tablet) twice a day up to a maximum dose of 2g daily in combination with tacrolimus or sirolimus and corticosteroids. Oral suspension (200mg/ml) was allowed and documented in the case report forms. MMF dose adjustments were at the discretion of the treating physician and were not based on drug concentration measurements. Patients with any medical condition (active or chronic) that could interfere with the pharmacokinetic behavior of MMF (absorption, distribution and elimination) were not eligible (e.g. liver transplantation). Concurrent use of antacids, cholestyramine and iron supplements was not allowed.

Pharmacokinetic sampling and MPA measurement

Four blood samples were obtained pre-dose (trough), at 20 min, 1h and 3 h post-dose for the MPA measurement from each of 18 pediatric kidney transplant recipients enrolled. In addition, fourteen pharmacokinetic profiles with same sampling schedule from a previous

pharmacokinetic study were included [32]. The sparse sampling schedule was based on existing guidelines [33, 34]. MPA was analyzed in plasma using a validated high performance liquid chromatography (HPLC) assay according to a modification of a previously described method [35, 36]. In short, plasma samples were extracted using solid phase extraction (Oasis HLB 15 μ m 2.1x20mm; Waters, Milford, MA) and separated on a SynergiTM reversed-phase column (4 μ m Hydro-RP 80 Å 250x3.0 mm; Phenomenex, Torrance, CA) using an Agilent 1100 HPLC system with diode array detection at 215nm with a semi gradient elution (Agilent Technologies, Santa Clara, CA). The method was selective and reproducible in the range of 0.25–25.0 mg/L for MPA and 1.0–250 mg/L for MPAG. Extraction efficiencies were between 91 % and 100 % over the working range for both analyses. The lower limit of quantification (LLOQ) was 0.25 mg/L for MPA and 1.0 mg/L for MPAG, respectively. The limit of detection (LOD) was 0.03 mg/L for both analysts. Intra- and inter-day precision and accuracy for QC samples was always <5%.

Pharmacokinetic analysis

Individual peak concentration (C_{max}), trough concentration (C_{trough}) and area under the concentration time curve (AUC_{0-12h}) were estimated by a web-based Bayesian algorithm developed and hosted by the Limoges University Hospital using four MPA concentrations (STAMP; <https://pharmaco.chu-limoges.fr/>). MMF actual dose and MPA equivalent dose were used as applicable. MPA equivalent dose was calculated from the MMF dose according to: MPA dose = 0.739*MMF dose, where 0.739 is the fractional difference in molecular mass between MPA and MMF. Body surface area (BSA) was calculated from height and weight according to the Mosteller's formula [37]. The estimated parameters, C_{max} , C_{trough} and AUC, and parameter estimates normalized by MPA-dose, MPA-dose/BSA or MPA-dose/kg body weight were considered as variables in the pharmacogenetic evaluation. Parameter estimates were normalized by MPA-dose, as is common in adult association studies. Next, they were also normalized for size (using BSA or weight) to account for age-related size differences according to method of Anderson and Holford for pediatric data [38].

Data Collection

Demographic data were collected including age, sex, weight, height, race/ethnicity, donor type, age at transplantation and date of transplantation. Clinical chemistry data such as serum creatinine, hemoglobin, albumin, AST and ALT were also collected as part of standard of care. Serum creatinine was obtained from all subjects and creatinine clearance (CrCL) was calculated using Schwartz' formula [39]. Concomitant medications were also recorded with their dosing regimen.

Genotyping

A blood sample for DNA isolation and genotyping was obtained during a regular clinic visits with routine blood work. Genomic DNA was extracted from blood using standard procedures. All recipients (n=32) were genotyped for UGT1A9 98T>C (*3), UGT1A9-2152C>T, UGT1A9-440C>T, UGT1A9-275T>A, UGT1A8 830G>A (*3), UGT2B7-900A>G (formerly -842 or -840) and MRP2-24C>T polymorphisms. Commercially available TaqMan assays (Applied Biosystems, Foster City, CA, USA) were used to determine these genotypes after cross-validation by direct sequencing. It has been confirmed that UGT1A9-440C>T is completely linked with UGT1A9-331T>C in our in-house validation process by direct sequencing. For the reason, only UGT1A9-440C>T was tested in this study.

