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Brief communication (Original)

Association of CYP3A5 and POR polymorphisms with the maintenance tacrolimus dosage requirement in Thai recipients of kidney transplants

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Background: Cytochrome P450 (CYP) 3A5 is a major isoform metabolizing tacrolimus. Individual variation in the metabolism may result from *CYP3A5* single nucleotide polymorphisms (SNPs). *CYP3A5*3* polymorphism is strongly associated with tacrolimus pharmacokinetic variations in 65%–85% of Asian populations. A minor polymorphism related to requirement for tacrolimus is the *POR*28* mutation, which increases in vivo CYP3A activity for tacrolimus. These two SNPs might affect individual maintenance dosages of tacrolimus.
Objectives: To determine the association of *CYP3A5*3* and *POR*28* SNPs with maintenance dosage requirements for tacrolimus in Thai recipients of kidney transplants.
Methods: We enrolled 150 Thai recipients of kidney transplants. Clinical laboratory data were recorded 3 months after first administration of tacrolimus. Two SNPs; rs776746 A > G (*CYP3A5*3* allele) and rs1057868 C > T (*POR*28* allele) were assessed. All 300 genotypes were analyzed by real-time polymerase chain reactions.
Results: Recipients were classified into 9 groups according to possible matching genotypes. The mean dosage required for the maintenance phase was significantly higher in the *CYP3A5*1* allele or CYP3A5 expressers (groups 1-6, 0.163, 0.167, 0.141, 0.128, 0.131, and 0.174 mg/kg/day, respectively) than those not expressing *CYP3A5*3/*3* or CYP3A5 (groups 7-9, 0.081, 0.073, and 0.069 mg/kg/day, respectively, $P < 0.05$). When the mean dosage was compared under *POR*28* one or two alleles in CYP3A5 expressers, *P* was significantly smaller than in CYP3A5 expressers with *POR*1/*1*.
Conclusions: CYP3A5 polymorphism is key to determining tacrolimus dosage requirements during the maintenance phase in kidney transplant recipients and *POR*28* may contribute to the interindividual variability.

Keywords: CYP3A5, kidney transplantation, maintenance dose, POR, single nucleotide polymorphisms, tacrolimus

Tacrolimus, a calcineurin inhibitor, was approved in 1997 to prevent acute rejection of kidney transplants, and its role remains crucial as an effective immunosuppressant in kidney transplant recipients [1]. Tacrolimus is characterized by its narrow therapeutic index. Therefore, close drug monitoring of plasma tacrolimus levels is essential to optimize efficacy and minimize toxicity. Achieving therapeutic trough levels (C_0) is important, especially in the initial period after transplantation during which the highest risk of organ rejection occurs. Large interindividual variability is found in tacrolimus pharmacokinetics, particularly in the dosage required to achieve target blood concentrations [2]. The recommended C_0 levels of tacrolimus are 10 to 20 ng/mL during the first 3 months after transplantation (induction phase), followed by C_0 levels of 5 to10 ng/mL during the maintenance phase. Significant toxicity is seen with C_0 levels of 15 ng/mL [3]. Subsequent trials often used for C_0 ranged between 7 to 8 ng/mL in the early post-transplantation period, and 5 to 7 ng/mL during the maintenance phase. An individualized dosage is required to achieve optimum C_0 levels. A major factor affecting dosage variations is genetic polymorphisms of the cytochrome P450 (CYP) enzymes.

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CYP3A5 is a major isoform responsible for the metabolism of tacrolimus. Many reports have indicated that the interindividual variations seen in pharmacokinetics of tacrolimus were from *CYP3A5* single nucleotide polymorphism [4-7]. CYP3A5 accounts for approximately 10% of CYP enzymes in the liver. It is also expressed in extrahepatic tissues, such as kidneys, lungs, and prostate glands [8]. A *CYP3A5*3* polymorphism (rs776746) is an A to G transition (6986; A > G) within intron 3, which results in alternate mRNA splicing, and a truncated and nonfunctional protein. This transition causes the most frequent functional polymorphism of CYP3A5 [9]. *CYP3A5*3* has been extensively studied and was found to be a predominant allele in many populations [10]. The frequency of the **3* allele is 85%–95% in white people of European ancestry, 27%–55% in African Americans, 65%-85% in Asians, and 75% in Mexicans [11]. CYP3A5 variations are the strongest predictor of tacrolimus dose requirements because individuals with the *CYP3A5*1* allele (CYP3A5 expressers) required a higher daily dose of tacrolimus than those with *CYP3A5*3/*3* (CYP3A5 nonexpressers) in order to maintain the target trough level [8, 12, 13].

