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Multidrug resistance-associated protein 2 (MRP2/*ABCC2*) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients

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Abstract

BACKGROUND AND OBJECTIVE—Tacrolimus is an immunosuppressive drug used for the prevention of the allograft rejection in the kidney allograft recipients. It exhibits a narrow therapeutic index and a large pharmacokinetic variability. Tacrolimus is mainly metabolized by cytochrome P450 (CYP) 3A4 and 3A5, and effluxed via ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp), encoded by *ABCB1* gene. The influence of *CYP3A5**3 on the pharmacokinetics of tacrolimus has been well characterized. On the other hand, the contribution of polymorphisms in other genes is controversial. In addition, the involvement of other efflux transporter than P-gp in tacrolimus disposition is uncertain. The present study was designed to investigate the effects of genetic polymorphisms of CYP3As and efflux transporters on the pharmacokinetics of tacrolimus.

SUBJECTS AND METHODS—A total of 500 blood concentrations of tacrolimus from 102 adult stable kidney transplant recipients were included in the analyses. Genetic polymorphisms in *CYP3A4* and *CYP3A5* genes as well as the genes of efflux transporters including P-gp (*ABCB1*), multidrug resistance-associated protein (MRP2/*ABCC2*) and breast cancer resistance protein (BCRP/*ABCG2*) were genotyped. For *ABCC2* gene, haplotypes were determined as follows: H1 (wild type), H2 (1249G>A), H9 (3972C>T) and H12 (−24C>T and 3972C>T). Population pharmacokinetic analysis was performed using nonlinear mixed effects modeling.

RESULTS—Analyses revealed that CYP3A5 expressers (*CYP3A5**1 carriers) and MRP2 high activity group (*ABCC2* H2/H2 and H1/H2) decreased the dose-normalized trough concentration of tacrolimus by 2.3-fold ($p<0.001$) and 1.5-fold ($p=0.007$), respectively. The pharmacokinetics of tacrolimus was best described using a two-compartment model with first order absorption and an absorption lag time. In the population pharmacokinetic analysis, CYP3A5 expressers and MRP2 high activity groups were identified as the significant covariates for tacrolimus apparent clearance expressed as $20.7 \times (\text{Age}/50)^{-0.78} \times 2.03$ (CYP3A5 expressers) × 1.40 (MRP2 high activity group). No other *CYP3A4*, *ABCB1* and *ABCG2* polymorphisms were associated with the apparent clearance of tacrolimus.

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CONCLUSIONS—This is the first report that MRP2/*ABCC2* has crucial impacts on the pharmacokinetics of tacrolimus in a haplotype specific manner. Determination of *ABCC2* as well as *CYP3A5* genotype may be useful for more accurate tacrolimus dosage adjustment.

INTRODUCTION

The calcineurin inhibitor tacrolimus is an immunosuppressive agent used in combination with mycophenolic acid or corticosteroids for the prevention of the allograft rejection in solid organ transplant recipients.[1] Tacrolimus exhibits a narrow therapeutic index and considerable interindividual pharmacokinetic variability.[2] Therefore, routine therapeutic drug monitoring is an integral part of tacrolimus immunosuppressive therapy.[3]

Tacrolimus is extensively metabolized by cytochrome P450 (CYP) 3A4 and 3A5 in the liver and small intestine.[4, 5] In addition, it is a substrate of P-glycoprotein (P-gp), encoded by *multidrug resistance (MDR) 1/ABCB1* gene.[6] Pharmacogenetic studies indicate that the interindividual variability in tacrolimus pharmacokinetics can be partly related to genetic polymorphisms in *CYP3A* and *ABCB1* genes.[7] Among them, the most frequently investigated polymorphism is *CYP3A5**3 (6986A>G, rs776746), which causes an alternative splicing and is associated with low levels of CYP3A5 functional protein.[8, 9] CYP3A5 expressers, who carried at least one *CYP3A5**1 (wild type) allele, showed a lower dose-adjusted tacrolimus concentration and a higher dose requirement compared to CYP3A5 non-expressers (*CYP3A5**3 homozygotes).[7] Several population pharmacokinetic analyses have demonstrated that *CYP3A5**3 had a significant impact on the apparent clearance of tacrolimus.[10, 11]

Accumulating evidences indicate that in the small intestine, CYPs and efflux transporters, mainly CYP3A and P-gp, cooperatively function as the absorption barrier.[12] Using the pig intestinal mucosa in an Ussing chamber, Lampen et al.[13] have demonstrated that when tacrolimus was added to the luminal side of the intestinal preparation, more than 90% of the metabolites were transported from the tissue back into the luminal chamber, most likely by active transport. The concentrations of tacrolimus were more than 100-fold higher than the concentrations of the formed metabolites and these findings suggested that the metabolites of tacrolimus either have a higher affinity for the P-gp than the parent or are substrates of a different transporter.[12] In the small intestine, multidrug resistance-associated protein 2 (MRP2: encoded by *ABCC2* gene) and breast cancer resistance protein (BCRP: encoded by *ABCG2* gene) as well as P-gp play an important role in the efflux of xenobiotics.[14] Compared to *ABCB1* polymorphisms, the information on the association between the pharmacokinetics of tacrolimus and polymorphisms in *ABCC2* and *ABCG2* genes are limited. Therefore, in this study, the effects of polymorphisms in *CYP3A4*, *CYP3A5*, *ABCB1*, *ABCC2* and *ABCG2* genes on the dose-normalized concentration of tacrolimus have been investigated in adult stable kidney transplant recipients. Furthermore, using a population pharmacokinetic approach, we have examined whether these polymorphisms can account for the interindividual variability in the population pharmacokinetic parameters of tacrolimus.

METHODS

Patients

The study protocol was reviewed and approved by the Institutional Review Board at Rhode Island Hospital (IRB#0159-03, 0054-05, 0066-06, 4060-10 and 4176-10), and all patients gave informed consent to participate. Patients were excluded if they were suffering from severe liver dysfunction, were pregnant, nursing or younger than 18 years of age. In addition, patients with pancreatic transplantation were excluded from the study. In total, 102

adult stable kidney transplant recipients were included in this study. Detail demographic information is presented in Table 1. All study participants received triple immunosuppressive drug regimens including tacrolimus oral tablets (Prograf, Astellas Pharma US Inc., Northbrook, IL, USA), prednisone and mycophenolic acid.

