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Association of CYP3A5 polymorphisms and parathyroid hormone with blood level of tacrolimus in patients with end-stage renal disease

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Abstract

Because tacrolimus is predominantly metabolized by CYP3A, the blood concentration/dose (C/D) ratio is affected by CYP3A5 polymorphism. Parathyroid hormone (PTH) expression increases in secondary hyperparathyroidism, which is frequently associated with end-stage renal disease. Recently, PTH has been shown to downregulate CYP3A expression at mRNA level. In this study, we examined the influence of CYP3A5 polymorphism on and association of serum intact-PTH (iPTH) level with blood tacrolimus concentration in patients with end-stage renal disease just before kidney transplantation. Forty-eight patients who satisfied the selection criteria were analyzed. Subjects were classified into two phenotype subgroups: CYP3A5 expressor (CYP3A5*1/*1 and *1/*3; $n = 15$) and CYP3A5 nonexpressor (CYP3A5*3/*3; $n = 33$). The blood tacrolimus C/D (per body weight) ratio was significantly lower in CYP3A5 expressors than that in CYP3A5 nonexpressors. A significant positive correlation was found between tacrolimus C/D and iPTH concentrations ($r = 0.305$, $p = 0.035$), and the correlation coefficient was higher after excluding 20 patients co-administered CYP3A inhibitor or inducer ($r = 0.428$, $p = 0.023$). A multiple logistic regression analysis by stepwise selection identified CYP3A5 polymorphism and serum iPTH level as significant factors associated with tacrolimus C/D. These results may suggest the importance of dose design considering not only the CYP3A5 phenotype but also serum iPTH level when using tacrolimus in patients who undergo renal transplantation.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Tacrolimus is primarily metabolized by cytochrome P450 (CYP) 3A4/5 and the pharmacokinetics is affected by CYP3A5 polymorphism. Recently, intact parathyroid hormone (PTH) has been shown to downregulate CYP3A expression at the mRNA level.

WHAT QUESTION DID THIS STUDY ADDRESS?
Do CYP3A5 polymorphism and serum intact PTH influence the tacrolimus concentration/dose per body weight before kidney transplantation in patients with end-stage renal failure?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
When designing dose of tacrolimus for patients scheduled to undergo renal transplantation, it may be important to consider not only the CYP3A5 phenotype but also the serum intact PTH level.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Confirming the CYP3A5 phenotype and serum intact PTH level could allow physicians to control blood concentration of tacrolimus from an earlier stage before transplantation. This may contribute to prevent rejection and graft-versus-host disease in patients who undergo renal transplantation and to prolong the posttransplant survival of the transplanted kidney.

INTRODUCTION

Kidney transplantation relieves patients with end-stage renal failure from dialysis, and the number of kidney transplantations has increased in recent years. For kidney transplantation, calcineurin inhibitors, such as tacrolimus and cyclosporine, are administered from before transplantation to avoid acute rejection and graft-versus-host disease after transplantation. According to an annual report from the Japanese Kidney Transplant Registry, 87% of recipients received tacrolimus compared with only 13% who received cyclosporine.[1] Individual administration design based on therapeutic drug monitoring is essential for tacrolimus because of the narrow effective therapeutic range and large individual differences in pharmacokinetics.[2,3]

Tacrolimus is primarily metabolized by cytochrome P450 (CYP) 3A4/5 that expresses in the small intestine and hepatocytes. The blood concentration of tacrolimus is affected by concomitant use of CYP3A inducer or inhibitor and individual differences in protein expression of CYP3A4/5.[4,5] The protein expression of CYP3A5 in hepatocytes and small intestine correlates strongly with the single nucleotide polymorphism 6986A>G in intron 3 of CYP3A5, designated CYP3A5*3.[6] Tacrolimus pharmacokinetics is affected by CYP3A5 polymorphism. Thus, a significantly lower dose of tacrolimus is required to maintain the same target blood concentration in patients with CYP3A5*3/*3 than in those with the CYP3A5*1 allele.[7]

