Population Pharmacokinetics and Pharmacogenetics of Mycophenolic Acid Following Administration of Mycophenolate Mofetil in De Novo Pediatric Renal-Transplant Patients

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The objective was to develop a population pharmacokinetic–pharmacogenetic model of mycophenolic acid following administration of mycophenolate mofetil (MMF) in de novo pediatric renal-transplant patients and identify factors that explain variability. The pharmacokinetic samples were collected from 89 de novo pediatric renal-transplant patients treated with MMF and studied during the first 60 postoperative days. All patients were genotyped for UGT1A8-A9, UGT2B7, and ABCC2. Population pharmacokinetic analysis was performed with the NONMEM and was validated using bootstrap visual predictive check. The pharmacokinetic data were best described by a 2-compartment model with Erlang distribution to describe the absorption phase. The covariate analysis identified body weight as an individual factor influencing central volume of distribution and concomitant immunosuppressive medication and identified body weight and UGT2B7 802C>T genotype as individual factors influencing apparent oral clearance (CL/F) of MMF. CL/F in cyclosporine-MMF–treated patients was 33% higher than in tacrolimus-MMF–treated patients. The CL/F was significantly lower in patients with UGT2B7 802 C/C genotype compared with patients with UGT2B7 802 C/T and 802T/T genotypes, and this effect was independent of concomitant immunosuppressive medication or body weight. The population pharmacokinetic–pharmacogenetic model of mycophenolic acid was validated. Body weight, concomitant medication, and UGT2B7 genotype contribute significantly to the interindividual variability of MMF disposition in pediatric renal-transplant patients.

Keywords: *Pediatric; mycophenolate mofetil; UDP-glucuronosyltransferase; pharmacokinetics; renal transplantation*

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Mycophenolate mofetil (MMF) is an immunosuppressive drug that prevents acute and chronic rejections after solid organ transplantation as well as hematopoietic stem cell transplantation.[1] MMF is an ester prodrug, which is rapidly and extensively (95%) hydrolyzed to the active metabolite mycophenolic acid (MPA). MPA decreases de novo purine biosynthesis by inhibiting inosine monophosphate dehydrogenase reversibly and noncompetitively. As a result, the proliferation of T and B lymphocytes is suppressed.[2] Large interpatient variation in MPA exposure has been reported, with a correlation between total MPA area under the curve and the risk of acute rejection as well as toxicity.[3,4] We previously performed a population pharmacokinetic study of MMF

in pediatric renal-transplant patients and demonstrated that body weight was an important covariate for clearance.[5] However, the derived population model still presented large interindividual and residual variability, which indicated the implication of unidentified factors influencing the pharmacokinetics of MMF. These findings emphasize the need to decipher the molecular and cellular events responsible for this variability with the objective of tailoring MMF therapy to individual patients.[6]

MPA is the substrate of UDP-glucuronosyltransferases (UGTs), a superfamily of phase 2 enzymes.[7] The main elimination pathway of MPA is through conjugation of its phenol group to the inactive hydroxyl-β-glucuronide, generally referred to as MPA-phenyl-glucuronide (MPAG). The conjugation of its carboxylic acid moiety leads to a second glucuronide, namely MPA-acyl-glucuronide (AcMPAG).[8,9] After glucuronidation, MPAG is excreted into the bile by the ABC transporter multidrug resistance protein 2 (ABCC2) and breast cancer resistance protein (BCRP)[10] and undergoes enterohepatic recirculation (EHC).

Pharmacogenetic variation has been reported in almost all UGT family members.[11,12] Three major UGT isoforms involved in the metabolism of MPAG and AcMPAG have been identified: UGT1A8, 1A9, and UGT2B7. UGT1A9 is known as the main enzyme involved in the formation of MPAG.[13] In human liver microsomes, the variants –275T/A and –2152C/T of the UGT1A9 promoter region were associated with a 2.3-fold higher hepatic expression of UGT1A9 and a 2.1-fold increase in glucuronidation activity.[14] Kuypers et al[15] reported that these 2 variants were associated with significantly lower MPA concentrations in adult renal-transplant recipients receiving high-dose MMF (2 g/d) but not in those treated with low doses (1 g/d). UGT1A8, expressed in the gastrointestinal tract, is also involved in MPAG formation. An in vitro metabolic study demonstrated that the presence of the variant 830G>A induced a drastic reduction in the formation of MPAG.[13] UGT2B7 is the key UGT responsible for the formation of the acyl-glucuronide metabolite of MPAG. It is predominantly expressed in the liver but also is expressed in the gastrointestinal tract, kidney, pancreas, and brain. An in vitro study demonstrated that the 802C>T variant did not affect the glucuronidation rate of the MPA.[16] Van Agteren et al[17] reported no influence of –840G>A on AcylMPAG exposure. Djebli et al[9] reported that –842G>A polymorphism, which is in a reverse linkage disequilibrium with 802C>T, was associated with higher AcMPAG area under the curve (AUC_{0-9})/MMF dose in a subgroup of adult patients (MMF-sirolimus treatment, n = 40) but not in adult patients receiving MMF-tacrolimus or MMF-cyclosporine (n = 52).

Multidrug-resistance protein 2 (ABCC2) is considered major transporter involved in MPAG excretion,[18,19] both in the liver and in the proximal renal tubule. Naesens et al[20] reported that the ABCC2 –24C>T polymorphism was associated with a lower oral clearance of MPA in adult steady-state renal-transplant patients.

To our knowledge, no pharmacokinetic–pharmacogenetic data of MMF in pediatric patients are available. Data evaluating the ontogeny of UGT activities are limited.[21,22] However, considering the available data, we hypothesized that UGT and ABCC2 polymorphisms, in addition to age[23] and covariates already identified in adults, might affect the MMF pharmacokinetic parameters in pediatric patients. Therefore, we conducted a population pharmacokinetic–pharmacogenetic study using nonlinear mixed-effects modeling (NONMEM) to evaluate the impact of demographic, clinical (weight, gender, age, time after transplantation, and concomitant medication), and pharmacogenetic covariates (polymorphisms of UGT1A9, UGT1A8, UGT2B7, and ABCC2) on MMF pharmacokinetics in 89 renal-transplant patients.

METHODS

Study Population

A total of 89 pediatric patients (<18 years old) undergoing renal transplantation were enrolled in this prospective multicenter trial. The patients were recruited in 9 French centers from 2005 to 2008. The clinical trial was designed in accordance with legal requirements and the Declaration of Helsinki and was approved by CCPPRB (Comité Consultatif de Protection des Personnes dans la Rechercher Biomédicale, Lyon B, France). The parents of our patients gave informed consent for the participation of their children. The patients received induction therapy by thymoglobulins or basiliximab and were treated with prednisone, MMF (Cellcept, Roche Pharma A.G, Grenzach-Wyhlen, Germany), and cyclosporine (n = 41, Neoral, Novartis Pharmaceutical Corporation, Basel, Switzerland) or tacrolimus (n = 47, Prograf, Astellas Pharmaceutical, Chicago, Illinois). (One patient did not receive any anticalcineurine drug.) Prednisone was started at a daily dose of 60 mg/m^2 and then tapered to 30 mg/m^2 at day 30, 15 mg/m^2 at day 60, and 7.5 mg/m^2 at 6 months post transplantation in all patients.