Pharmacokinetic study

On the day of pharmacokinetic study, subjects underwent routine physical examination including blood pressure, height and weight measurement. After collecting the pre-dose (trough) blood sample (4.0 mL) into ethylenediaminetetraacetic acid (EDTA) vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA), immunosuppressive drugs were administered. Blood samples (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12-hours post-dose) were then collected up to 12 hours from 31 patients, whereas two blood samples (pre-dose and 2-hours post-dose) were collected from 71 patients. We have selected 0 and 2 hour post dose because trough concentration usually reflect the elimination phase whereas concentration at 2 hour post dose, although is not monitored, but it is around the absorption phase of the drug. The whole blood samples were immediately stored at −80°C until further analysis.

Bioanalytical assay

Quantitative analysis of tacrolimus was performed using a previous published and utilized assay using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled to an API 4000 tandem mass spectroscopy system (AB Sciex, Foster City, CA, USA) equipped with a turbo electrospray ion source.[15] In brief, sample preparation involved the addition of 800 μL of ZnSO4 (17.28 g/L): methanol (30:70, v/v) containing the internal standard (ascomycin, 100 ng/mL) to a 200 μL aliquot of EDTA anticoagulated whole blood, calibration standards or quality control samples. Samples were vortex mixed, centrifuged (13,000 rpm for 10 minutes at 4°C), and 50 μL of supernatant was injected onto an HPLC column (4.6 × 150 mm, 3.5 μm, Eclipse Zorbax XDB -C8, Agilent Technologies) maintained at 65°C. The mobile phase that consisted of (A) HPLC grade methanol with 0.1% v/v formic acid and (B) 0.1% v/v formic acid was pumped at a flow rate of 1.0 mL/min. The mass spectrometer was run in the single ion mode and focused on the $[M+Na]^+$ of tacrolimus and ascomycin (internal standard). The lower limit of quantification for tacrolimus in human EDTA whole blood was at 62.5 pg/mL and the range of reliable response was from 62.5 pg/mL to 25.0 ng/mL. Inter-day accuracies for tacrolimus was within 85–115% and total imprecision was <15%.

Genotyping of CYP3A4, CYP3A5, ABCB1, ABCC2 and ABCG2 genes

Genomic DNA from patients' peripheral blood sample was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions and was stored at −80°C until analysis. *CYP3A4**22 (rs35599367), *CYP3A5**3, *ABCB1* 1236C>T (rs1128503), 2677G>T,A (rs2032582) and 3435C>T (rs1045642), *ABCC2* −24C>T (rs717620), 1249G>A (rs2273697) and 3972C>T (rs3740066), and *ABCG2* 421C>A (rs2231142) were determined by TaqMan® allelic discrimination assay (Life Technologies, Foster, CA, USA) using an Applied Biosystems 7500 Real-Time PCR system (Life Technologies) according to manufacturer's instructions. *CYP3A4**1B (rs2740574) was genotyped by polymerase chain reaction amplification and the subsequent direct sequencing using Applied Biosystems 3130xl Genetic Analyzer (Life Technologies). The primers for the amplification of *CYP3A4* gene were described previously.[16] *ABCC2* haplotypes were determined using Haploview version 4.1.[17]

Data analysis

Statistical analysis was carried out using the SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA) and GraphPad Prism (version 4.0, GraphPad Software, Inc., La Jolla, CA, USA). Normal distribution of log transformed tacrolimus concentration was verified using Shapiro-Wilk test and significance level was tested using independent samples t test or analysis of variance (ANOVA) followed by Bonferroni correction. Comparisons of tacrolimus dose were carried out using Mann-Whitney U test or Kruskal-Wallis test followed by Dunn's test.

Population pharmacokinetic modeling was performed using nonlinear mixed effects modeling with NONMEM (version 7.2.0, ICON Development Solutions, Ellicott City, MD, USA). PLT Tools (P Less Than, San Francisco, CA, USA) was used as an interface for NONMEM. Double-precision, first-order conditional estimation (FOCE) with interaction and subroutines ADVAN4 TRANS4 were used. The model selection was based on goodness-of-fit criteria, including diagnostic plots, minimum objective function value (MOFV) after accounting for the number of fitted parameters, precision and the physiological plausibility of the estimates. The inter-subject variability in the pharmacokinetic parameters was modeled assuming an exponential distribution. Additive, proportional and combined proportional and additive models were investigated but a proportional error model has best described the variability in the data.

Covariates (sex, ethnicity, diabetes, age, weight, time post-transplantation, hematocrit, hemoglobin A1c, glucose, alanine aminotransferase, alkaline phosphatase, albumin, total bilirubin, blood urea nitrogen, creatinine, creatinine clearance, concomitant prednisone dose, concomitant mycophenolic acid dose and genetic polymorphisms) were examined graphically via plots of η vs. covariate values, where η is the individual random effect with a mean of 0 and a variance of ω^2. The candidate covariates identified during graphical screening were then tested in NONMEM. The influence of continuous covariates on the pharmacokinetic parameters was modeled according to a power model scaled to the population median covariate value. The influence of categorical covariates on the pharmacokinetic parameters was modeled using a proportional relationship.

The statistical significance of the covariates was assessed using a likelihood ratio test corresponding to a decrease in MOFV of <3.84 (with one degree of freedom: $p<0.05$) in comparisons between two hierarchical models. These covariates were then included in the basic population model using forward stepwise method until objective function no longer improved. A covariate was retained in the model if the objective function changed by 6.63 or greater ($p<0.01$) when it was removed from the full model. This was repeated and continued until all remaining covariates were significant in the final model.