P450 oxidoreductase (POR) is a protein containing both flavin adenine dinucleotide and flavin mononucleotide. It transfers electrons from NADPH to microsomal cytochrome P450 enzymes, enabling their activity [14]. Recently, polymorphisms in the POR genes have been reported to modulate the activity of various CYP enzymes including those from CYP1A2, CYP2C19, and CYP3A families [15, 16]. An important variant identified in the POR gene is **28* SNP, rs1057868 (1508; C > T), which varies in frequency of 26.4% in white Americans of European ancestry, 19.1% in African Americans, 31% in Mexicans, and 36% in Chinese Americans [17]. The mechanism of *POR*28* SNP leads to a loss of function in CYP1A2 and a gain of function CYP2C19. However, its effects on CYP3A function is still unclear. One study found that *POR*28/*28* increased CYP3A activity in vivo, demonstrated by a 1.6-fold increase in midazolam metabolic ratio compared with *POR*1/*1* [18].

The present study sought to investigate the associations of *CYP3A5*3* and *POR*28* SNPs with the maintenance dosage requirements of tacrolimus in Thai recipients of kidney transplants.

Materials and methods

Approval for this cross-sectional study was granted by the Institutional Review Board (IRB) for Human Research of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (certificate of approval No.422/2014, IRB No.205/57). All transplant recipients provided their written informed consent before their participation in this study.

Study population

We recruited 150 kidney transplant recipients at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, prescribed with tacrolimus as an immunosuppressant from October 2014 to June 2015. Clinical laboratory data including C_0 levels of tacrolimus were recorded at 3 months after administration of the first dose. Tacrolimus levels were quantified using a chemiluminescent microparticle immunoassay (Architect i1000 system, Abbott Laboratories, Abbott Park, IL, USA). Recipients taking medications that are known to interact with CYP3A, including CYP3A inducers (rifabutin, rifampin, phenytoin, carbamazepine, phenobarbital and chloramphenicol), and CYP3A inhibitors (verapamil, diltiazem, clotrimazole, ketoconazole, itraconazole, fluconazole, voriconazole, danazol, nifedipine, nicardipine, clarithromycin, troleandomycin, erythromycin, cimetidine, metoclopramide, cisapride, and bromocriptine) were excluded from the study.

Identification of CYP3A5 and POR genotypes

Whole blood samples (2 mL) were collected in ethylene diamine tetraacetic acid tubes. Genomic DNA samples were extracted using DNA extraction kits (Invitrogen, Carlsbad, CA, USA). CYP3A5 and POR genotypes were assessed using real-time polymerase chain reactions (PCR) (7500 Applied Biosystems PCR thermocycler apparatus; Forster City, CA, USA). Analysis of *CYP3A5*3* and *POR*28* were performed using a TaqMan single nucleotide polymorphism genotyping assay (Applied Biosystems). Reaction mixtures (20 mL) consisted of 6 μL purified water, 3 μL of purified DNA (30 ng yield), 10 μL of TaqMan Genotyping Master Mix and 1 μL TaqMan minor groove binder primer specific *CYP3A5*3*/*POR*28* probes. For *CYP3A5*3* (rs776746) polymorphism, the forward primer was 5′-CATGACTTAGTAGACAG ATGA-3′ and reverse primer was 5′-GGTCCAAA CAGGGAAGAAATA-3′. For *POR*28* (rs1057868) polymorphism, the forward primer was 5′-TACTCCA

TCGCCTCATCCTC-3′ and a reverse primer was 5′-AAGCCTATGAAGGGTGCCAC-3′. Amplification was performed for 40 cycles under optimal conditions consisting of denaturation at 92°C for 15 s, followed by annealing at 60°C for 90 s and primer extension at 60°C for 90 s. Genotypes were analyzed using allelic discrimination plots and amplification graph plots of each sample.