Exploration of a responsible factor to the PK parameters:

In an initial exploratory analysis, recursive partitioning [40] was performed by using the JMP software (a SAS-based statistic program) for AUC, C_{trough} and C_{max} as dependent variables. Recursive partitioning is a multivariable statistical analysis method which creates a decision tree to classify patients based on several dichotomous dependent variables. Since recursive partitioning is rule based such as “If a patient has this factor, they probably have such phenomena”, it can be used for grouping a population to generate more intuitive models within potential covariates. By recursive partitioning, decision trees were created with the best available and reasonable cutting values from possible covariates (listed in Table 1) (Figure, Supplemental Digital Content 2, <http://links.lww.com/TDM/A20>). A split was created with a *cutting value* which most significantly separates the means by examining the sums of squares of mean differences for all variables considered.

Statistical Analysis

Descriptive statistics were used to characterize group demographics. Non-normally distributed variables were expressed as median and quartiles and normally distributed variables as mean and SD. Continuous variables were analyzed by parametric analysis of variance (ANOVA), Welch t-test or non-parametric tests (Kruskal-Wallis or Wilcoxon-Mann-Whitney rank sum tests) as applicable. Categorical variables were reported using actual numbers or proportions, and the exact Chi-square test was used for small sample counts. General information about each SNP, such as allele frequency, genotype frequency and Hardy-Weinberg equilibrium, was gathered. Genotype differences in the generated subgroups were tested using the exact Chi-square test. Statistical significance was defined as $p < 0.05$. Two-sided or one-sided statistical testing was used as appropriate. Because of the exploratory nature of this study, no adjustments were made for multiple testing.

RESULTS

Pharmacokinetic and genetic data were obtained from 32 pediatric kidney transplant recipients. Patient demographics, clinical chemistry data, and pharmacokinetic parameters are summarized in Table 1. The mean age ($\pm$ SD) was 13 years (± 4.6). The three youngest children (2.1, 2.1 and 3.7 years old) were identified out of the 2-SD range for mean age (Figure, Supplemental Digital Content 1, <http://links.lww.com/TDM/A19>) and showed high heterogeneity in their clinical data and in their genetic backgrounds. From the genotyping assays, the UGT1A9-275T>A SNP, an increased activity allele, was found in 5 recipients as heterozygous, but 2 out of the 5 recipients also had UGT1A8*3, a decreased activity allele. To minimize the heterogeneity of this population, the three youngest children and five children with UGT1A9-275T>A (one met both, 7 in total) were accordingly excluded from the initial pharmacogenetic exploratory analysis.

MPA-dose/BSA normalized AUC and C_{trough} were plotted with combined genotypes using three genetic factors, UGT1A9-440C>T, UGT2B7-900A>G and MRP2-24C>T (Figure 1). Since a non-linear relationship was observed between MMF-dose/BSA and MPA-dose/BSA-normalized AUC, MMF-dose/BSA was considered as an important covariate (Figure, Supplemental Digital Content 3, <http://links.lww.com/TDM/A21>). To reduce the potential influence of large differences in MMF dose, the genetic effects on the MPA-dose/BSA-normalized AUC and C_{trough} were evaluated by using two dosing groups. A MMF dose of 354.7 mg/m² was identified by recursive partitioning and used for dividing patients into the two groups (see Methods, Figure, Supplemental Digital Content 2, <http://links.lww.com/TDM/A20>).