The predictability of the final model was evaluated using visual predictive check. Data for 1000 datasets were simulated and compared with the observed data set. Within the 2.5th and 97.5th percentiles of the simulated concentration, a 95% prediction interval was constructed and plotted along with the observed data and the median of the simulated concentration. A bootstrap procedure was performed to determine the stability and robustness of the final model. Thousand bootstrap re-samples of the original dataset were created, and these were evaluated using the final model.

RESULTS

Patients' characteristics

Demographic characteristics, doses of immunosuppressive agents and biochemical indices of study participants are presented in Table 1. None of the study subjects were administered drugs that are known to inhibit or induce tacrolimus disposition.

Effects of genetic polymorphisms on C_0 and C_2 of tacrolimus

Allele frequencies of genetic polymorphisms in *CYP3A4*, *CYP3A5*, *ABCB1*, *ABCC2* and *ABCG2* genes are shown in Table 2. We investigated the effects of these polymorphisms on dose-normalized tacrolimus concentrations at pre-dose (C_0/dose) and 2-hour post dose (C_2/dose) (Table 3). CYP3A5 expressers (*CYP3A5**1 carriers) showed 2.3-fold lower C_0/dose and C_2/dose of tacrolimus compared to CYP3A5 non-expressers, who were *CYP3A5**3 homozygotes ($p<0.001$). *CYP3A4**1B, which is linked with *CYP3A5**1, significantly decreased C_0/dose ($p=0.004$) and C_2/dose ($p=0.003$) of tacrolimus, while *CYP3A4**22 did not have significant effects on them. No *ABCB1* and *ABCG2* polymorphisms affected C_0/dose and C_2/dose of tacrolimus. *ABCB1* haplotypes did not appear to be a significant factor on tacrolimus dose normalized concentrations (data not shown). As for *ABCC2* polymorphisms, 1249G>A significantly decreased C_0/dose by 1.41-fold ($p=0.011$) and C_2/dose by 1.59-fold ($p=0.003$), while 3972C>T significantly increased C_0/dose by 1.33-fold ($p=0.023$) and C_2/dose by 1.47-fold ($p=0.012$). *ABCC2* −24C>T did not influence them. For further investigation of the effects of *ABCC2* polymorphisms, haplotype analysis was carried out according to Laechelt et al.[18] As shown in Table 4, four haplotypes were determined: H1 (wild type, 54.4%), H2 (1249G>A, 20.6%), H9 (3972C>T, 12.3%) and H12 (−24C>T and 3972C>T, 12.7%). *ABCC2* haplotype H2 is reported to have a higher protein expression and transport activity of MRP2, while haplotypes H9 and H12 are reported to have a lower protein expression and transport activity of MRP2 compared to wild type (haplotype H1).[18] Therefore, in the following study, the patients were divided into 3 groups according to *ABCC2* haplotypes as follows: MRP2 high activity group (H2/H2 and H1/H2), MRP2 low activity group (H9/H9, H12/H12, H1/H9 and H1/H12) and MRP2 reference group (H1/H1, H2/H9 and H2/H12). Tacrolimus C_0/dose and C_2/dose in MRP2 high activity group were 1.54-fold and 1.80-fold lower than those in MRP2 low activity group and reference group ($p=0.007$ and $p<0.001$, respectively, Table 3). There were no significant differences in the concentrations between MRP2 low activity group and reference group. These results suggest that 1249G>A but not 3972C>T has a significant effect on the dose-normalized tacrolimus concentration. In *CYP3A4**1B carriers, CYP3A5 expressers or MRP2 high activity group, significant higher tacrolimus doses were required compared to the other group, respectively (Table 3).

Combined effects of CYP3A5*1 and ABCC2 haplotype on C_0 and C_2 of tacrolimus

To investigate the combined effects of *CYP3A5**1 and *ABCC2* haplotype on C_0/dose and C_2/dose of tacrolimus, the patients were divided into 4 groups with respect to CYP3A5 expressers or non-expressers and MRP2 high activity group or low activity and reference group. CYP3A5 expressers with MRP2 high activity group showed a significant lower C_0/dose and C_2/dose of tacrolimus compared to other three groups (CYP3A5 expressers with MRP2 low activity + reference group, CYP3A5 non-expressers with MRP2 high activity group, CYP3A5 non-expressers with MRP2 low activity + reference group) (Fig. 1A–B). Tacrolimus dose in CYP3A5 expressers with MRP2 high activity group was significantly higher than that in CYP3A5 non-expressers with MRP2 high activity group or MRP2 low activity + reference group (Fig. 1C).

Population pharmacokinetics of tacrolimus

To understand the relative impacts of these polymorphisms on the pharmacokinetics of tacrolimus, population pharmacokinetic analysis was carried out. The pharmacokinetics of tacrolimus was best described using a 2-compartment model with first order absorption and an absorption lag time. Random effect parameters were estimated for the interindividual variability in apparent central volume of distribution (V_1/F) and apparent clearance (CL/F). Residual variability was best described by a proportional model. The population pharmacokinetic parameters of the structural model were as follows: absorption rate constant (k_a), 0.486 h^{-1}; V_1/F, 214 L; CL/F, 27.1 L/h; apparent peripheral volume of distribution (V_2/F), 1863 L; apparent inter-compartmental clearance (Q/F), 68.7 L/h; and absorption lag time, 0.339 h.