Apart from genetic factors, a multitude of environmental and physiologic factors are known to affect CYP3A expression and activity.[8] Several studies in rats and patients have revealed that renal failure suppresses the expression and function of CYP3A.[9–13] Parathyroid hormone (PTH) is one of the middle molecular weight uremic toxins, and the serum level increases by a decrease in kidney function. PTH has been shown to influence the expression and activity of CYP3A. Using primary rat hepatocytes and a human colon carcinoma cell line, secondary hyperparathyroidism (SHPT) model rats, Watanabe et al.[14] showed that elevation of serum PTH caused a reduction in CYP3A expression in both the small intestine and liver, resulting in a significant reduction in CYP3A activity and a marked increase in the area under the curve (AUC) of midazolam, a typical substrate of CYP3A. Furthermore, Hirata et al.[15] showed a significant positive correlation between the serum intact PTH (iPTH) level and blood tacrolimus concentration in 12 patients who underwent kidney transplantations who were not receiving potent CYP3A inhibitors. However, this previous report has not investigated the genotype of CYP3A5 polymorphism, a pivotal factor affecting the blood concentration of tacrolimus.

In this study, we examined the effects of CYP3A5 polymorphism on and association of iPTH level with blood tacrolimus concentration in patients with end-stage renal disease just before kidney transplantation.

METHODS

Subjects

In this retrospective study, patients with end-stage renal failure who underwent kidney allograft transplantation and CYP3A5 polymorphism analysis between September 2012 and August 2017 in the Department of Urology, Faculty of Medicine at Oita University, were enrolled. We excluded patients who met any of the following criteria: no measurement of serum iPTH level before transplantation; no use of tacrolimus (oral Prograf: twice daily) for immunosuppressive therapy; no measurement or only outpatient measurement of blood tacrolimus concentrations before transplantation; and measurement of pre-transplantation blood tacrolimus

concentration less than 3 days after admission. All recruited patients underwent triple-therapy immunosuppression protocol consisting of tacrolimus, corticosteroids, and mycophenolate mofetil. Using electronic medical records, the patient's demographic characteristics and the following clinical data before kidney transplantation were collected: gender, age, body weight, daily dose of tacrolimus, trough concentration of tacrolimus, serum albumin, alanine aminotransferase (ALT), serum creatinine (SCr), white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, and iPTH level. For analyses, we used the blood concentration of tacrolimus measured 3 days or later after admission, because outpatient adherence to tacrolimus was unknown. After trough level blood sampling in the morning into tubes containing ethylenediaminetetraacetic acid anticoagulant, the blood tacrolimus concentration was quantified by antibody-conjugated magnetic immunoassay using Dimension Xpand plus. The measurement was conducted according to the manufacturer's instructions. Serum iPTH level was measured by electro-chemiluminescence immunoassay as a routine clinical test, and the data were collected retrospectively from the electronic medical records. For analyses, we used the serum iPTH levels measured before transplantation, on the day closest to measurement of blood tacrolimus concentration. The study was conducted in accordance with the ethical standards of our institute and with the Helsinki Declaration of 1975, as revised in 2013. The protocol for this study was approved by the Oita University Faculty of Medicine Ethics Committee (review reference number: 1924) before the study was started. Each subject gave written informed consent.

Genotyping procedures for CYP3A5

A venous blood sample was obtained from each patient. Using 400 μl of blood, total DNA was prepared using the Maxwell 16 DNA Purification Kit (Promega). To determine the CYP3A5 mutant alleles, all samples were analyzed for the single nucleotide polymorphism A6986G (CYP3A5*3). Allelic discrimination reaction was performed using TaqMan genotyping assay (C_26201809_30) with a LightCycler Nano System (Roche Applied Science). Subjects were classified into two phenotype subgroups: CYP3A5 expressor (CYP3A5*1/*1 and *1/*3) and CYP3A5 nonexpressor (CYP3A5*3/*3).

Statistical analysis

Statistical analyses were performed using Predictive Analysis Software (PASW) Statistics version 26 (SPSS Inc.). Demographic and clinical data of patients are expressed as number (%) for categorical variables and as median

[interquartile range] for continuous variables. Categorical variable was analyzed by one-sided Fisher's exact test. Data normality was tested using the Shapiro-Wilk test. Parametric data were analyzed by Student's t-test, and nonparametric data by Mann–Whitney U test. Correlation between variables was analyzed by Spearman rank correlation coefficient. A p value less than 0.05 was considered statistically significant.