A full concentration–time profile was determined during the initial posttransplant period. Blood samples were obtained before and 0.5, 1, 2, 3, 4, 6, 8, and 12 hours after administration of MMF in 18 patients.

Because we have to limit the number of samples drawn from children, for the next 71 children, blood samples were obtained before and 0.5, 1, 2, 3, and 12 hours after administration. Target therapy was initiated at a dosage of 600 mg/m^2 twice daily. Doses were adjusted to maintain MPA AUC between 30 and 60 h·mg/L.[24] Individual AUCs were calculated by the linear-trapezoidal rule.

Concentrations of cyclosporine and tacrolimus were monitored and doses adjusted to the following target concentrations in the initial posttransplant period: for cyclosporine, trough concentration 150 to 250 ng/mL; for tacrolimus, trough concentration 5 to 15 ng/mL.[25]

Assay of Mycophenolic Acid

The plasma MPA concentrations were measured either by high-performance liquid chromatography (HPLC) or by enzyme-multiplied immunoassay technique (EMIT). The HPLC-UV system consisted of a quaternary P1000 XR pump (ThermoQuest TQ, Riviera Beach, Florida), a TQ auto sampler, and a TQ UV 6000 detector (detection wavelength, 254 nm) linked to a TQ Spectranet for recording and storing throughout analysis. The system used a Hypersil BDS C18, 5 µm (250 × 4.6 mm) analytic column (CIL Cluzeau, Sainte Foix la Grande, Gironde, France) with a mobile phase of acetonitrile and 0.05% aqueous phosphoric acid (38:62 vol/vol, increased to 43/57 vol/vol at 10 minutes and decreased at 28/72 vol/vol at 18 minutes). The assay showed linearity from 0.1 to 40 µg/mL. We determined intra-assay precision by analyzing, on the same day, 3 replicates of quality control samples at 3 different levels (0.25, 2.5, and 30 µg/mL). We evaluated interassay precision by analyzing the quality controls each day for 5 days. Intraday and interday coefficients of variation (CVs) were less than 10%. The lower limit of quantification was 0.1 µg/mL for MPA.[5]

The second method was an EMIT mycophenolic acid assay (EMIT 2000 Mycophenolic Acid Assay, Dade Behring Diagnostics, Milton Keynes, UK) and was operated on a Cobas Mira Plus System (Roche Diagnostic, Neuilly-sur-Seine, France), according to the manufacturer's guidelines. Intraday and interday CVs were less than 6.5%.The lower limit of quantification was 0.5 µg/mL.

The effect of using 2 different analytical methods was considered a possible systematic bias between assays[26] and was tested in the model using different scale parameter values, Scale = V for the HPLC-UV assay and Scale = $h \times V$ for the EMIT assay, with h measuring the proportional bias of the other assay. This method was described in the NONMEM user's guide[27] and applied in the population pharmacokinetic study of cyclosporine by Saint-Marcoux et al.[26]

Blood concentrations of cyclosporine and tacrolimus were measured by EMIT (Dade Behring Diagnostics).

Pharmacogenetic Analysis

Genomic DNA was extracted from blood samples using a QIAamp DNA Blood Midi kit (Qiagen, Chatsworth, California) following the manufacturer's instructions. Total genomic DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Labtech, Palaiseau, France) at 260 nm. Polymorphisms in UGTs and ABCC2 were determined using a real-time TaqMan allelic discrimination method with 3′-minor groove binding (MGB) quencher probes (ABI Prism 7700 SDS; Applied Biosystems, Foster City, California). For ABCC2, UGT1A8, 1A9, and 2B7 polymorphisms, primers and probes were obtained from Applied Biosystems (UGT1A9, 98T>C, –2152C>T rs 17868320; UGT1A8, 830G>A rs 17863762, 802C>T rs 7439366; ABCC2, –24C>T rs 717620, 1249G>A rs 2273697, 3563T>A rs 8187694, 3972C>T rs 3740066).

For UGT1A9 –275T>A mutation, primer and probe design and determination of the technical conditions were carried out in our laboratory.

Forward primer: 5′-GAACCTTCAAGGTCCAAAAG CAT-3′
Reverse primer: 5′-AACATGCCCTGTGCTGCAA-3′
MGB probes: 5′-(FAM)-AATTCTGCTtCTAAACTTA-3′ and 5′-(VIC)-AATTCTGCTaCTAAACTTAA-3′

After a first step that consisted of AmpErase (50°C for 2 minutes) and AmpliTaq Gold enzyme activation (90°C for 10 minutes), 50 polymerase chain reaction cycles were performed for 15 seconds at 92°C (95°C for UGT1A9 –275T>A) for denaturation and for 60 to 90 seconds at 60°C for hybridation and elongation.

Pharmacokinetic Analysis

Pharmacokinetic analysis was carried out using the nonlinear mixed-effects modeling program NONMEM (GloboMax, Hanover, Maryland). The first-order conditional estimation method was used to estimate pharmacokinetic parameters and their variability.

Different 2-compartment open models with first-order elimination were compared, each with a different input to describe the absorption phase: (1) a zero-order input with or without a lag time, (2) a

first-order input with or without a lag time, and (3) an Erlang distribution. The Erlang distribution is the analytical solution for a chain of *n* compartments between the depot and central compartment. The number of serial compartments *n* was estimated by interposing an increasing number of compartments until no improvement was noted.[26-28] Five parameters had to be estimated: the constant transfer rate between 2 absorption compartments (K_t), the apparent oral clearance (CL/F), the apparent volume of the central compartment (V_1/F), the apparent volume of the peripheral compartment (V_2/F), and the intercompartment clearance (Q/F).

Interindividual variability of the pharmacokinetic parameters was estimated by use of an exponential model and could be expressed as follows:

$$\theta_{Aj} = \theta_A \times \exp(\eta_j^{\theta_A})$$

where θ_A is the typical population value of a pharmacokinetic parameter; $\eta_j^{\theta_A}$ represents the difference between the *j*th individual's θ_A values and the predicted values; $\eta_j\theta^A$ values are independent, identically distributed random variables and are normally distributed around 0 with variance ω^2; and *j* is the variable for the *j*th individual. A combined proportional and additive error model was used for the residual random effects.

Covariate Analysis

The individual pharmacokinetic parameter estimates were plotted against demographic factors (age, weight, gender, time after transplantation, creatinine clearance, and concomitant immunosuppressive medication) for visual inspection according to the method described by Hesselink et al[29] and Maitre et al.[30] Covariates that showed a correlation with a pharmacokinetic parameter were entered into the model for further tests.

The influence of the different genotypes on the pharmacokinetic parameters was evaluated with the following equation:

$$TV_{pop} = \theta1 \times \theta2^{\text{heterozygous}} \times \theta3^{\text{homozygous}}$$

where TV_{pop} is the typical value of the pharmacokinetic parameter in the population, θ1 is the typical value for homozygous wild-type patients, θ2 is the fractional change for heterozygous patients, and θ3 is the fractional change for homozygous mutant patients. The population pharmacokinetic and pharmacogenetic analysis was in accordance with the method described by Hesselink et al.[29]

The likelihood ratio test was used to test the effect of each covariate.