During the preliminary covariate screening (univariate analysis), CYP3A5 expressers, *CYP3A4**1B carriers, MRP2 high activity group, Caucasian and age were found to be a significant covariate for CL/F (Table 5). We have previously reported slightly longer tacrolimus T_{max} (time to reach the maximum blood concentration following drug administration) in the diabetic patients,[19] which is most likely due to the delayed gastric emptying.[20] Incorporation of diabetes into the model as a covariate for absorption lag time significantly reduced the objective function by 12.96. Therefore, diabetes was included in the following analysis. All the significant covariates were then included in the base model in a forward stepwise manner until there was no further reduction in the objective function. The full model included CYP3A5 expressers, MRP2 high activity group and age on CL/F and diabetes on the lag time. After backward elimination, they were still retained as covariates on CL/F and lag time. The covariates reduced interindividual variability from 66% to 44% for CL/F. CL/F was expressed as: $CL/F = 20.7 \times (Age/50)^{-0.78} \times 2.03^{CYP3A5} \times 1.40^{MRP2}$, where CYP3A5 = 0 for CYP3A5 non-expressers; CYP3A5 = 1 for CYP3A5 expressers; MRP2 = 0 for MRP2 low activity + reference group; MRP2 = 1 for MRP2 high activity group. The parameters of the final model, including bootstrap medians and 95% confidence intervals for the pharmacokinetic parameters, are presented in Table 6. Interindividual variability for V_1/F and proportional error in the final model were 157% and 18%, respectively. Figures 2 and 3 present goodness-of-fit plots for the final model, and the visual predictive check for tacrolimus dose-normalized concentration, respectively. The visual predictive check (Figure 3) supports the finding that *ABCC2* haplotype as well as *CYP3A5* polymorphism significantly affected the pharmacokinetics of tacrolimus.

DISCUSSION

In the present study, it is demonstrated that genetic polymorphisms of *CYP3A5* and *ABCC2* affect the pharmacokinetics of tacrolimus. Dose-normalized tacrolimus concentration was 2.3-fold lower in CYP3A5 expressers than CYP3A5 non-expressers. In addition, population pharmacokinetic model showed that CL/F was 2-fold higher in CYP3A5 expressers than CYP3A5 non-expressers. There are numerous reports on a strong relationship between *CYP3A5* polymorphism and tacrolimus pharmacokinetics in kidney, heart and liver transplant recipients,[7] which are consistent with the finding of the present study. Patients with MRP2 high activity, who is the homozygote of H2 (1249G>A) and the heterozygote of H2 and H1 (wild type), showed a significant lower dose-normalized concentration of tacrolimus compared to MRP2 low activity group and reference group. Furthermore, MRP2 high activity group was an independent covariate for CL/F of tacrolimus. Although 3972C>T had a significant effect on dose-normalized concentration of tacrolimus, haplotype analysis demonstrated that there is no difference in tacrolimus pharmacokinetics between the reference group and MRP2 low activity group, which included 3972C>T. This is probably due to the linkage disequilibrium in *ABCC2* gene: 81% (30 out of 37) of the patient population that has at least one 1249G>A did not have 3972C>T. These results suggest that

ABCC2 haplotypes, not each individual polymorphism, should be taken into account when characterizing the effects of *ABCC2* polymorphisms on tacrolimus pharmacokinetics.

According to the routine practice of our transplant centre, tacrolimus doses are optimized to achieve a blood concentration of 10–12 ng/mL for the first 6 months and 4–6 ng/mL thereafter. CYP3A5 expressers have been reported to show a higher dose requirement compared to CYP3A5 non-expressers,[7] which is in accordance with our findings. Among CYP3A5 expressers, tacrolimus dose in MRP2 high activity group had the tendency to increase compared with MRP2 low activity and reference group although there was no significant difference (median [25th–75th percentiles]: 11.0 mg [8.5–12.0] and 6.0 mg [5.0–9.5], respectively). This finding suggests that characterization of *ABCC2* haplotype as well as *CYP3A5* genotype may be informative for adjusting tacrolimus dose in the early post-operative period. To evaluate the usefulness of *ABCC2* haplotypes in dose adjustment of tacrolimus, further studies using much larger sample size are needed.

This is the first report on the significant influence of *ABCC2* polymorphism on the pharmacokinetics of tacrolimus. The association between *ABCC2* polymorphism and tacrolimus concentrations was reported in only two studies in transplant recipients in Europe.[11, 21] A population pharmacokinetic approach of tacrolimus in pediatric transplant recipients did not identify *ABCC2* polymorphism as a significant covariate.[11] Renders et al.,[21] in adult transplant recipients, found a combined effect of *ABCC2* −24C>T and 1249G>A on tacrolimus trough concentration although there were no influences of individual *ABCC2* polymorphisms. The discrepancies between the present study and the previous studies can be because of the difference in the environmental factor or the ratio of ethnic groups and diabetes among the transplant recipients. Further studies are needed to confirm the effects of *ABCC2* polymorphisms on the tacrolimus disposition.

The mechanism underlying the effect of *ABCC2* haplotype on tacrolimus pharmacokinetics is not known. It was recently shown that erythromycin is a substrate for human MRP2.[22, 23] Erythromycin is another macrolide lactone with comparable physicochemical properties to tacrolimus (molecular weight, LogP, topological polar surface area). The metabolism of erythromycin via CYP3A4 was increased in Mrp2 knockout mice and human carrying *ABCC2* −24C>T, suggesting the interplay between the metabolism and MRP2.[23] It was suggested that the metabolites of tacrolimus are substrates of other transporters than P-gp.[12] We hypothesize that tacrolimus metabolites or tacrolimus are substrates for MRP2. Thus, MRP2 may be involved in the clearance of tacrolimus in cooperation with CYP3A in the small intestine. Further in vitro studies are warranted to elucidate the transport of tacrolimus and its metabolites via MRP2.

Although *CYP3A4**1B significantly reduced the dose-normalized tacrolimus concentration, *CYP3A4**1B was not the covariate for any parameters in population pharmacokinetic model. This could be because of a possible linkage between *CYP3A4**1B and *CYP3A5**1. The frequencies of most of SNPs except for *CYP3A4**1B and *CYP3A5**3 perfectly agree with those predicted by the Hardy-Weinberg equation. As for *CYP3A5**3, Hardy-Weinberg equation predicts *1/*1: n=3 (observed: n=8), *1/*3: n=28 (observed: n=18), *3/*3: n=71 (observed: n=76). This discrepancy is because there is the ethnic difference in the allele frequency of *CYP3A5**3 (Caucasian: 91.1%; Hispanic: 75.0%; African-American: 45.5%) and because the ratios of ethnic group were different in our population (73 Caucasian, 14 Hispanic, 11 African-American, 4 others). In this study, we have observed that 81% of the patient population that express at least one *CYP3A4**1B allele are also expressing a functional CYP3A5. Our finding that *CYP3A4**1B does not have an independent effect on tacrolimus pharmacokinetics is consistent with the previous result.[24] Elens et al.[25] have reported that *CYP3A4**22 was associated with reduced tacrolimus clearance. In this study,