Factors associated with blood tacrolimus concentration/dose (per body weight) ratio (C/D) were analyzed by multiple logistic regression, using the following as independent variables: CYP3A5 expressor, serum iPTH level, age, ALT level, and concomitant use of CYP3A inhibitor/inducer. Other than CYP3A5 expressor, serum iPTH level, and concomitant use of CYP3A inhibitors, we selected age and ALT level as covariates for the following reasons. Older age may influence the clearance of CYP3A substrates due to reduced metabolic activity.[16] The activity of CYP3A decreases in patients with liver dysfunction and ALT is a typical biomarker of liver dysfunction.[17] Using the Mann–Whitney U test, χ^2 test, and Spearman rank correlation coefficient, we confirmed no association among the covariates. For multiple regression analysis, independent variables were inserted stepwise in the model using Schwarz's Bayesian information criterion and the variable remained in the model if p was less than 0.05.

RESULTS

Patient characteristics

Forty-eight patients who satisfied the selection criteria were analyzed. The demographic and laboratory data of the patients are shown in Table 1. All recruited patients were Japanese. The male to female ratio was 3:1, and median age and body weight were 42.0 years and 61.1 kg, respectively. The median dose/body weight was 0.114 mg/kg and median blood tacrolimus concentration was 9.1 ng/ml. Most of the recruited patients underwent kidney transplantation from living donors. The most frequent primary diseases were immunoglobulin A nephropathy and chronic glomerulonephropathy. The median iPTH level was 166.5 pg/ml, and the levels varied from 76.5 to 243.8 pg/ml (interquartile range). Table S1 shows tacrolimus C/D, CYP3A5 genotype, iPTH level, and co-administered CYP3A inhibitors or inducer[15] in individual patients. Among the 48 patients, 19 received CYP3A inhibitors and one had CYP3A inducer.

Comparison of tacrolimus C/D between CYP3A5 expressors and nonexpressors

Based on CYP3A5 phenotypes, 15 of 48 patients were classified as CYP3A5 expressors and 33 as nonexpressors

TABLE 1 Demographic and clinical characteristics of patients

Characteristics	All	Expressor group	Nonexpressor group	p value
Patients; n	48	15	33	
Gender (male/female); n (%)	36 (75.0)/12 (25.0)	10 (66.7)/5 (33.3)	26 (78.8)/7 (21.2)	0.476[a]
Age (year)	42.0 [37.8–59.8]	49.0 [36.5–63.5]	41.0 [38.0–39.0]	0.327[b]
Body weight (kg)	61.1 [53.8–70.0]	57.3 [50.1–69.1]	63.9 [56.9–69.8]	0.322[b]
Dose/body weight (mg/kg)	0.114 [0.094–0.143]	0.133 [0.111–0.153]	0.106 [0.092–0.141]	0.161[b]
Tacrolimus concentration (ng/ml)	9.1 [6.8–11.3]	8.7 [6.6–10.5]	9.6 [7.0–11.5]	0.415[c]
Albumin (g/dl)	4.28 [3.93–4.45]	3.98 [3.66–4.45]	4.28 [4.00–4.45]	0.296[b]
Alanine aminotransferase (U/L)	9.8 [7.9–14.3]	9.8 [8.5–14.7]	10.1 [7.8–13.7]	0.903[b]
Serum creatinine (mg/dl)	9.75 [8.36–10.82]	10.09 [7.18–11.39]	9.50 [8.53–10.78]	0.647c
White blood cell ($\times 10^3/mm^3$)	5.38 [4.52–6.22]	4.78 [3.95–5.60]	5.49 [4.59–6.60]	0.033[b]
Red blood cell ($\times 10^6/mm^3$)	3.87 [3.56–4.28]	3.63 [3.33–4.19]	3.98 [3.63–4.38]	0.157[c]
Hemoglobin (g/dl)	11.6 [10.6–13.2]	11.1 [10.4–12.7]	11.9 [10.8–13.3]	0.075[c]
Hematocrit (%)	35.7 [32.5–38.7]	34.3 [31.1–37.4]	36.5 [33.6–39.9]	0.063[c]
Platelet count ($\times 10^3/\mu l$)	184.5 [150.3–212.8]	182.0 [135.5–213.5]	185.0 [162.0–211.0]	0.416[c]
Intact parathyroid hormone level (pg/ml)	166.5 [76.5–243.8]	191.0 [79.0–256.0]	161.0 [81.0–232.0]	0.556[b]
Race; n (%)				
Japanese	48 (100)	15 (100)	33 (100)	
Donor; n (%)				
Living	46 (95.8)	14 (93.3)	32 (97.0)	
Deceased	2 (4.2)	1 (6.7)	1 (3.0)	
Primary disease; n (%)				
Immunoglobulin A nephropathy	11 (22.9)	4 (26.7)	7 (21.2)	
Chronic glomerulonephritis	10 (20.8)	3 (20.0)	7 (21.2)	
Polycystic kidney disease	4 (8.3)	0 (0)	4 (12.1)	
Nephrosclerosis	2 (4.2)	1 (6.7)	1 (3.0)	
Diabetic nephropathy	2 (4.2)	0 (0)	2 (6.1)	
Thin basement membrane disease	1 (2.1)	1 (6.7)	0 (0)	
Membranoproliferative glomerulonephritis	1 (2.1)	0 (0)	1 (3.0)	
Membranous nephropathy	1 (2.1)	0 (0)	1 (3.0)	
Unclear chronic renal failure	16 (33.3)	6 (40.0)	10 (30.3)	