A covariate was selected only if a significant ($P < .05$) decrease (reduction exceeded 3.8) in the objective function (OFV) from the covariate-free model was obtained. This criterion is based on the objective function having an approximate chi-square distribution with 1 *df.*

The importance of each covariate was reevaluated by backward selection. Each covariate was independently removed from the full model to confirm its relevance. A significant increase in the OFV was required to confirm that the covariate was significant. The resulting model was called the "final" population pharmacokinetic model, which included all the significant covariates.

Model Validation

The bootstrap visual predictive check method was used to assess the stability and performance of the final model. A degenerate bootstrap visual predictive check was performed by computing a nonparametric bootstrapped median including its 5th and 95th percentiles based on the available data per elapsed time point.[31] The 50th percentile concentration (as an estimator of the population-predicted concentration) and the 5th and 95th percentile concentrations, processed by R for NONMEM (v.20070911), were then plotted against elapsed time. The bootstrap procedure was repeated 200 times in an automated fashion using Wings for NONMEM (http://wfn_sourceforge.net/).

Statistical Analysis

The distribution of continuous data was evaluated, and parametric or nonparametric tests were consequently applied when appropriate. The patient genotypes were used as categorical independent variables. Hardy-Weinberg equilibrium was evaluated using the Fisher exact test. These statistical analyses were performed using SPSS for Windows 15.0 (SPSS, Chicago, Ill). We considered $P < .05$ as statistically significant.

RESULTS

Eighty-nine renal-transplant children, 53 males and 36 females, with a mean ± standard deviation (SD) age of 10.1 ± 5.3 years (range, 1.6-18.3 years) and a mean ± SD weight of 32.7 ± 16.2 kg (range, 7-73 kg) were enrolled in this prospective, multicenter trial. The patients' characteristics are presented in Table I. The pharmacogenetic results and Hardy-Weinberg equilibrium are presented in Table II.

Table I Characteristics of the 89 De Novo Pediatric Renal-Transplant Patients

No. of patients	89
Male/female, n	53/36
Age at time of transplantation, y[a]	10.1 ± 5.3 (1.6-18.3)
Time after transplantation, d[a]	23.0 ± 11.9 (7-60)
Body weight, kg[a]	32.7 ± 16.2 (7.1-73)
Creatinine clearance, mL/min[a,b]	95.8 ± 25.7 (38.1-160.3)
MMF dose, mg/d[a]	1078.0 ± 511.8 (200-2000)
MMF dose, mg/kg/d[a]	36.3 ± 15.2 (4.1-104.3)
Co-medication	
Tacrolimus-MMF, n	47
Tacrolimus dose, mg/d[a]	7.6 ± 3.7 (1.5-20)
Tacrolimus dose, mg/kg/d[a]	0.28 ± 0.16 (0.08-0.89)
Cyclosporine-MMF, n	41
Cyclosporine dose, mg/d[a]	252.7 ± 115.7 (100-600)
Cyclosporine dose, mg/kg/d[a]	7.9 ± 3.1 (2.1-15.8)

MMF, mycophenolate mofetil.
a. Mean ± standard deviation (range).
b. Calculated with the Schwartz formula.

Table II Genotype Frequencies and Hardy-Weinberg Equilibrium of UDP-Glucuronosyltransferases (UGTs) and ABC Transporter Multidrug Resistance Protein 2 (ABCC2) in 89 Patients

Gene	Variation	Genotype	Frequency	%	*P* Value[a]
UGT1A9	98T>C	T/T	85	95.5	1.00
		T/C	4	4.5	
	–275T>A	T/T	80	89.9	.19
		T/A	8	9	
		A/A	1	1.1	
	-2152C>T	C/C	82	92.1	1.00
		C/T	7	7.9	
UGT1A8	830G>A	G/G	87	97.8	
		G/A	2	2.2	
UGT2B7	802C>T	C/C	24	27	
		C/T	44	49.4	
		T/T	21	23.6	
ABCC2	–24C>T	C/C	60	67.4	.49
		C/T	25	28.1	
		T/T	4	4.5	
	1249G>A	G/G	58	65.2	.72
		G/A	29	32.6	
		A/A	2	2.2	
	3563T>A	T/A	17	19.1	1.00
		T/T	72	80.9	
	3972C>T	C/C	39	43.8	.64
		C/T	38	42.7	
		T/T	12	13.5	

a. Fisher's exact test (Hardy-Weinberg equilibrium).

Population Modeling

A total of 89 pharmacokinetic profiles (497 samples) were available for population modeling and were best described with a 2-compartment model with first-order elimination and Erlang distribution to describe the absorption phase (ADVAN5, SS5). The optimal number of serial compartment placed upstream to the central one was 4. This model led to significant improvement (lower residual variability and OFV value) compared with classical zero-order or first-order absorption models (Table III). Interindividual variability was best described by an exponential model and was then estimated for K_r, V_1/F, Q/F, and CL/F. Residual variability was best described by a combined proportional and additive model. All the measured concentrations were above the lower limit quantification. Mean population pharmacokinetic parameters of structure model were K_r 7.81 h^{-1}, CL/F 15 L/h, V_1/F 33.2 L, V_2/F 183 L, and Q/F 18.6 L/h.

The effect of using 2 different analytical methods for MPA concentrations was tested using different scale parameter values (25% samples were measured by HPLC-UV and 75% samples by EMIT). Significant improvement in the OFV (7.8 U decrease) was obtained when the concentrations were rescaled by a constant, which was estimated separately for EMIT assay, HPLC being considered as the reference. Then systematic bias between assays was included in this model.[26]

Plots of the individual pharmacokinetic parameters K_r, V_1/F, Q/F, and CL/F versus the covariates age, gender, weight, time after transplantation, creatinine clearance, and concomitant immunosuppressive medication indicated the following relationships: age, weight, and concomitant immunosuppressive medication with CL/F and weight with V_1/F. These potential covariates were evaluated in the model. We then divided the patients into 2 groups: cyclosporine-MMF–treated and tacrolimus-MMF–treated patients. The plot of CL/F versus the 2 groups indicated an increased CL/F in cyclosporine-treated patients. This covariate caused a significant decrease of the OFV by 4.2 points ($P < .05$, likelihood ratio test). CL/F in cyclosporine-MMF–treated patients was 33% higher than in tacrolimus-MMF–treated patients (18.1 ± 10.0 L/h vs 13.5 ± 6.6 L/h, *t* test $P = .014$). Furthermore, the OFV continued to decrease by 10.7 points when a nonlinear relationship between weight and CL/F was introduced in

Table III Comparison of the Different Basic Models Tested for Modeling MMF Pharmacokinetics

Models	Objective Function	Residual Variability	
		Proportional, %	Additive
Two-compartment zero-order absorption without lag time	1694	42	1.42
Two-compartment zero-order absorption with lag time	1689	29	1.69
Two-compartment first-order absorption without lag time	1630	19	1.52
Two-compartment first-order absorption with lag time	1602	10	1.36
Two-compartment Erlang absorption	1540	6	1.19

the model ($P < .001$, likelihood ratio test), $CL/F = \theta 3 \times (\text{body weight/median})^{\theta 4}$, where the median body weight is 30.3 kg. Mean weight was not different between cyclosporine-MMF–treated and tacrolimus-MMF–treated patients ($P = .627$, t test). The OFV further decreased by 14.7 points when another nonlinear relationship between weight and V_1/F was introduced in the model ($P < .001$, likelihood ratio test). $V_1/F = \theta 1 \times (\text{body weight/median})^{\theta 2}$, where the median body weight is 30.3 kg. Introduction of the relationship between age and CL/F did not further improve the model.