Data and statistical analysis

Statistical analysis of data was conducted using SPSS Statistics for Windows (version 20; IBM Corp, Armonk, NY, USA). Genotypes of 150 Thai recipients of kidney transplants are presented as percentages of each incident of 6 genotypes (*CYP3A5*1/*1*, *CYP3A5*1/*3*, *CYP3A5*3/*3*, *POR*1/*1*, *POR*1*28*, and *POR*28/*28*) and daily dosage requirements of tacrolimus are presented as mean ± standard deviation (SD). Maintenance dosage requirements of each group were compared using a one-way analysis of variance (ANOVA). Multiple comparison analysis between groups was conducted using a Student *t* test with Bonferroni adjustment. $P < 0.05$ (95% confidence interval) was considered to be statistically significant.

Results

Baseline characteristics of the recipients are summarized in **Table 1**. All 300 allele frequencies of 150 recipients were categorized according to 2 SNPs (*CYP3A5*3* and *POR*28*). Average tacrolimus C_0 plasma concentrations in all recipients was 7.65 ng/mL. CYP3A5 nonexpressers (*CYP3A5*3/*3*) showed higher C_0 plasma concentrations (8.53 ng/mL) compared with CYP3A5 expressers (*CYP3A5*1/*1* and *CYP3A5*1/*3*; 6.53 ng/mL ($P = 0.013$) and 7.15 ng/mL ($P = 0.008$), respectively). When we considered *POR*28* polymorphism, tacrolimus C_0 plasma concentrations in *POR* mutations (*POR*1/*28* and *POR*28/*28*) showed no statistical difference when compared with POR nonexpressers (*POR*1/*1*) (7.71 ng/mL, 7.34 ng/mL versus 7.68 ng/mL, respectively, $P = 1$).

Average tacrolimus dose requirements (mg/kg/day) in the maintenance phase were compared in each polymorphism. With focus on CYP3A5, mean doses for *CYP3A5*1/*1* and **1/*3* were significantly higher than for *CYP3A5*3/*3* (0.164, 0.134, and 0.075 mg/kg/day, respectively, $P < 0.0001$). However, when comparing *POR*1/*1*, **1/*28*, and **28/*28*, the mean doses were not different (0.114, 0.114, and 0.106 mg/kg/day, respectively, $P > 0.99$).

All recipients were further classified into 9 groups using the combination of the genotypes of their 2 SNPs containing 1. *CYP3A5*1/*1-POR*1/*1*; 2. *CYP3A5*1/*1-POR*1/*28*; 3. *CYP3A5*1/*1-POR*28/*28*; 4. *CYP3A5*1/*3-POR*1/*1*; 5. *CYP3A5*1/*3-POR*1/*28*; 6. *CYP3A5*1/*3-POR*28/*28*; 7. *CYP3A5*3/*3-POR*1/*1*; 8. *CYP3A5*3/*3-POR*1/*28*, and 9. *CYP3A5*3/*3-POR*28/*28*. The frequency of genotype distribution is revealed in **Table 2**. The maximum number of recipients was 36 in group 5 showing the highest percentage of both intermediate alleles (*CYP3A5*1/*3* = 46% and *POR*1/*28* = 51.3%). The minimum number of recipients was 1 in group 3 showing the lowest percentage of both alleles (*CYP3A5*1/*1* = 12% and *POR*28/*28* = 12.7%).

Because the normal oral dosage range of tacrolimus in clinical practice is from 0.075 to 0.2 mg/kg/day in 2 divided doses, dot plots between low, normal, and high dose ranges of tacrolimus were performed for 9 possible genotypes to depict the distribution (**Figure 1**). We detected a higher range of tacrolimus daily dosage requirements in recipients in groups 1, 2, 4, 5, and 6 because they were *CYP3A5*1* expressers, while recipients in groups 4, 5, 7, 8, and 9 showed a lower range of tacrolimus daily dose requirements.