In the high dosing group (MMFdose/BSA $\geq 354.7\text{mg/m}^2$), recipients heterozygous for MRP2-24C>T coupled with UGT1A9-440T or UGT2B7-900G had clearly higher AUC values than the others (Figure 1). Carriers of both UGT1A9-440T and UGT2B7-900G showed distinctly higher AUC than recipients carrying the wild-type of all three SNPs or heterozygotes for either one of the two UGT-SNPs. A posterior statistical analysis revealed that not only dose/BSA-normalized AUC and C_{trough} but also non-normalized AUC and C_{trough} were significantly different ($P<0.05$) between the combined genotype groups while the other demographics did not show a significant effect (Table 2). These trends were not observed in the low dosing group (MMFdose/BSA $< 354.7\text{mg/m}^2$). On the other hand, MMDose/BSA-normalized peak concentration (C_{max}) appeared to be influenced by the UGT1A9-440C>T allele rather than the combined genotypes (Figure 2). Carriers of UGT1A9-440T had a higher dose/BSA-normalized C_{max} compared to non-carriers in the low dosing group and in the high dosing group ($P<0.05$) (Table 3).

DISCUSSION

To the best of our knowledge, there are only few reports describing genetic contributions to MPA pharmacokinetics in pediatric kidney transplant recipients. Recently, a French group reported UGT2B7-900A>G SNP as an informative genetic covariate in their population pharmacokinetic analysis in pediatric kidney transplant recipients [41]. We previously reported that the UGT1A9-440C>T/-331T>C and UGT2B7-900A>G SNPs were associated with MMF-related adverse drug reactions [26]. In the present exploratory study, the MRP2-24 SNP was considered as a potentially additional influential genetic variant to predict variability in MPA pharmacokinetics.

In this non-interventional study, MMF dose adjustments were at the physicians' discretion, resulting in a wide dose range used in this patient cohort. In line with a recent observation in adult transplant patients [42], we observed a non-linear relationship between individual MPA exposure (AUC) and dose (Figure, Supplemental Digital Content 3, <http://links.lww.com/TDM/A21>). Based on recursive partitioning, we therefore stratified patients into high or low dosing groups based on BSA-normalized dose and subsequently explored the genetic effects on normalized pharmacokinetic parameter estimates. A decrease in bioavailability with higher doses may be due to a saturable transporter-mediated absorption process of MMF or MPA from the gut. Another possible explanation might be saturable enterohepatic circulation, which is responsible for the reabsorption of MPA in the gut after bacterial deglucuronidation of MPAG. At higher doses, MPAG transport may become saturated with a lower MPAG fraction being recirculated and excreted by the kidney, resulting in lower overall MPA exposure.

In the high dose group, we observed a significant contribution of the MRP2 variant in combination with UGT2B7-900A>G and UGT1A9-440C>T to the variability in AUC and C_{trough} normalized by MMDose/BSA (Figure 1). This was not seen in the low dosing group. In contrast, the presence of UGT1A9-440C>T could in part explain C_{max} variability in both high and low dose groups (Figure 2). The UGT1A9-440C>T SNP also appeared to explain in part the AUC variability in the low dose group (Figure, Supplemental Digital Content 2, <http://links.lww.com/TDM/A20>). A similar trend was observed in MPA pharmacokinetic parameters normalized by MMF dose per body weight (kg). This dose-dependent genetic contribution seems to be in line with previous reports in adult kidney transplant patients. The dose of 450mg/m^2 in the high dose group corresponds to a MMF dose of around 800 mg for a 70 kg subject with a height of 175 cm. In the first study to show an impact of MRP2-24C>T on C_{trough} [31], patients received MMF doses of 500mg ($n=63$) or 1g ($n=32$) in combination with tacrolimus and corticosteroids which is quite similar to the high dose group in our study. Notably, a contribution of MRP2-24C>T to MPA pharmacokinetic

parameters was not observed in a single-dose study of 1.5g in healthy volunteers [43], suggesting a potential effect of dose or dosing regimen. However, and regarding UGT1A9 and UGT2B7 variants, significant contributions of *UGT2B7**2 (linked with UGT2B7-900A>G) and UGT1A9-2152C>T/-275T>A to the variability of MPA exposure were detected in this study [43]. Interestingly, the influence of the UGT1A9-2152/-275 SNPs on MPA exposure was also observed in patients on a 1g MMF BID regimen, but not in patients on 500 mg BID [11, 13]. In contrast, an influence of the UGT1A9-440C>T/-331T>C variant on MPA-PK was found, while no contribution of UGT1A9-2152C>T/-275T>A was observed, most likely as a result of the lower MMF dose [12]. Based on these data, it is tempting to speculate that the MMF dosing regimen itself may influence the pharmacogenetic findings from MPA association studies. Our observations correspond with the findings in adult populations, although our subject number is too small to fully confirm them.