To evaluate the influence of the different genotypes on the pharmacokinetic parameters, individual Bayesian estimates of these parameters were generated on the basis of the intermediate model and plotted versus the different genotypes.[29] The plot of CL/F versus UGT2B7 polymorphism indicated a lower CL/F in patients with 802C/C genotype compared with those with 802C/T and 802T/T. Evaluation of this hypothesis in the intermediate model demonstrated a significant reduction of OFV by 6.3 points ($P < .05$, likelihood ratio test). CL/F values among the 3 genotype groups were 12.3 ± 5.5, 16.4 ± 10.0, and 17.9 ± 7.8 L/h, respectively (Figure 1). Body weights were not significantly different between genotype groups (31.4 ± 13.8, 32.9 ± 17.4, and 33.7 ± 16.9 kg, respectively, analysis of variance [ANOVA] $P = .891$). Furthermore, CL/F appeared to be lower in –275T/A and –2152T/C individuals compared with wild-type. However, this difference was not statistically significant. No other significant changes in any of the pharmacokinetic parameters were detected. The analysis of covariates was reevaluated by the backward selection processes: all the covariates found to be significant were added simultaneously into the model, and then each one was independently removed. The removal of body weight, concomitant immunosuppressive medication, or UGT2B7 genotype led to a significant increase of OFV (increase >3.8, $P < .05$, likelihood ratio test). The independence of body weight, concomitant immunosuppressive medication, and UGT2B7 genotype was confirmed in backward selection processes. Therefore, these covariates were retained in the final model. The interindividual variability of CL/F and V_1/F decreased from 62.4% to 53.5% and from 104% to 61.6%, respectively. The forward and backward covariate selection processes are summarized in Table IV. The final population pharmacokinetic parameters of MMF are presented in Table V. The weight-normalized and concomitant immunosuppressive medication–normalized CL/F, determined using a formula developed in a population model,

$$\frac{CL/Fif}{(\text{Body weight}/30.3)^{0.424} * 0.778^{\text{comedication}}}$$

cyclosporine comedication = 0;
if tacrolimus comedication = 1

were significantly different between genotype groups (14.4 ± 6.7 for UGT2B7802C/C, 18.1 ± 8.7 for UGT2B7802C/T, and 20.7 ± 9.8 for UGT2B7802T/T; ANOVA $P = .047$), confirming the influence of UGT2B7 genotype.

Model Validation

Routine diagnostic individual-weighted residuals versus individual model-predicted values (Figure 2B) were symmetrically distributed and were mostly within about 1 U of the null ordinate, indicating a good fit of the model to the data. Plots of individual residuals versus time (Figure 2C) were distributed symmetrically in a band with no obvious trend and were mostly within approximately 3 U of the null ordinate, indicating that no time-related factor affected the data and that no subject's data contributed to any marked deviation from the model.

For the bootstrap visual predictive check, the 5th and 95th percentiles as well as the median 50th percentile of these bootstraps and the patients' data over a 12-hour interval are shown in Figure 3. This graph, representing a visual internal validation of the model, shows that approximately 90% of the data of MPA fit well within the 5th to 95th percentiles (exact binomial test, 7.4% out of limits observed; 95% confidence interval, 5.3-10.1) and

Table IV Covariates Forward and Backward Selection Processes

		Objective Function	
	Pharmacokinetic Parameter	**Forward (*P* Value)**	**Backward (*P* Value)[a]**
Two-compartment Erlang absorption, no covariate		1540.4	
Systemic bias between assays		1532.7	
Concomitant immunosuppressive medication (tacrolimus or cyclosporine)	CL/F	1528.5 (<.05)	+4.2 (<.05)
Body weight	CL/F	1517.8 (<.001)	+15.3 (<.001)
	V/F	1503.2 (<.001)	+15.0 (<.001)
UGT2B7 genotype[b]	CL/F	1496.9 (<.05)	+6.3 (<.05)

CL/F, apparent oral clearance; V/F, apparent volume.
a. The independence of body weight, concomitant immunosuppressive medication, and UGT2B7 genotype was confirmed in backward selection processes.
b. Body weights were not significantly different between genotype groups (31.4 ± 13.8 kg, 32.9± 17.4 kg, and 33.7 ± 16.9 kg, respectively, analysis of variance $P = .891$).

Table V Population Pharmacokinetic Parameters of Mycophenolate Mofetil

	Final Model	
Parameters	**Final Estimate**	**RSE, %**
K_r, h^{-1}	6.2	10
V_1/F, L		
$V_1/F = \theta1 \times (\text{body weight}/30.3)\theta^2$		
θ1	23.0	19
θ2	1.35	21
V_2/F, L	158	19
Q/F, L/h	25.6	23
CL/F, L/h		
$CL/F = \theta3 \times (\text{body weight}/30.3)\theta^4 \times \theta5^{\text{comedication}} \times \theta6^{\text{heterozygous}} \times \theta7^{\text{homozygous}}$		
θ3	12.9	16
θ4	0.424	32
θ5 if cyclosporine comedication = 0; if tacrolimus comedication = 1	0.778	16
θ6 if UGT2B7 –802 C/T, heterozygous = 1 and homozygous = 0	1.29	15
θ7 if UGT2B7 –802 T/T, homozygous = 1 and heterozygous = 0	1.51	21
Interindividual variability, %		
K_r	74.9	24.8
V_1/F	61.6	46.0
Q/F	115.3	31.9
CL/F	53.5	23.4
Residual proportional (EMIT method), %	8.9	62.1
Residual additive (EMIT method)	1.27	7.9
Residual additive (HPLC method)	0.69	44.8

CL/F, apparent oral clearance; Kr, constant transfer rate between 2 absorption compartments; Q/F, intercompartment clearance; RES, Standard Error; V_1/F, apparent volume of the central compartment; V_2/F, apparent volume of the peripheral compartment.