dose-normalized concentration of tacrolimus in *CYP3A4**1/*22 had the tendency to increase compared with *CYP3A4**1/*1, but this difference was not significant. This might be because allele frequency of *CYP3A4**22 is low and our sample size (n=102) was not enough to evaluate the effect of *CYP3A4**22 on the pharmacokinetics of tacrolimus. There were no linkage between *CYP3A4**22 and *CYP3A5**1 or *CYP3A4**1B. Further studies are needed to elucidate the contribution of *CYP3A4**22 on the disposition of tacrolimus. *ABCB1* 1236C>T, 2677G>T, A and 3435C>T and *ABCG2* 421C>A had no effects on dose-normalized concentration and any population pharmacokinetic parameters of tacrolimus in this study. Influences of *ABCB1* polymorphisms on the pharmacokinetics of tacrolimus are still controversial, but most studies have failed to find an association between them.[7]

The values of the population pharmacokinetic parameters of tacrolimus in this study are comparable to the values reported previously.[26–29] Age was also identified as a covariate for CL/F and the model demonstrated that the apparent clearance of tacrolimus decreases with age. Older age is reported to be associated with higher tacrolimus dose-normalized concentration or lower tacrolimus dose requirement,[30–32] which is in accordance with our finding. Diabetes significantly reduced the objective function by 12.96 as a covariate for an absorption lag time. Patients with long-term diabetes exhibit delayed gastric emptying due to diabetes-induced autonomic neuropathy that may affect the rate of drug absorption.[20] This result support the longer T_{max} of tacrolimus in diabetic patients.[19]

Similar to cyclosporine, tacrolimus is highly bound to red blood cells, and the reduced binding to blood cells or plasma proteins increases the values of unbound fraction and apparent clearance.[33, 34] Tacrolimus apparent clearance was higher in patients with low hematocrit levels (< 33 or 35%) as compared to those with normal hematocrit levels.[11, 29, 34, 35] However, in the current study, hematocrit levels did not have any significant influence on tacrolimus CL/F. This discrepancy might be because there were a few patients with low hematocrit levels in our study (only 12 patients have lower hematocrit levels than 35%). Xue et al.[36] reported no effects of hematocrit on tacrolimus clearance in healthy volunteers with normal hematocrit levels, which is consistent with our results.

CONCLUSIONS

ABCC2 haplotype as well as *CYP3A5* polymorphism have significant impacts on the pharmacokinetics of tacrolimus. These findings suggest that MRP2 is involved in the efflux of tacrolimus or its metabolites into the lumen in cooperation with CYP3A in the small intestine. Determination of *ABCC2* as well as *CYP3A5* genotype may be useful for more accurate tacrolimus dosage adjustment.

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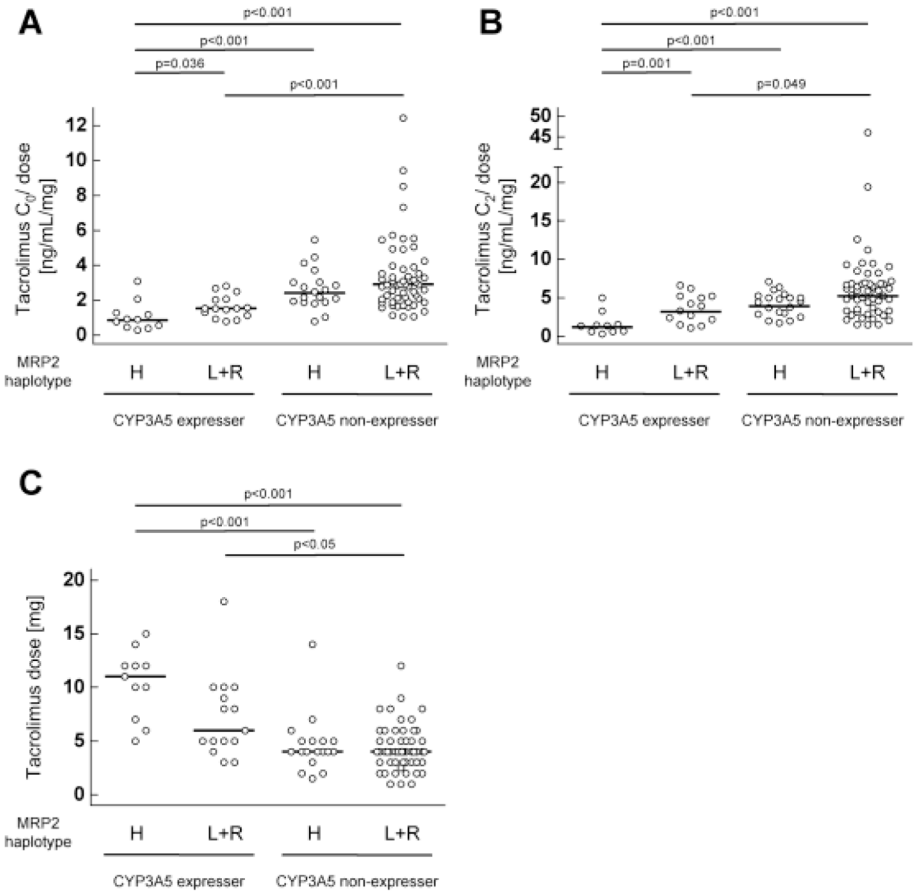

Fig. 1. Combined effects of *CYP3A5* polymorphism and *ABCC2* haplotypes on dose-normalized tacrolimus concentration at pre-dose and 2-hour post-dose

Dose-normalized trough concentrations **(A)**, 2-hour post-dose concentrations **(B)** and dose **(C)** of tacrolimus in stable kidney transplant recipients separated according to *CYP3A5* polymorphism (CYP3A5 expressers or non-expressers) and *ABCC2* haplotypes (MRP2 high activity group (H) or low activity group (L) + reference group (R)). The bars show the geometric means of dose-normalized tacrolimus concentration **(A and B)** and the median of tacrolimus dose **(C)** in each group. CYP, cytochrome P450; MRP2, multidrug resistance-associated protein 2.