When evaluating the effect of MRP2 polymorphisms on MPA trough concentrations and total MPA exposure and comparing results with other studies, calcineurin inhibitor co-medication was confirmed as an important consideration. In patients on cyclosporine (CsA) and MMF, the effect of the MRP2 variants has not been observed due to the CsA-transporter interaction [12]. In the present study, all patients were on a MMF/tacrolimus immunosuppressive regimen (with some on MMF in combination with sirolimus), allowing us to observe the MRP2 genetic effect on C_{trough} and AUC. Concomitant sirolimus administration did not result in differences in MPA pharmacokinetics under the consideration of the genotypes observed.

In the present study, the recursive partitioning method was used for screening important covariates and their combinations [40]. This is a powerful approach in a pharmacogenetic association study. This method effectively provides insight in how to categorize subjects with a candidate factor in studies of limited sample size. MMFdose/BSA was recognized as the most influential factor for MPAdose/BSA- normalized AUC, followed by genetic variants of MRP2-24C>T, UGT2B7-900A>G and UGT1A9-440C>T (Figure, Supplemental Digital Content 3, <http://links.lww.com/TDM/A21>). A recessive model was suggested for both UGT1A9-440C>T and UGT2B7-900A>G in recursive partitioning. Although recipients homozygous for UGT1A9-440C>T and UGT2B7-900A>G were present in our population, they did not clearly show higher MPA exposure compared to heterozygotes. This might be due to a natural selection bias of MMF treatment discontinuation of those patients at risk for increased MPA exposure. On the other hand, the MPA metabolic capacity may have been up-regulated to at least the level of the heterozygous phenotype by the induction of the main metabolic enzymes. Several of the UGT isozymes and transporters contributing to the disposition of MPA can be up-regulated [44]. Considering concentrations of metabolites such as Acyl-MPAG and MPAG may further strengthen the MPA pharmacokinetics pharmacogenetic association. It would be of interest to also include acyl-MPAG and MPAG concentration data in a future analysis.

Finally, we have to discuss our youngest subjects (less than 4 years old, n=3) and patients heterozygous for UGT1A9-275T>A (n=5). There was an age gap between these three youngest subjects and those older than 7 years, which could be captured in a bimodal distribution (Figure, Supplemental Digital Content 1, <http://links.lww.com/TDM/A19>). In addition, the three youngest subjects were genetically and clinically quite heterogeneous. One recipient showed mild liver dysfunction with the highest values in terms of AST/ALT of all, another recipient had the highest creatinine clearance of all, and the rest had the UGT1A9-275T>A polymorphism. Furthermore, all three young patients were on a steroid-free regimen, representing a difference from the majority of our study population. On the other hand, five recipients heterozygous for the UGT1A9-275T>A polymorphism were also

unique in terms of combined polymorphisms such as *UGT1A8*3* and MRP2-24C>T status, intake of calcineurin inhibitor, age and actual dose. This means that this genotype group was too heterogeneous to be handled properly as one group, although mean AUC values tended to be lower in the heterozygotes for UGT1A9-275T>A than the others (Figure, Supplemental Digital Content 3, <http://links.lww.com/TDM/A21>).

In conclusion, this exploratory study demonstrates that the variability in MPA exposure in pediatric kidney transplant recipients can be explained in part by combined pharmacogenetic factors constituting polymorphisms in the UGT1A9-440C>T, UGT2B7-900A>G and MRP2-24C>T. These findings are in line with the change of activity that we expect based on previous studies of these SNPs. Our data also show an influence of the MMF dose. Further larger studies are warranted to confirm our observations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Relationship between the genotype group and A) Estimated MPA AUC₀₋₁₂ and B) Estimated C_{trough}

Sixteen recipients are included in this analysis, who are 7 years old, on more than 354.7mg/m² MMF dose and not a carrier of UGT1A9-2152 C>T and *UGT1A8**3. They are divided into three groups as follows: Group 1: Recipients with no or only one UGT-SNP, and no MRP2-24T>C, Group 2: MRP2-24T>C-negative recipients having both UGT1A9-440C>T and UGT2B7-900A>G, Group 3: Heterozygotes for MRP2-24T>C who also had UGT1A9-440C>T and/or UGT2B7-900A>G. Horizontal lines indicate 25, 50 and 75 percentiles.