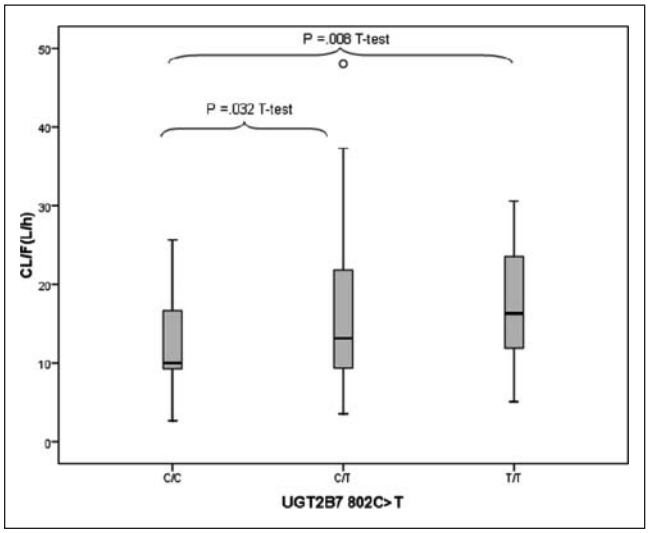

Figure 1. Relationship between apparent oral clearance (CL/F) of mycophenolate mofetil and UGT2B7 802C>T genotype.

were symmetrically distributed around the median (Pearson's chi-square test, $P = .82$).

DISCUSSION

The pharmacokinetics of MMF are known to exhibit considerable interindividual variability. In this study we have developed and validated a population pharmacokinetic model of MMF to individualize the dosage regimen in de novo pediatric renal-transplant patients. Furthermore, we have examined the impact of the UGTs and ABCC2 polymorphisms on the pharmacokinetic parameters of MMF. The pharmacokinetic samples were drawn in the initial posttransplant period, and individual MMF dose was adjusted to maintain target AUC between 30 and 60 h·mg/L.

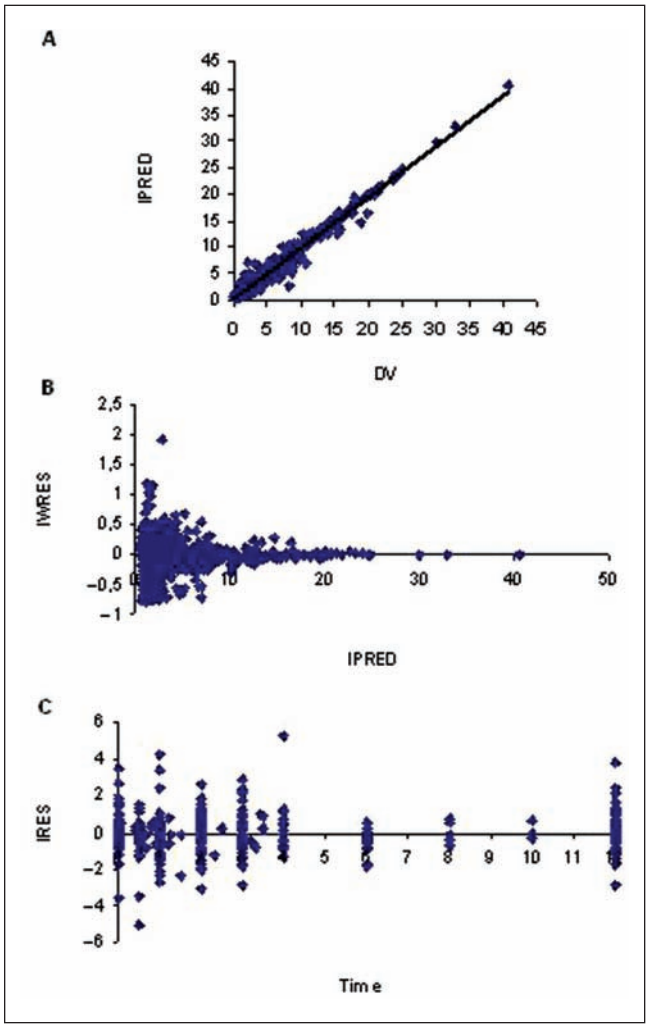

Figure 2. Goodness-of-fit plots for the final model. (A) Individual model-predicted concentration (IPRED) versus observed concentration (DV). (B) Individual weighted residuals (IWRES) versus individual model-predicted concentration (IPRED). (C) Individual residuals (IRES) versus time.

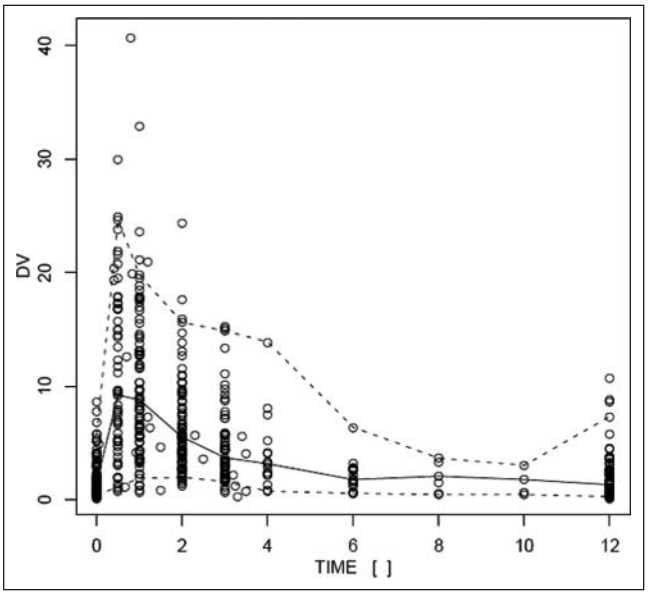

Figure 3. Bootstrap visual predictive check. DV, observed concentrations (μg/mL). Dashed lines, 5th and 95th percentiles; solid line, 50th percentile.

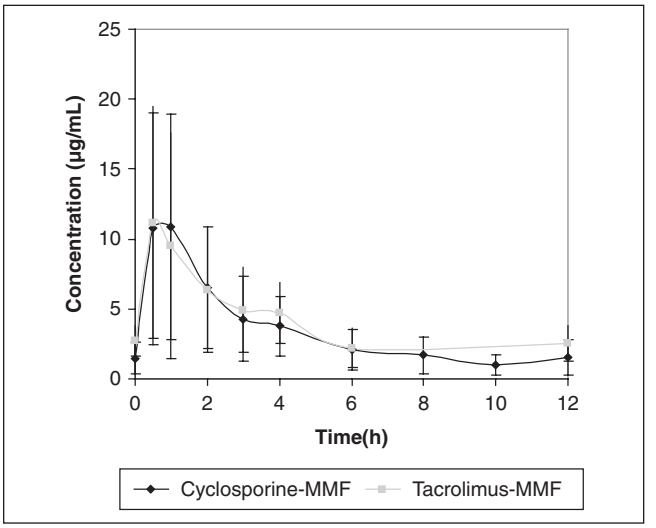

Figure 4. MPA pharmacokinetic profiles obtained between cyclosporine- and tacrolimus-treated patients (mean ± SD). MMF, mycophenolate mofetil.