Note: Data are expressed as numbers (%) for categorical variables, and median [interquartile range] for continuous variables. The *p* values represent the results of univariate analyses between expressor and nonexpressor groups.

[a]Categorical variable was analyzed by Fisher's exact test. Data normality was analyzed by Shapiro–Wilk test.

[b]Nonparametric data were analyzed by Mann–Whitney *U* test.

[c]Parametric data were analyzed by Student's *t*-test.

(Table 1). A significant difference in white blood cell count between two phenotypes was found, but no significant differences in the other clinical characteristics. As shown in Figure 1, the median tacrolimus C/D was 55.9 (IQR: 50.5–80.0) ng/ml/(mg/kg) in CYP3A5 expressors and 77.0 (IQR: 63.5–109.4) ng/ml/(mg/kg) in nonexpressors. The tacrolimus C/D was significantly lower in CYP3A5 expressors than that in nonexpressors (*p* = 0.029).

When only subjects who were not co-administered CYP3A inhibitor or inducer (*n* = 28) were analyzed, tacrolimus C/D did not differ significantly between CYP3A5 expressors and nonexpressors, although the expressors tend to have higher C/D than non-expressors (*p* = 0.054; Table S2). Furthermore, in subjects co-administered CYP3A inhibitors (*n* = 19), no significant difference in tacrolimus C/D between CYP3A5 expressor and non-expressor was found (*p* = 0.315; Figure S1), suggesting a possibility of phenoconversion by administration of CYP3A inhibitors.
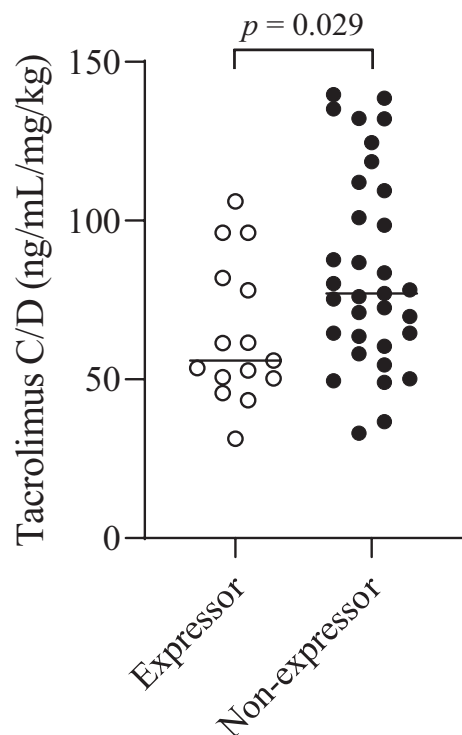
FIGURE 1 Comparison of tacrolimus concentration/dose per body weight (C/D) between CYP3A5 expressor and nonexpressor groups. Shapiro–Wilk test showed that the data were not normally distributed, and the nonparametric data were analyzed by Mann–Whitney U test. Bar indicates median

Correlation with tacrolimus C/D and iPTH level

As shown in Figure 2a, serum iPTH level just before kidney transplantation correlated significantly and positively with tacrolimus C/D in all patients ($r = 0.305$, $p = 0.035$). However, no significant correlation was observed in CYP3A5 expressors ($r = 0.449$, $p = 0.093$) and in non-expressors ($r = 0.286$, $p = 0.106$; Figure 2b,c). Next, we examined the correlation between serum iPTH level and tacrolimus C/D in patients who were not co-administered CYP3A inhibitor or inducer ($n = 28$). Figure 3 shows a significant correlation between the two factors in this sub-group, with a higher correlation coefficient compared with all patients.