The mean dose of tacrolimus required during the maintenance phase (mg/kg/day) is shown in **Table 2**. Between CYP3A5 expressers (groups 1-6), mean dosages were not significantly different and not significantly different from those of nonexpressers (groups 7-9). When we compared CYP3A5 expressers and nonexpressers, dosage requirements were significantly higher in recipients in groups 1-6 than those in groups 7-9 ($P < 0.05$). When we compared the *POR*28* allele in those recipients expressing the same *CYP3A5* genotype, the variance between *POR*1/*28** and *28*/28** was more highly significant than *POR*1/*1* (**Figure 2**). When comparing the *CYP3A5*1/*1* genotype (groups 1-2) with *POR*1/*1* and *POR*1/*28* variances, there was higher significance in groups 7 (0.010 to <0.0001), 8 (0.002 to < 0.0001), and 9 (0.004 to < 0.0001). Similarly, variances in the *CYP3A5*1/*3* genotype (groups 4-6) *POR*1/*1*, *POR*1/*28*, and *POR*28/*28*, were more highly significant when compared with groups 7–9.

Table 1. Baseline characteristics of 150 Thai recipients of kidney transplants, defined by *CYP3A5* and *POR* genotype

Parameters	***CYP3A5* (n = 150)**			***POR* (n = 150)**			**Total**
	***1* expressers**		***3***	***1***		***28* mutation**	
Genotype	**1/*1*	**1/*3*	**3/*3*	**11/**	**1/*28*	**28/*28*	N/A
n (%)	18 (12)	69 (46)	63 (42)	54 (36)	77 (51.3)	19 (12.7)	150 (100)
Male/female (n)	11/7	31/38	35/28	24/30	46/31	7/12	77/73
Age (years)	43.3	42.9	45.2	43.9	44.5	41.3	43.9
Weight (kg)	59.7	56.0	59.0	58.4	57.8	55.4	57.7
Living donors (n)	11	30	34	28	37	10	7575
Cadaveric donors (n)	7	39	29	26	40	9	
Blood urea nitrogen (mg/dL)	16.4 (7.87)	20.2 (12.9)	19.9 (9.21)	20.3 (14.1)	19.1 (8.16)	19.6 (11.2)	19.6 (11.0)
Serum creatinine (mg/dL)	1.38 (0.47)	1.37 (0.40)	1.48 (0.72)	1.33 (0.41)	1.48 (0.65)	1.41 (0.57)	1.42 (0.56)
Tacrolimus C_0 (ng/mL)	*6.53 (2.59)	**7.15 (2.52)	8.53 (2.79)	7.68 (2.77)	7.71 (2.67)	7.34 (2.62)	7.65 (2.69)

*P = 0.013 and ** P = 0.008 compared with *CYP3A5*3/*3*. Values are presented as mean (SD). C_0: trough concentration.

Table 2. Frequency of genotype distribution and mean dosage requirements of tacrolimus defined by 9 matching groups of genotypes.

Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
Genotype	*CYP3A5*1/*1-POR*1/*1*	*CYP3A5*1/*1-POR*1/*28*	*CYP3A5*1/*1-POR*28/*28*	*CYP3A5*1/*3-POR*1/*1*	*CYP3A5*1/*3-POR*1/*28*	*CYP3A5*1/*3-POR*28/*28*	*CYP3A5*3/*3-POR*1/*1*	*CYP3A5*3/*3-POR*1/*28*	*CYP3A5*3/*3-POR*28/*28*	
N	6	11	1	27	36	6	21	30	12	150
%	4	7.3	0.7	18	24	4	14	20	8	100
Mean dose (mg/kg/day)	0.163	0.167	0.141	0.128	0.131	0.174	0.081	0.073	0.069	0.113
SD	0.050	0.065	–	0.057	0.049	0.065	0.051	0.031	0.023	0.059