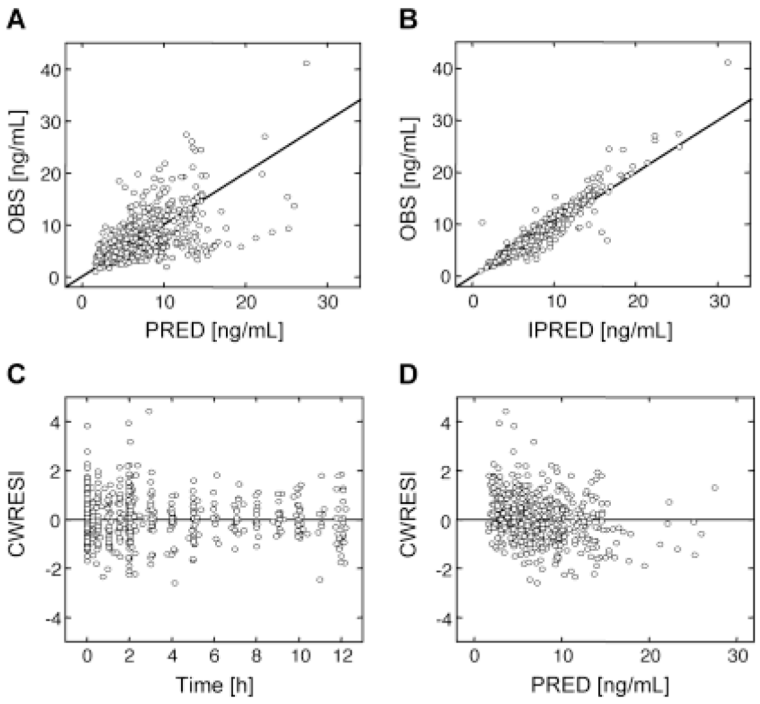

Fig. 2. Goodness of fit plots of the final model

(A) Observed tacrolimus concentration (OBS) vs. population-predicted tacrolimus concentration (PRED), **(B)** OBS vs. individual-predicted tacrolimus concentration (IPRED), **(C)** conditional weighted residual with interaction (CWRESI) vs. time and **(D)** CWRESI vs. PRED.

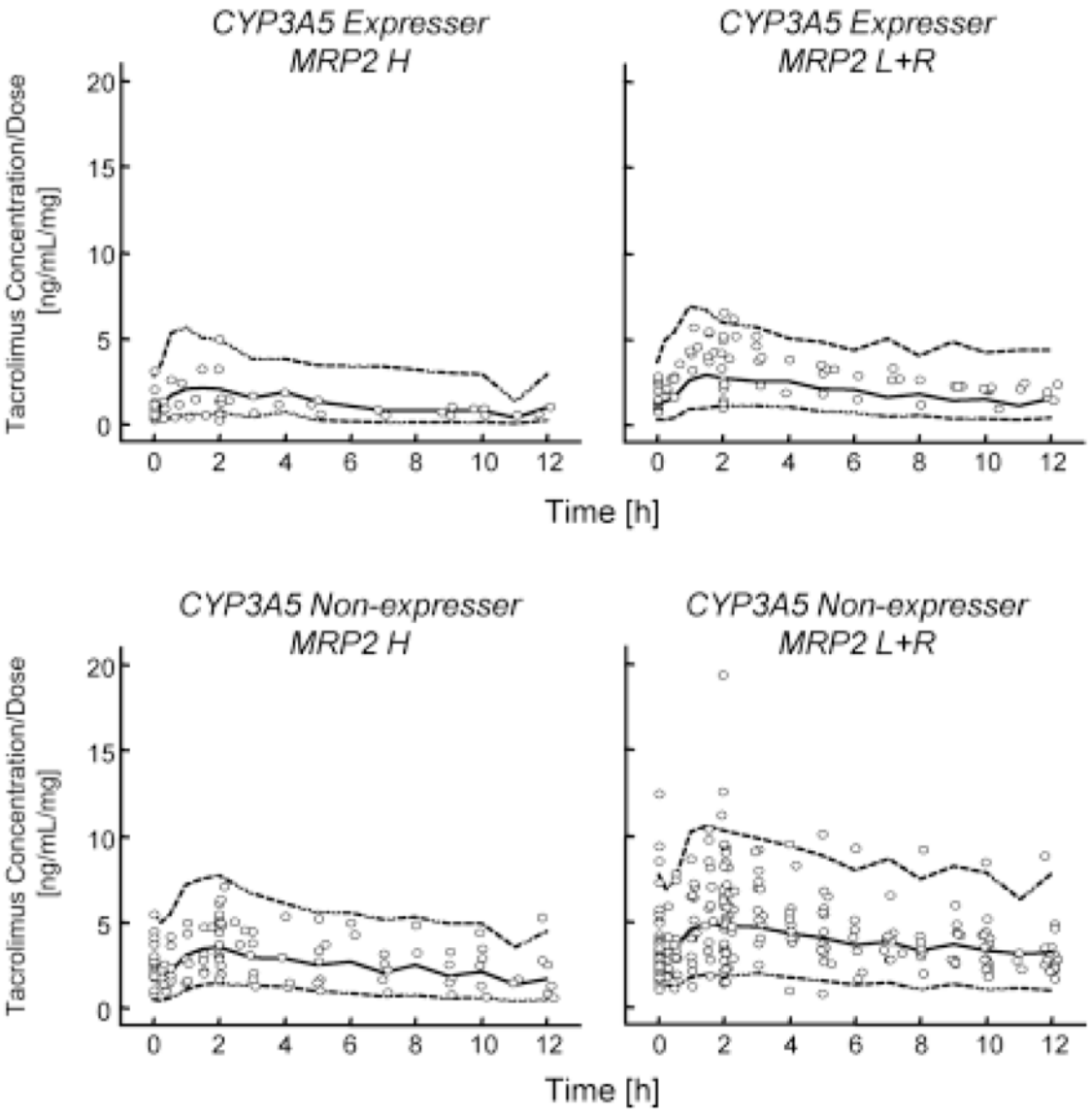

Fig. 3. The visual predictive check for dose-normalized tacrolimus concentrations

Comparison of observed dose-normalized tacrolimus concentrations with the 97.5th (upper dashed line), 50th (middle solid line) and 2.5th (lower dashed line) percentiles of the 1000 simulated datasets.