Identification of factors influencing on tacrolimus C/D by multivariate regression analysis

Multiple logistic regression analysis by stepwise selection was conducted using the following independent variables: CYP3A5 expressor, serum iPTH level, age, ALT level, and concomitant use of CYP3A inhibitor. The analysis
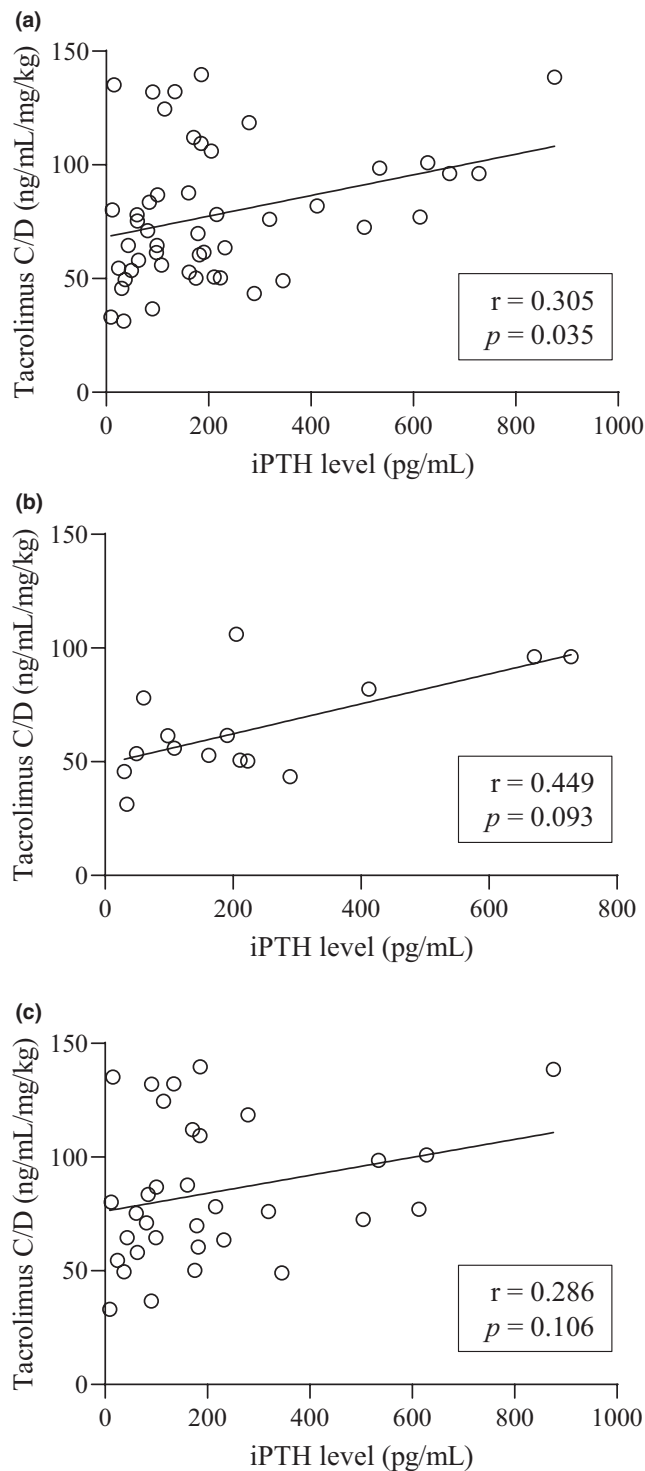

FIGURE 2 Correlation between tacrolimus concentration/dose per body weight (C/D) and intact parathyroid hormone (iPTH) level in (a) all patients ($n = 48$), patients who were (b) CYP3A5 expressors ($n = 15$) and (c) CYP3A5 nonexpressors ($n = 33$). Shapiro–Wilk test showed that the data were not normally distributed, and the correlation between nonparametric data was analyzed by Spearman rank correlation coefficient. Solid line represents regression line

identified CYP3A5 expressor and serum iPTH levels as independent factors associated with tacrolimus C/D, whereas age, ALT level, and concomitant use of CYP3A inhibitor

were not significant factors (Table 2). The parameter estimates (95% confidence interval [CI]) for CYP3A5 expressor and serum iPTH levels were 0.048 (CI, 0.010–0.086, p = 0.015) and −21.178 (CI, −38.11 to −4.247, p = 0.015), respectively.