Figure 2. Association between estimated MPA C_{max} and UGT1A9-440/-331 genotype
 Twenty five recipients who are 7 years old and not a carrier of UGT1A9-275T>A and/or UGT1A8*3 are included in this analysis. Sixteen recipients were on more than 354.7mg/m^2 MMF dose (left) and 9 recipients were less than 354.7mg/m^2 MMF dose (right).

Table 1

Clinical Characteristics of this study population.

	n	Mean	SD	Range		Percentiles		
				Min	Max	25%	50%	75%
Age at sampling (years)	32	13.9	4.6	2.1	19.9	13.4	15.1	17.0
Sex (Male : Female)	32	18 : 14						
Race (CA : AA)	32	29 : 3						
Body weight (kg)	32	52.5	23.8	12.7	107.5	39.9	49.4	68.7
Height (cm)	32	148.1	24.6	86.4	177.5	141.7	156.0	163.8
BSA (m ²)	32	1.45	0.44	0.55	2.25	1.28	1.47	1.77
Clinical Chemistry								
Creatinine clearance (mL/min per 1.73m ²)	32	96.2	34.0	53.2	237.6	76.2	93.7	103.3
Hemoglobin (g/dl)	32	11.8	1.3	9.0	14.4	10.9	11.8	12.7
AST (unit / L)	23	44.7	38.0	15	204	26	34	48
ALT (unit / L)	25	41.0	67.3	6.2	350	16	23	37
Albumin (g/dl)	26	4.21	0.57	3.10	5.60	3.80	4.25	4.60
Transplantation & Medication								
Age at transplantation (years)	32	11.0	6.0	1.4	18.7	3.7	13.2	15.4
Donor (living : diseased)	32	19 : 13						
Days after transplantation (days)	32	1053	1437	39	4470	115	243	1512
Tacrolimus : Sirolimus	32	25 : 7						
Prednisone (on : off)	32	25 : 7						
Prednisone Daily Dose (mg)	25	12.8	12.4	2.0	60.0	5.0	10.0	15.0
Dose MMF (mg)	32	592	276	140	1500	500	500	750
Dose MMF/BSA (mg /m ²)	32	403	109	234	666	324	395	495
Pharmacokinetic parameters estimated by Bayesian								
C _{trough} (mg / L)	32	2.5	1.4	0.3	6.4	1.4	2.4	3.5
C _{max} (mg / L)	32	16.0	9.2	3.8	37.9	9.2	13.6	18.0
AUC ₀₋₁₂ (mg hr / L)	32	43.7	18.4	8.9	92.9	29.7	40.7	53.4

	n	Mean	SD	Range		Percentiles		
				Min	Max	25%	50%	75%
Pharmacokinetic parameters estimated by Bayesian (MPA dose/BSA-normalized)								
C _{trough} (mg / L) per gMPA/m ²	32	8.9	5.4	1.0	25.2	5.7	8.8	11.7
C _{max} (mg / L) per gMPA/m ²	32	60.5	44.0	11.5	201.7	29.0	50.1	72.6
AUC ₀₋₁₂ (mg hr / L) per gMPA/m ²	32	156.7	76.5	26.8	365.3	99.3	149.5	190.7
Genotypes	n	Major homozygous		Heterozygous		Minor homozygous		
<i>UGT1A9-2152 C>T</i>	32	28		4		0		
<i>UGT1A9-440 C>T</i>	32	13		16		3		
<i>UGT1A9-275 T>A</i>	32	27		5		0		
<i>UGT1A9 (*3)</i>	32	32		0		0		
<i>UGT2B7-900 A>G</i>	32	12		14		6		
<i>UGT1A8 (*3)</i>	32	30		2		0		
<i>MRP2-24C>T</i>	32	25		7		0		