The pharmacokinetics of MMF in 89 de novo pediatric renal-transplant patients were described with a 2-compartment model. Because simple time-lagged first-order absorption exhibited a large inter-individual variability, 207%, a more complex Erlang distribution was used to describe the absorption phase, which has shown greater flexibility in modeling the flat or delayed absorption profiles.[26,28,32] We observed a significant improvement when using the Erlang distribution compared with classical zero-order or first-order absorption models. Plots of observed concentrations versus individual model-predicted concentrations demonstrated an adequate goodness of fit. The mean apparent clearance was estimated to be 15 L/h. It was similar to the value previously found in other pediatric study: 17 L/h, reported by Payen et al.[5]

EMIT and HPLC-UV are 2 concurrent methods used to quantify MPA concentrations. Recently, Irtan et al[33] reported that EMIT overestimated MPA concentrations compared with HPLC-UV in pediatric renal-transplant patients, attributable to cross-reactivity with the active acyl-glucuronide metabolite. In our study, significant

improvement in the OFV was obtained when systematic bias between the 2 assays was included in the model. The developed population pharmacokinetic model took that into account by introducing a scaling factor considering the HPLC method as reference and attributing a different residual variability model to EMIT. Thus, this model could be applied to MMF pharmacokinetic profiles obtained with these 2 methods.[26,27]

Previously, we reported that the central volume distribution of MMF was related to body weight in stable pediatric renal-transplant patients.[5] In this study, we found that the V_1/F and CL/F of MMF could be better described with the nonlinear relationship as follows:

$$V_1/F = \theta 1 \times (\text{body weight} / \text{median})^{\theta 2}$$
$$CL/F = \theta 3 \times (\text{body weight} / \text{median})^{\theta 4}$$

where the median body weight is 30.3 kg and θ2 and θ4 were estimated by the final model. Body weight was considered as a regular continuous covariate. However, the allometric scaling (1/4 power models) used in a number of population pharmacokinetic studies in children was not used in the present study because of imprecise estimates of typical pharmacokinetic parameters. Indeed, given the potential impact of UGT maturation,[21,22] MMF metabolic clearance might not be correlated with weight via a standard fixed power term.[34]

Another interesting finding was the observed effect of concomitant immunosuppressive medication on MMF CL/F. Cyclosporine has been reported to interact with MPA and to decrease MMF AUC.[35,36] In the present study, a significant improvement in the OFV was obtained when concomitant immunosuppressive medication was included in the model. CL/F was 33% higher in cyclosporine-MMF–treated patients than in tacrolimus-MMF–treated patients. Concomitant immunosuppressive medication has a differential effect on MPA CL/F, which was due to inhibition of MPA EHC via inhibition of ABCC2 by cyclosporine rather than to inhibition of UGT by tacrolimus.[37-39]

The MPA EHC has been identified as a second peak occurring 6 to12 hours after MMF intake. To characterize this EHC in children, a full pharmacokinetic profile was planned in the initial protocol. We studied 18 patients (n = 14 cyclosporine-MMF; n = 4 tacrolimus-MMF) and then decided to reduce both the number of samples and blood volume in the pediatric patients; a "relatively sparse profile" based on 6 samples was obtained in the 71 additional patients. This modification considered different limited sampling strategies already published[40-42] and validated using full pharmacokinetic profiles, because the influence of EHC was minor for these limited sampling strategies. Both sparse and full pharmacokinetic profiles were included in the final analysis.

We next investigated the potential influence of the pharmacogenetic polymorphisms of UGTs and ABCC2 on the pharmacokinetic parameters of MMF described by an intermediate model to obtain a refined population model.

UGTs exist as a superfamily of enzymes, and pharmacogenetic variants have been characterized in almost all UGT family members.[11,12] Among them, UGT2B7 and UGT1A9 have been identified as the main enzymes involved in the formation of AcMPAG and MPAG, respectively.[43,44]

In vitro, complementary DNA expression studies failed to demonstrate that the UGT2B7 802C>T variant affected the glucuronidation rate of various UGT2B7 substrates, including morphine.[16,45-48] A possible explanation is that microsomal systems do not reflect the in vivo activity of UGT2B7, particularly if the polymorphism affects the transcriptional activation of UGT2B7.[47,48]

In an in vivo study conducted in adult patients, Djebli et al[9] reported that UGT2B7 842G>A polymorphism, which is in a reverse linkage disequilibrium with UGT2B7 802C>T, was associated with higher AcMPAG exposure in renal-transplant adult treated with MMF and sirolimus but not in patients receiving MMF-tacrolimus or MMF-cyclosporine. In contrast, a study in Japanese renal-transplant adults showed that dose-adjusted $AUC_{0\text{-}12}$ and maximum concentration of MPA tended to be lower, but not significantly different, in patients carrying 802T/T genotype.[49] The authors suggested that their study did not reach significance because of the low frequency (6.9%, n = 5) of patients carrying the 802T/T genotype. Kuypers et al[15] reported that –275T/A and –2152C/T single nucleotide polymorphisms (SNPs) of the UGT1A9 gene were associated with significantly lower MPA exposure AUC in de novo renal-transplant adults treated with 2 g of MMF per day but not in patients treated with 1 g of MMF per day. Here again, the small number of patients carrying these 2 variants might result in misinterpretation of the impact of UGT1A9 polymorphisms on MPA pharmacokinetics in renal-transplant patients, as discussed by Van Schaik et al.[50] Altogether, these data demonstrate that in adults, the impact of UGB2B7 on MMF metabolism is limited and although hepatic UGT1A9 is mostly responsible for the formation of MPAG, the impact of UGT1A9 polymorphism remains controversial and difficult to investigate in vivo, because the number of patients carrying mutant alleles is limited, requiring large

study populations. In contrast to adult data, our results obtained in renal-transplant children using modeling technique to describe population pharmacokinetics demonstrated a significant effect of the UGT2B7 polymorphisms on MPA pharmacokinetics, because MMF oral clearance was significantly lower in children with UGT2B7 802C/C than in children with UGT2B7 802C/T and 802T/T genotypes. The influence of UGT2B7 genotype was independent of concomitant immunosuppressive medication or body weight. This was confirmed in the backward covariate selection processes. The weight-normalized CL/F and concomitant immunosuppressive medication–normalized CL/F, determined using a formula developed in a population model, were also significantly different between UGT2B7 genotype groups.

We also investigated the impact of UGT1A9 and observed that MMF CL/F tended to be lower in patients carrying 1 mutated −2152C/T allele (n = 7), Here again, and similar to adult studies, the small number of patients carrying −275T/A and −2152C/T UGT1A9 variants might result in misinterpretation.[50]

The role of UGT2B7 remains largely unexplained. The activity of UGT2B7 has been characterized in vivo and in vitro using morphine as prodrug and is present in early life.[51] Prausa et al[52] studied renal-transplant children experiencing MMF-associated leukopenia and diarrhea and showed that the 3 patients (out of 38) who were homozygous for UGT1A9 −331T>C developed leukopenia, whereas heterozygotes had significantly more adverse events, but UGT2B7 −900G>A had no influence. AcMPAG formation could not be quantified in the present study but was shown to be highly variable in pediatric renal-transplant patients.[53] Whether the different maturation rates between the UGT1A9 and UGT2B7 isoforms could lead to differences in MPA metabolic profile and pharmacokinetic–pharmacogenetic relationship of MMF should be evaluated in further research taking into consideration newly identified polymorphisms (UGT1A9−331T>C, UGT2B7−842G>A).