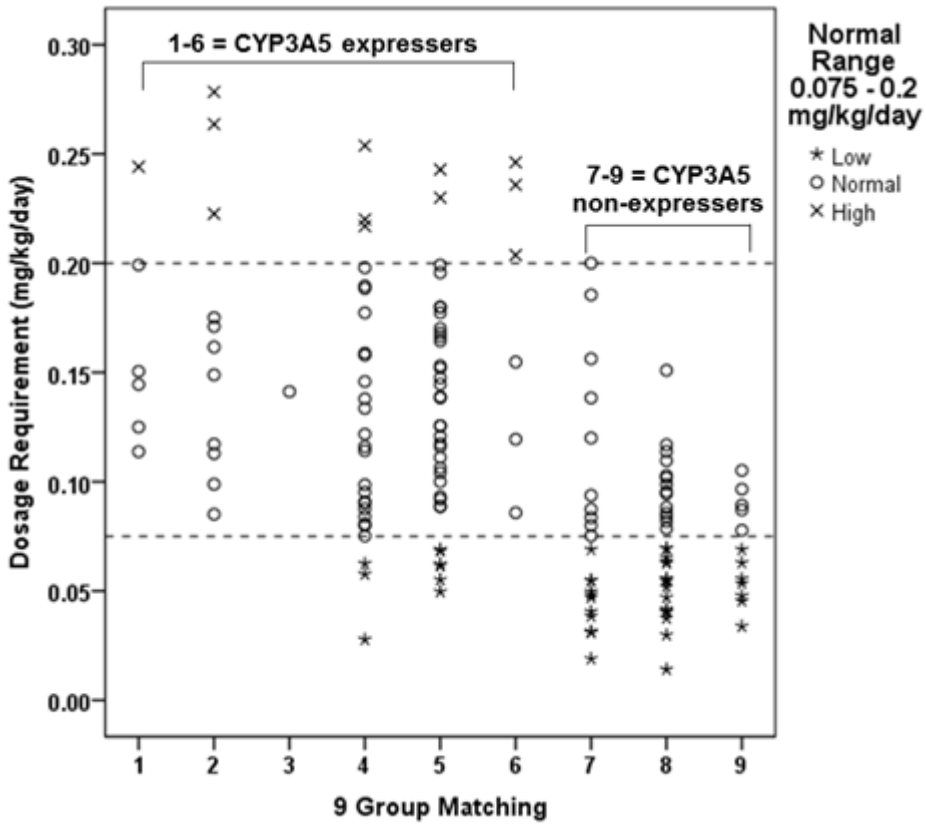

Figure 1. Scatter dot plots of tacrolimus requirement doses, defined by 9 matching group genotypes. The groups are 1. *CYP3A5*1/*1-POR*1/*1*; 2. *CYP3A5*1/*1-POR*1/*28*; 3. *CYP3A5*1/*1-POR*28/*28*; 4. *CYP3A5*1/*3-POR*1/*1*; 5. *CYP3A5*1/*3-POR*1/*28*; 6. *CYP3A5*1/*3-POR*28/*28*; 7. *CYP3A5*3/*3-POR*1/*1*; 8. *CYP3A5*3/*3-POR*1/*28*, and 9. *CYP3A5*3/*3-POR*28/*28*.