CA: Caucasian; AA: African American; BSA: body surface area; AST: aspartate aminotransferase; ALT: alanine aminotransferase; C_{trough} trough MPA concentration; C_{max} maximum MPA concentration. BSA was calculated using the following equation: BSA = (weight*height /3600)^{1/2}

Table 2

Effect of combined genotypes on MPA-PK parameters in higher dosing sub-group.

Genotype group	1	2	3	P value ANOVA ^{#2}
Group Descriptions	MRP2 -24 wild		MRP2 -24 heterozygous	
	Carriers of no SNP or either UGT1A9-440T or UGT2B7-900G	Carriers of both UGT1A9-440T and UGT2B7-900G	Carriers of UGT1A9-440T or UGT2B7-900G ^{#1}	
Number of subject	7	5	4	
Estimated Pharmacokinetic Parameters				
Estimated AUC ₀₋₁₂ (mg*hr/L)	30.7 ± 12.0	43.5 ± 8.8	66.8 ± 4.9 ***††	<0.001
Estimated C _{trough} (mg/L)	1.45 ± 1.02	2.90 ± 0.64 *	4.06 ± 0.30 ***	<0.001
Estimated C _{max} (mg/L)	11.1 ± 8.4	13.0 ± 4.8	17.7 ± 8.3	0.39
Normalized by MPAdose				
AUC ₀₋₁₂ (mg*hr/L per g)	63.9±33.1	81.3±21.1	135.1±28.0 ***†	<0.01
C _{trough} (mg/L per g)	2.9±2.2	5.4±1.5	8.1±1.1 **	<0.01
C _{max} (mg/L per g)	25.2±25.0	23.9±8.4	35.1±14.4	0.64
Normalized by MPAdose/body weight				
AUC ₀₋₁₂ (mg*hr/L per mg/kg)	3.54 ± 1.83	4.51 ± 1.17	7.50 ± 1.55 ***†	<0.01
C _{trough} (mg/L per mg/kg)	0.16 ± 0.12	0.30 ± 0.09	0.45±0.07 **	<0.01
C _{max} (mg/L per mg/kg)	1.40 ± 1.39	1.32 ± 0.47	1.95 ± 0.79	0.64
Normalized by MPAdose/BSA				
AUC ₀₋₁₂ (mg*hr/L per g/m ²)	85.6 ± 27.5	141.8 ± 30.3 *	190.7 ± 41.6 ***	<0.001
C _{trough} (mg/L per g/m ²)	3.8 ± 2.2	9.4 ± 2.1 **	11.5 ± 1.8 ***	<0.001
C _{max} (mg/L per g/m ²)	34.8 ± 34.4	42.3 ± 16.4	49.1 ± 18.3	0.69
Patient demographics				
Creatinine clearance (mL/min per 1.74m ²)	92.4± 37.6	96.6 ± 27.6	95.5 ± 4.7	0.97
Age at sampling (years)	14.4 ± 3.5	16.7 ± 1.6	15.3 ± 1.5	0.34
Body weight (kg)	59.6 ± 33.0	67.0 ± 12.3	46.4 ± 6.4	0.45
BSA (m ²)	1.56 ± 0.53	1.77 ± 0.21	1.41 ± 0.15	0.39
Creatinine clearance (mL/min)	104.8 ± 37.5	96.6 ± 27.6	108.9 ± 19.5	0.83
Hemoglobin (g/dl)	11.4 ± 0.74	11.2 ± 1.66	12.6 ± 1.51	0.23
AST (unit / L)	44.8 ± 24.1	38.0 ± 14.7	33.3 ± 18.0	0.73
ALT (unit / L)	24.2 ± 9.7	35.3 ± 30.1	39.4 ± 50.1	0.72
Albumin (g/dl)	4.4 ± 0.4	4.5 ± 1.1	4.0±0.8	0.63
Dose MMF/BSA (mg/m ²)	483.3 ± 98.0	419.1±57.6	488.6 ± 92.9	0.39
Dose MMF (mg)	771.4 ± 380.6	750.0 ± 176.8	687.5 ± 125.0	0.89
Donor (Living : Diseased)	5 : 2	4 : 1	2 : 2	0.61