Table 1

Demographic characteristics and clinical data of 102 adult kidney transplant recipients.

Characteristic	Value
Sex (n)	
Male	74
Female	28
Ethnicity (n)	
Caucasian	73
Hispanic	14
African American	11
Asian	2
American Indian	2
Patients with Diabetes Mellitus (n)	
Non-diabetics	38
Diabetics	64
Age (y)	50 (18–74)
Body weight (kg)	85.2 (47.7–145.0)
Tacrolimus dose (mg/day)	4 (1–18)
Prednisone dose (mg/day)	5.0 (2.5–50.0)
Mycophenolic acid dose (mg/day)	739.0 (369.5–1477.9)
Time post transplantation (mo)	24 (2–123)
Hematocrit (%)	40.5 (26.1–54.0)
Hemoglobin A1c (%)	6.9 (4.0–11.8)
Glucose (mg/dL)	115 (36–601)
Aspartate aminotransferase (IU/L)	19 (9–333)
Alanine aminotransferase (IU/L)	18 (4–98)
Alkaline phosphatase (IU/L)	76 (23–291)
Albumin (g/dL)	4.3 (3.3–4.8)
Total bilirubin (mg/dL)	0.4 (0.1–1.3)
Blood urea nitrogen (mg/dL)	21 (9–53)
Creatinine (mg/dL)	1.3 (0.6–2.5)
Creatinine clearance (mL/min)[a]	78.3 (33.7–190.0)

Values are expressed as median (range) unless specified otherwise.

[a]Creatinine clearance was estimated using Cockcroft-Gault formula.

Table 2

Allele frequencies of genetic polymorphisms in *CYP3A4, CYP3A5, ABCB1, ABCC2* and *ABCG2* genes and *ABCC2* haplotypes in 102 adult kidney transplant recipients.

Gene	dbSNP	Position	Allele	Allele frequency	Genotype	Number
CYP3A4	rs2740574	*1B	*1	0.892	*1/*1	86
			*1B	0.108	*1/*1B	10
					*1B/*1B	6
	rs35599367	*22	*1	0.966	*1/*1	95
			*22	0.034	*1/*22	7
					*22/*22	0
CYP3A5	rs776746	*3	*1	0.167	*1/*1	8
			*3	0.833	*1/*3	18
					*3/*3	76
ABCB1	rs1128503	1236	C	0.554	C/C	36
			T	0.446	C/T	41
					T/T	25
	rs2032582	2677	G	0.534	G/G	29
			T	0.451	G/T	48
			A	0.015	G/A	3
					T/T	22
	rs1045642	3435	C	0.515	C/C	25
			T	0.485	C/T	55
					T/T	22
ABCC2	rs717620	−24	C	0.873	C/C	78
			T	0.127	C/T	22
					T/T	2
	rs2273697	1249	G	0.794	G/G	64
			A	0.206	G/A	34
					A/A	4
	rs3740066	3972	C	0.750	C/C	56
			T	0.250	C/T	41
					T/T	5

Gene	dbSNP	Position	Allele	Allele frequency	Genotype	Number
ABCG2	rs2231142	421	C	0.907	C/C	85
			A	0.093	C/A	15
					A/A	2

ABC adenosine triphosphate-binding cassette, *CYP* cytochrome P450, *dbSNP* the Single Nucleotide Polymorphism database

Table 3

Effects of genetic polymorphisms on tacrolimus dose and dose-normalized tacrolimus concentration at pre-dose and 2-hour post-dose.