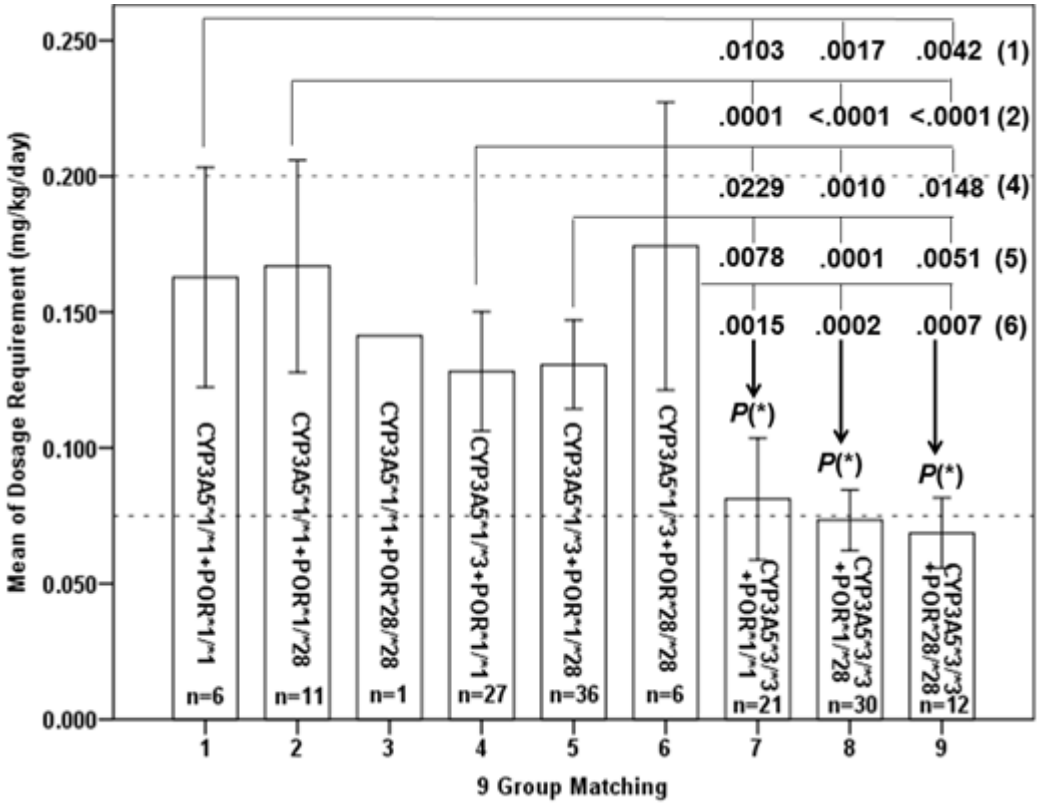

Figure 2. Bar charts of mean ± standard error of tacrolimus dose requirements and *P*, defined by 9 matching group genotypes. **P* < 0.05.

Discussion

In this study, the frequencies of *CYP3A5*3* (65%) and *POR*28* alleles (38.3%) were similar to those found in a previous study of Asian populations. In a study of 71 Chinese men, the frequency of the *CYP3A5*3* was 73.3% and that of the *POR*28* alleles 29.6% [19]. In a study of 240 Chinese renal transplant recipients, the frequencies were 69.8% and 35.6% respectively [20]. From baseline characteristics, results showed no difference in sex, age, body weight, or kidney function. Differences were found only in C_0 level in patients with the *CYP3A5* allele and none with *POR* alleles. This difference may be the result of variations in CYP enzyme activity resulting from its polymorphism. This is consistent with a study of *CYP3A5* genotyping on determining initial dosages in 76 Chinese by Zhang et al. [21], which found that CYP3A5 polymorphism plays an important role in influencing tacrolimus blood levels. Initial tacrolimus dosage selection based on *CYP3A5* genotyping can improve drug blood levels in early stages.

To our knowledge, we are the first to present 9 matching groups of 2 SNPs (*CYP3A5* and *POR*) in an Asian population. Other studies of Asians, classified recipients by expression and nonexpression of *CYP3A5*1* and *POR*28* into 4 groups, such as the study of *POR*28* polymorphism on the pharmacokinetics of tacrolimus in 71 Chinese healthy male volunteers by Zhang et al. [19]. That study did not find any *POR*28/*28* volunteers in CYP3A5 expressers and classified 71 volunteers into only 4 groups because of this limitation. The importance of the present study of 9 groups is that it provides better, clearer depictions of variations than previous studies with only 4 groups. We examined the effect of intermediate alleles including *CYP3A5*1/*3* and *POR*1/*28*. With 9 matching groups, we compared CYP3A5 from each genotype with variations of POR polymorphisms, thereby reporting more concise findings in the differences of mean dosage requirements.As seen by dot plots, most patients with *CYP3A5*1/*1* and *CYP3A5*1/*3* alleles required a high dosage (0.128 to 0.174 mg/kg/day of tacrolimus), but those with the *CYP3A5*3/*3* allele did not (0.069 to 0.081 mg/kg/day).