UGT1A8 is also involved in MPAG formation. An in vitro metabolic study demonstrated that the presence of the variant 830G>A induces a drastic reduction in the formation of MPAG.[13] In a small group of adult healthy individuals (n = 17), a higher MPA exposure was reported to be associated with the variant 830G>A.[6] In our study, because only 2 children were heterozygotes, its impact could not be confirmed.

Multidrug-resistant protein 2 (ABCC2) is considered a major transporter involved in MPAG excretion.[18,19] In our pediatric population, none of the ABCC2 SNPs studies was found to influence pharmacokinetic parameters. Recently, in a pharmacokinetic–pharmacogenetic study of 338 adult renal-transplant patients, Van Schaik et al[50] also reported that ABCC2 SNPs did not strongly affect MPA exposure.

In our pediatric patients, the posttransplant delay had no impact on MPA clearance, whereas in previous reports MMF exposure increased with postoperative delay.[54] This might reflect the impact of renal function on MPA pharmacokinetics, as suggested by Van Hest et al.[55] In patients with impaired renal function, acidosis and uremia decrease MPA binding to albumin[10] and the accumulation of the glucuronidated metabolite MPAG displaces MPA from its albumin binding sites,[56] resulting in high concentrations of free MPA and an increase in MPA clearance in the initial posttransplant period. As renal function improves, MPA clearance progressively increases over the first months after transplantation.[55] Van Hest et al[38] also demonstrated that the impact of renal function was significant only in adult kidney-transplant patients having a creatinine clearance lower than 25 mL/min. In our study, all the patients had a creatinine clearance greater than 25 mL/min, and this might explain why neither posttransplant delay nor renal function significantly influenced MMF CL/F. When we designed this trial, albumin concentration was not identified as a covariate influencing MPA pharmacokinetics, and therefore we could not investigate its impact here.

Interindividual and residual variability remained large even when the genetic information available on MMF metabolism was taken into account. The range of postoperative delay might contribute, at least partly, to the unexplained variability in the final model, because it is an important predictor of interoccasion variability and high variability of MMF pharmacokinetics in the initial posttransplant period.[54]

In a previous study, Cattaneo et al[57] reported that the withdrawal of steroids reduced MMF CL/F in adult kidney-transplant patients. All our patients received prednisone in the initial posttransplant period, and dosage reduction was conducted according to a unique protocol; therefore, withdrawal could not be tested.

Using a population pharmacokinetic–pharmacogenetic model, we found a statistically significant effect of the UTG2B7 802C>T genotype on the pharmacokinetics of MMF in de novo pediatric renal-transplant patients. We demonstrated that 3 covariates—concomitant immunosuppressive medication, body weight, and UGT2B7 802C>T genotype—significantly influenced MMF clearance. Body weight was also a significant covariate influencing MMF central volume of distribution. The

influence of the UGT maturation process on MPA metabolism across a wide range of age and body weight is key for further research evaluating their impact on the clinical response to immunosuppressant therapy with MMF.

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REFERENCES

1. Sollinger HW. Mycophenolates in transplantation. *Clin Transplant*. 2004;18:485-492.

2. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology*. 2000;47:85-118.

3. Shaw LM, Korecka M, Venkataramanan R, Goldberg L, Bloom R, Brayman KL. Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. *Am J Transplant*. 2003;3:534-542.

4. Knight SR, Morris PJ. Does the evidence support the use of mycophenolate mofetil therapeutic drug monitoring in clinical practice? A systematic review. *Transplantation*. 2008;85:1675-1685.

5. Payen S, Zhang D, Maisin A, et al. Population pharmacokinetics of mycophenolic acid in kidney transplant pediatric and adolescent patients. *Ther Drug Monit*. 2005;27:378-388.

6. Lévesque E, Delage R, Benoit-Biancamano MO, et al. The impact of UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in healthy volunteers. *Clin Pharmacol Ther*. 2007;81:392-400.

7. Miles KK, Kessler FK, Smith PC, Ritter JK. Characterization of rat intestinal microsomal UDP-glucuronosyltransferase activity toward mycophenolic acid. *Drug Metab Dispos*. 2006;34:1632-1639.

8. Shipkova M, Armstrong VW, Wieland E, et al. Identification of glucoside and carboxyl-linked glucuronide conjugates of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. *Br J Pharmacol*. 1999;126:1075-1082.

9. Djebli N, Picard N, Rérolle JP, Le Meur Y, Marquet P. Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comedications on Acyl-MPAG production in vitro and in adult renal transplant patients. *Pharmacogenet Genomics*. 2007;17:321-330.

10. Miura M, Kagaya H, Satoh S, et al. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients [published online ahead of print August 7, 2008]. *Ther Drug Monit*.

11. Miners JO, McKinnon RA, Mackenzie PI. Genetic polymorphisms of UDP-glucuronosyltransferases and their functional significance. *Toxicology*. 2002;181-182:453-456.

12. Argikar UA, Iwuchukwu OF, Nagar S. Update on tools for evaluation of uridine diphosphoglucuronosyltransferase polymorphisms. *Expert Opin Drug Metab Toxicol*. 2008;4:879-894.

13. Bernard O, Guillemette C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. *Drug Metab Dispos*. 2004;32:775-778.

14. Girard H, Court MH, Bernard O, et al. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. *Pharmacogenetics*. 2004;14:501-515.

15. Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. *Clin Pharmacol Ther*. 2005;78:351-361.

16. Bernard O, Tojcic J, Journault K, Perusse L, Guillemette C. Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. *Drug Metab Dispos*. 2006;34:1539-1545.

17. Van Agteren M, Armstrong VW, Van Schaik RH, et al. AcylMPAG plasma concentrations and mycophenolic acid-related side effects in patients undergoing renal transplantation are not related to the UGT2B7-840G>A gene polymorphism. *Ther Drug Monit*. 2008;30:439-444.

18. Kobayashi M, Saitoh H, Kobayashi M, Tadano K, Takahashi Y, Hirano T. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. *J Pharmacol Exp Ther*. 2004;309:1029-1035.

19. Hesselink DA, van Hest RM, Mathot RA, et al. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. *Am J Transplant*. 2005;5:987-994.

20. Naesens M, Kuypers DR, Verbeke K, Vanrenterghem Y. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. *Transplantation*. 2006;82:1074-1084.

21. Zaya MJ, Hines RN, Stevens JC. Epirubicin glucuronidation and UGT2B7 developmental expression. *Drug Metab Dispos*. 2006;34:2097-2101.

22. Strassburg CP, Strassburg A, Kneip S, et al. Developmental aspects of human hepatic drug glucuronidation in young children and adults. *Gut*. 2002;50:259-265.

23. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology—drug disposition, action, and therapy in infants and children. *N Engl J Med*. 2003;349:1157-1167.

24. Le Meur Y, Shaw LM, et al. Therapeutic drug monitoring of mycophenolate mofetil in transplantation. *Ther Drug Monit*. 2006;28:145-154.