Genotype group	1	2	3	P value ANOVA ^{#2}
Group Descriptions	MRP2 -24 wild		MRP2 -24 heterozygous	
	Carriers of no SNP or either UGT1A9-440T or UGT2B7-900G	Carriers of both UGT1A9-440T and UGT2B7-900G	Carriers of UGT1A9-440T or UGT2B7-900G ^{#1}	
Sex (Male : Female)	2 : 5	5 : 0	2 : 2	0.05
Prednisone (on : off)	4 : 3	4 : 1	4 : 0	0.27
Tacrolimus : Sirolimus	5 : 2	5 : 0	2 : 2	0.22

Note: Data are expressed as mean $\pm$ standard deviation or ratio. All patients were more than 7 years old and administered more than the MMF dose of 354.7 mgMMF/m². No patient was heterozygous nor homozygous for *UGT1A9-275T>A*.

^{#1} Two out of four patients possessed both *UGT1A9-440 C>T* and *UGT2B7-900 A>G* as a heterozygous, another patient were heterozygous for *UGT1A9-440 C>T* and the other one was heterozygous for *UGT2B7-900 A>G*.

^{#2} Mean comparisons were performed for each pair using the Tukey-Kramer post-hoc test;

* p<0.05,

** p<0.01,

*** p<0.001 vs. group 1;

[†] p<0.05,

^{††} p<0.01 vs. group 2.

Table 3

Association of UGT1A9-440 C>T genotype with estimated maximum MPA concentrations.

	354.7 mgMMF / m ²		< 354.7 mgMMF / m ²	
<i>UGT1A9-440C>T</i>	Non carrier	Heterozygous or Homozygous	No mutation	Heterozygous or Homozygous
Number of subject	5	11	4	5
Estimated C _{max} (mg/mL per gMPA/m ²)	24.1±9.0	48.3±27.1 *	56.7 ± 16.9	112.0 ± 40.5 *
Estimated C _{max} (mg/L)	9.5 ± 2.8	15.1 ± 8.4	11.9 ± 2.7	26.0 ± 9.7 *
Creatinine clearance (mL/min per 1.74m ²)	96.7 ± 40.8	93.4 ± 22.3	81.4 ± 14.5	82.4 ± 14.6
Age at sampling(years)	14.4 ± 4.1	15.8±1.8	14.6 ± 2.0	15.7 ± 1.8
BSA (m ²)	1.48 ± 0.53	1.64 ± 0.31	1.49 ± 0.30	1.62 ± 0.19
Dose MMF/BSA (mg/m ²)	546 ± 75	428 ± 64 *	290 ± 43	313 ± 34
Dose MMF (mg)	830 ± 404	705 ± 188	438 ± 125	500
Donor (living : diseased)	2 : 3	9 : 2	1 : 3	2 : 3
Sex (Male : Female)	2 : 3	7 : 4	2 : 2	2 : 3
Race(CA : AA)	3 : 2	11 : 0	4 : 0	5 : 0
Prednisone (on : off)	1 : 4	3 : 8	4 : 0	5 : 0
Tacrolimus : Sirolimus	3 : 2	9 / 2	3 / 1	4 / 1
<i>UGT2B7-900A>G</i> (non- : carrier)	2 : 3	4 : 7	1 : 3	1 : 4
<i>MRP2-24C>T</i> (non- : carrier)	4 : 1	8 : 3	4 : 0	4 : 1

Note: Data are expressed as mean ± standard deviation or ratio. All patients were more than 7 years old. No patient was heterozygous nor homozygous for *UGT1A9-275T>A*. Two-side Wilcoxon-Mann-Whitney rank sum test, a chi-square or the Fisher exact tests were applied.

*
p<0.05