In a previous report with a cohort of 52 and 45 patients [22], CYP3A5 expressers (**1/*3* and **1/*1*) required around a 2.2- to 2.6-fold higher daily tacrolimus dosage to reach the targeted C_0 concentrations at all studied time points (1, 3, 6, and 12 months) compared with CYP3A5 nonexpressers (**3/*3*). In the present study, *CYP3A5*1/*1* and *CYP3A5*1/*3* had 2.19- and 1.78-fold higher dose requirements compared with *CYP3A5*3/*3*. We found that the effects of CYP3A5 polymorphisms revealed different capabilities of the enzyme on dosage requirement. An A to G transition (position 6986) in CYP3A5 has sequence variability in intron 3 (**3* allele) that encodes an aberrantly spliced mRNA with a premature stop codon. The study of Kuehl et al. [9] explains the molecular defect responsible for one of the most common polymorphisms in CYP3A5 drug-metabolizing enzymes.

A significant effect of the *POR*28* allele was also reported. deJonge et al. [23] reported a gain of CYP3A5 activity linked to the *POR*28* genotype. They found that kidney transplant recipients who expressed CYP3A5 and carried at least one *POR*28* variant allele displayed significantly lower tacrolimus exposure early after transplantation. Elens et al. [24] showed the effects of combined genotypes *CYP3A5* and *POR*. They revealed that CYP3A5 expressers carrying one or two *POR*28* alleles had higher tacrolimus dose requirements throughout the first year compared with CYP3A5 expressers without a *POR*28* allele, such as *POR*1/*1*. However, in CYP3A5 nonexpressers, the *POR*28* allele had no effect on tacrolimus pharmacokinetics [24]. By contrast, Lunde et al. [25] did not support this finding because they did not show any significant impact of the *POR*28* allele on the tacrolimus C_0/dose ratio in a subgroup of patients expressing functional CYP3A5. The present study showed the effects of POR polymorphisms on mean dose requirements only in CYP3A5 expressers with significantly different levels *POR*28* allele. The result in *CYP3A5*1/*1* had presented a consequently smaller *P* when compared with groups 7, 8, and 9 (0.010 to < 0.0001, 0.002 to < 0.0001, and 0.004 with < 0.0001, respectively). A similar difference was also seen in these genotypes.

de Jonge et al. [23] found at least one *POR*28* allele was associated with a 25% higher requirement for tacrolimus throughout the first year of treatment compared with *POR*1/*1* CYP3A5 expressers. However, that study could detect a trend of increasing daily dose requirements among *POR*1/*1* and **28/*28* as 35.96% (0.128 to 0.174 mg/kg/day) in the *CYP3A5*1/*3* group.

Nevertheless, it remains unknown as to how exactly the *POR*28*; C > T SNP can affect tacrolimus metabolism. POR mutations possibly alter the distribution of charge in the electron-donating domain, which might have quite different effects on the interaction of POR with different cytochrome P450 enzymes [26, 27]. The *POR*28* allele has the potential to explain interindividual variability in CYP3A capacity.

Here, we recommend physicians first focus on the CYP3A5 polymorphism and then additionally classify patients according to *POR*28*. To clarify the influence of POR polymorphisms on dose requirements of tacrolimus, further study of the mechanisms common to *CYP3A5* and *POR*28* alleles should be performed. More patients may be required to increase the numbers of those with the *POR*28/*28* genotype.

A limitation of this study is that there were insufficient participants in group 3 *CYP3A5*1/*1-POR*28/*28* in which only one participant presented with these genotypes. This group could not be included in the statistical analysis.

Conclusion

CYP3A5 is the key polymorphism to determine optimal tacrolimus dose requirements for the maintenance phase in kidney transplant recipients. POR is an adjunctive genetic polymorphism, which affects only CYP3A5 expressers. Apart from *CYP3A5*3*, *POR*28* might also contribute to the interindividual variability seen in Thai recipients of kidney transplants receiving tacrolimus.

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Conflict of interest statement

The author declares that there is no conflict of interest in this research.

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