25. Fernández Del Mar, De Gatta M, Santos-Buelga D, Domínguez-Gil A, García MJ. Immunosuppressive therapy for paediatric transplant patients: pharmacokinetic considerations. *Clin Pharmacokinet*. 2002;41:115-135.

26. Saint-Marcoux F, Marquet P, Jacqz-Aigrain E, et al. Patient characteristics influencing ciclosporin pharmacokinetics and accurate Bayesian estimation of ciclosporin exposure in heart,

lung and kidney transplant patients. *Clin Pharmacokinet*. 2006;45:905-922.

27. Boeckmann AJ, Sheiner LB, Beal SL. *NONMEM User's Guide: Introductory Guide*. San Francisco, Calif: University of California; 1992.

28. Irtan S, Saint-Marcoux F, Rousseau A, et al. Population pharmacokinetics and bayesian estimator of cyclosporine in pediatric renal transplant patients. *Ther Drug Monit*. 2007;29:96-102.

29. Hesselink DA, van Gelder T, van Schaik RH, et al. Population pharmacokinetics of cyclosporine in kidney and heart transplant recipients and the influence of ethnicity and genetic polymorphisms in the MDR-1, CYP3A4, and CYP3A5 genes. *Clin Pharmacol Ther*. 2004;76:545-556.

30. Maitre PO, Bührer M, Thomson D, Stanski DR. A three-step approach combining Bayesian regression and NONMEM population analysis: application to midazolam. *J Pharmacokinet Biopharm*. 1991;19:377-384.

31. Post TM, Freijer JI, Ploeger BA, Danhof M. Extensions to the visual predictive check to facilitate model performance evaluation. *J Pharmacokinet Pharmacodyn*. 2008;35:185-202.

32. Djebli N, Rousseau A, Hoizey G, et al. Sirolimus population pharmacokinetic/pharmacogenetic analysis and bayesian modelling in kidney transplant recipients. *Clin Pharmacokinet*. 2006;45:1135-1148.

33. Irtan S, Azougagh S, Monchaud C, Popon M, Baudouin V, Jacqz-Aigrain E. Comparison of high-performance liquid chromatography and enzyme-multiplied immunoassay technique to monitor mycophenolic acid in paediatric renal recipients [published online ahead of print July 2, 2008]. *Pediatr Nephrol*. 2008;23:1859-1865.

34. Hu TM, Hayton WL. Allometric scaling of xenobiotic clearance: uncertainty versus universality. *AAPS pharmSci*. 2001;3:E29.

35. Gregoor PJ, de Sévaux RG, Hené RJ, et al. Effect of cyclosporine on mycophenolic acid trough levels in kidney transplant recipients. *Transplantation*. 1999;68:1603-1606.

36. Smak Gregoor PJ, van Gelder T, Hesse CJ, van der Mast BJ, van Besouw NM, Weimar W. Mycophenolic acid plasma concentrations in kidney allograft recipients with or without cyclosporin: a cross-sectional study. *Nephrol Dial Transplant*. 1999;14:706-708.

37. Van Gelder T, Klupp J, Barten MJ, Christians U, Morris RE. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. *Ther Drug Monit*. 2001;23:119-128.

38. Van Hest RM, van Gelder T, Vulto AG, Mathot RA. Population pharmacokinetics of mycophenolic acid in renal transplant recipients. *Clin Pharmacokinet*. 2005;44:1083-1096.

39. Mino Y, Naito T, Otsuka A, et al. Cyclosporine concentration-dependent increase in concentration ratio of mycophenolic acid acyl and phenol glucuronides to mycophenolic acid in stable kidney transplant recipients. *Clin Biochem*. 2009;42:595-601.

40. Le Guellec C, Bourgoin H, Büchler M, et al. Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in stable renal transplant patients. *Clin Pharmacokinet*. 2004;43:253-266.

41. Prémaud A, Le Meur Y, Debord J, et al. Maximum a posteriori bayesian estimation of mycophenolic acid pharmacokinetics in renal transplant recipients at different postgrafting periods. *Ther Drug Monit*. 2005;27:354-361.

42. Weber LT, Hoecker B, Armstrong VW, Oellerich M, Tönshoff B. Validation of an abbreviated pharmacokinetic profile for the estimation of mycophenolic acid exposure in pediatric renal transplant recipients. *Ther Drug Monit*. 2006;28:623-631.

43. Picard N, Ratanasavanh D, Prémaud A, Le Meur Y, Marquet P. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. *Drug Metab Dispos*. 2005;33:139-146.

44. Jude AR, Little JM, Bull AW, Podgorski I, Radominska-Pandya A. 13-hydroxy- and 13-oxooctadecadienoic acids: novel substrates for human UDP-glucuronosyltransferases. *Drug Metab Dispos*. 2001;29:652-655.

45. Innocenti F, Iyer L, Ramírez J, Green MD, Ratain MJ. Epirubicin glucuronidation is catalyzed by human UDP-glucuronosyltransferase 2B7. *Drug Metab Dispos*. 2001;29:686-692.

46. Coffman BL, King CD, Rios GR, Tephly TR. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). *Drug Metab Dispos*. 1998;26:73-77.

47. Bhasker CR, McKinnon W, Stone A, et al. Genetic polymorphism of UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significance. *Pharmacogenetics*. 2000;10:679-685.

48. Sawyer MB, Innocenti F, Das S, et al. A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. *Clin Pharmacol Ther*. 2003;73:566-574.

49. Kagaya H, Inoue K, Miura M, et al. Influence of UGT1A8 and UGT2B7 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol*. 2007;63:279-288.

50. Van Schaik R, van Agteren M, de Fijter J, et al. UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients [published online ahead of print June 3, 2009]. *Clin Pharmacol Ther*. 2009;86:319-327.

51. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Glucuronidation in humans. Pharmacogenetic and developmental aspects. *Clin Pharmacokinet*. 1999;36:439-452.

52. Prausa SE, Fukuda T, Maseck D, et al. UGT genotype may contribute to adverse events following medication with mycophenolate mofetil in pediatric kidney transplant recipients. *Clin Pharmacol Ther*. 2009;85:495-500.

53. Shipkova M, Armstrong VW, Weber L, et al. Pharmacokinetics and protein adduct formation of the pharmacologically active acyl glucuronide metabolite of mycophenolic acid in pediatric renal transplant recipients. *Ther Drug Monit*. 2002;24:390-399.

54. Hale MD, Nicholls AJ, Bullingham RE, et al. The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. *Clin Pharmacol Ther*. 1998;64:672-683.

55. Van Hest RM, Van Gelder T, Bouw R, et al. Time-dependent clearance of mycophenolic acid in renal transplant recipients. *Br J Clin Pharmacol*. 2007;63:741-752.

56. Nowak I, Shaw LM. Mycophenolic acid binding to human serum albumin: characterization and relation to pharmacodynamics. *Clin Chem*. 1995;41:1011-1017.

57. Cattaneo D, Perico N, Gaspari F, et al. Glucocorticoids interfere with mycophenolate mofetil bioavailability in kidney transplantation. *Kidney Int*. 2002;62:1060-1067.