Gene	SNP	Genotype	Dose (mg)				C_0/dose (ng/mL/mg dose)				C_2/dose (ng/mL/mg dose)			
			N	Median (25–75% Percentile)		*P*-value	N	Geometric Mean (95% CI)		*P*-value	N	Geometric Mean (95% CI)		*P*-value
CYP3A4	*1B	*1/*1	86	4.0	(3.0, 5.0)	<0.001††	85	2.50	(2.22, 2.82)	0.004††	81	4.49	(3.91, 5.15)	0.003††
		*1/*1B + *1B/*1B	16	10.0	(8.8, 12.0)		16	1.23	(0.80, 1.91)		15	1.83	(1.07, 3.12)	
	*22	*1/*1	95	5.0	(3.5, 7.0)	0.172	94	2.18	(1.91, 2.51)	0.213	89	3.82	(3.24, 4.49)	0.312
		*1/*22 + *22/*22	7	4.0	(3.5, 4.0)		7	3.01	(2.31, 3.91)		7	5.16	(3.41, 7.81)	
CYP3A5	*3	*1/*1 + *1/*3	26	8.5	(3.0, 10.8)	<0.001†††	26	1.20	(0.94, 1.54)	<0.001†††	24	2.09	(1.46, 2.99)	<0.001†††
		*3/*3	76	4.0	(3.0, 5.0)		75	2.77	(2.46, 3.12)		72	4.80	(4.18, 5.53)	
ABCB1	1236C>T	C/C	36	5.0	(3.8, 8.0)	0.440	36	2.11	(1.63, 2.74)	0.531	34	3.47	(2.51, 4.81)	0.320
		C/T + T/T	66	4.0	(3.3, 6.0)		65	2.30	(1.99, 2.66)		62	4.16	(3.53, 4.89)	
	2677G>T,A	G/G	29	5.0	(4.0, 10.0)	0.064	29	2.07	(1.49, 2.87)	0.537	27	3.22	(2.17, 4.77)	0.203
		G/T + G/A + T/T	73	4.0	(3.0, 6.0)		72	2.30	(2.02, 2.62)		69	4.21	(3.61, 4.90)	
	3435C>T	C/C	25	5.0	(4.0, 10.0)	0.386	25	2.23	(1.72, 2.90)	0.994	24	3.53	(2.62, 4.75)	0.457
		C/T + T/T	77	4.0	(3.0, 6.0)		76	2.23	(1.92, 2.60)		72	4.03	(3.36, 4.84)	
ABCC2	−24C>T	C/C	78	5.0	(3.3, 6.0)	0.873	77	2.20	(1.87, 2.58)	0.669	72	3.66	(3.05, 4.38)	0.147
		C/T + T/T	24	4.0	(3.8, 6.5)		24	2.35	(1.93, 2.85)		24	4.74	(3.53, 6.35)	
	1249G>A	G/G	64	4.0	(3.0, 6.0)	0.020†	64	2.53	(2.19, 2.93)	0.011†	60	4.64	(3.90, 5.52)	0.003††
		G/A + A/A	38	5.0	(4.0, 9.5)		37	1.80	(1.42, 2.29)		36	2.92	(2.22, 3.85)	
	3972C>T	C/C	56	5.0	(4.0, 7.3)	0.235	55	1.96	(1.60, 2.42)	0.023†	53	3.28	(2.61, 4.11)	0.012†
		C/T + T/T	46	4.0	(3.0, 5.8)		46	2.61	(2.28, 2.98)		43	4.83	(4.00, 5.84)	
	Haplotype	Low + Reference	71	4.0	(3.0, 6.0)	0.044†	71	2.54	(2.22, 2.90)	0.007††	66	4.69	(3.99, 5.51)	<0.001†††
		High	31	5.0	(4.0, 10.0)		30	1.65	(1.25, 2.19)		30	2.60	(1.92, 3.53)	
ABCG2	421C>A	C/C	85	5.0	(4.0, 6.0)	0.094	84	2.15	(1.89, 2.46)	0.218	81	3.73	(3.16, 4.41)	0.193
		C/A + A/A	17	4.0	(2.0, 6.0)		17	2.67	(1.73, 4.11)		15	4.93	(3.26, 7.45)	

One blood sample at pre-dose and 6 samples at 2-hour post-dose were not obtained among 102 patients. MRP2 high activity group includes H2/H2 and H1/H2; MRP2 low activity group includes H9/H9, H12/H12, H1/H9 and H1/H12); and MRP2 reference group includes H1/H1, H2/H9 and H2/H12. CYP, cytochrome P450.

ABC adenosine triphosphate-binding cassette, *CYP* cytochrome P450, *SNP* Single Nucleotide Polymorphism

† p<0.05,

$\dagger\dagger$ $p<0.01$,

$\dagger\dagger\dagger$ $p<0.001$, significant difference between groups.

Table 4

Frequencies of *ABCC2* haplotypes in 102 adult kidney transplant recipients.

Haplotype	−24	1249	3972	Frequency	Diplotype	Number
H1	C	G	C	0.544	H1/H1	25
H2	C	A	C	0.206	H1/H2	27
H9	C	G	T	0.123	H1/H9	17
H12	T	G	T	0.127	H1/H12	17
					H2/H2	4
					H2/H9	4
					H2/H12	3
					H9/H9	1
					H9/H12	2
					H12/H12	2

ABC adenosine triphosphate-binding cassette

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Table 5

Significant covariates for the univariate and multivariate analysis.

Significant covariates	ΔMOFV	*P*-value
Univariate analysis		
Effect of CYP3A5 expressers on CL/F	−27.64	< 0.001
Effect of age on CL/F	−16.42	< 0.001
Effect of *CYP3A4**1B carriers on CL/F	−12.95	< 0.001
Effect of MRP2 high activity group on CL/F	−11.45	< 0.001
Effect of Caucasian on CL/F	−7.24	< 0.01
Effect of diabetes mellitus on lag time	−12.96	< 0.001
Multivariate analysis		
Effect of CYP3A5 expressers on CL/F	+37.02	< 0.001
Effect of MRP2 high activity group on CL/F	+10.02	< 0.005
Effect of age on CL/F	+22.42	< 0.001
Effect of diabetes mellitus on lag time	+25.69	< 0.001

MOFV, minimum objective function value; CYP, cytochrome P450; CL/F, apparent oral clearance; MRP2, multidrug resistance-associated protein 2

Table 6

Population pharmacokinetic parameters of tacrolimus and bootstrap validation.

Model Parameter	Estimate (%RSE)	Bootstrap median (95% CI)
Fixed effects		
V_1/F (L)	234 (31.6)	228 (99.2, 420)
CL/F (L/h)	20.7 (6.77)	20.3 (15.0, 23.2)
V_2/F (L)	1319 (41.9)	1328 (574, 4141)
Q/F (L/h)	70.7 (15.7)	69.0 (40.2, 93.7)
k_a (h^{-1})	0.544 (25.8)	0.539 (0.241, 1.20)
Lag time (h)	0.183 (23.4)	0.184 (0.102, 0.308)
Effect of CYP3A5 on CL/F	2.03 (10.7)	2.05 (1.62, 2.64)
Effect of MRP2 on CL/F	1.40 (9.54)	1.41 (1.15, 1.72)
Effect of age on CL/F	−0.780 (21.1)	−0.805 (−1.17, −0.480)
Effect of diabetes on lag time	2.60 (23.9)	2.61 (1.55, 4.91)
Random effects		
Interindividual variability		
V_1/F (%)	157 (24.9)	156 (111, 197)
CL/F (%)	43.9 (15.9)	43.9 (36.4, 56.1)
Residual variability		
Proportional error (%)	18.4 (17.0)	18.0 (15.1, 21.4)

V_1/F, apparent central volume of distribution after oral administration; CL/F, apparent oral clearance; V_2/F, apparent peripheral volume of distribution after oral administration; Q/F, apparent inter-compartmental clearance; k_a, absorption rate constant; CYP, cytochrome P450; MRP2, multidrug resistance-associated protein 2; RSE, relative standard error; CI, confidence interval