DISCUSSION

In this study, we investigated the association of blood concentration of tacrolimus with serum iPTH level and CYP3A5 polymorphism in patients with end-stage renal failure just before kidney transplantation and obtained the following novel findings: (1) serum iPTH level correlated significantly and positively with tacrolimus C/D; (2) multiple regression analysis identified serum iPTH level and CYP3A5 phenotype as independently related to tacrolimus C/D.

Several studies in rats and human patients have revealed that the expression and function of CYP3A are suppressed in renal failure.[9-13] According to a previous review, hepatic
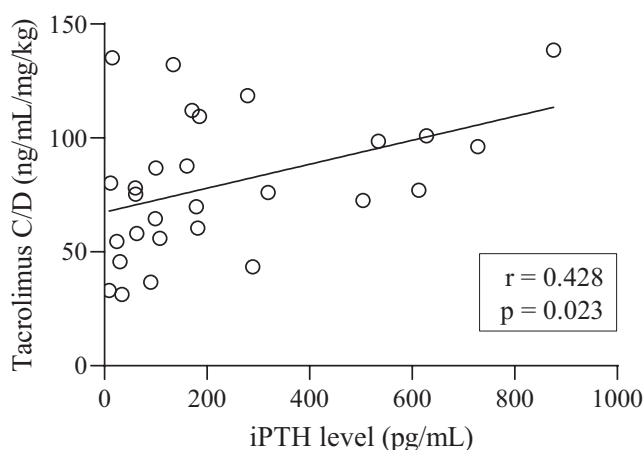

FIGURE 3 Correlation between tacrolimus concentration/dose per body weight (C/D) and intact parathyroid hormone (iPTH) level in patients who were not co-administered a CYP3A inhibitor or inducer (n = 28). Shapiro–Wilk test showed that the data were not normally distributed, and the correlation between nonparametric data was analyzed by Spearman rank correlation coefficient. Solid line represents regression line

CYP3A activity is reduced by 35–75%, and CYP3A protein expression is reduced by 45–91% in renal failure.[18] Moreover, the AUC of ~ 30% of nonrenal excreted drugs approved by the US Food and Drug Administration (FDA) between 2003 and 2007 increased in patients with chronic kidney disease (CKD).[19] Although the reduced extrarenal clearance in patients with CKD is considered to be caused by some uremic toxins that accumulate in renal failure,[20-22] the detailed mechanism has not been completely elucidated. Recently, Watanabe et al.[14] demonstrated that elevation of serum iPTH, one of the uremic toxins, caused a reduction in CYP3A expression in both the small intestine and liver through multiple signaling pathways, including the PI3 K/PKC/PKA/NF-κB pathway, following elevation of intracellular cAMP. Hence, downregulation of CYP3A expression in the liver and small intestine by accumulating iPTH may reduce the metabolism of tacrolimus. This would account for the high tacrolimus C/D in patients with high serum PTH (Figure 2). In contrast, Suzuki et al.[23] studied stable kidney transplant recipients with CYP3A5*1 allele, and reported that only plasma indoxyl sulfate concentration, but not IL-6, TNF-α, or iPTH, was significantly higher in recipients with CYP3A phenoconversion (genotypic extensive/intermediate metabolizer exhibiting CYP3A activity below the cutoff value differentiating extensive/intermediate from poor metabolizers) compared with those without phenoconversion. Watanabe et al.[14] created the experimental SHPT model by feeding a high phosphorus diet (Ca: 0.6%, P: 1.2%) to 5 of 6 nephrectomy rats for 56 days. In these SHPT rats, CYP3A protein expression in the homogenates of the liver and small intestine decreased significantly compared with sham-operated rats. However, the mean serum iPTH level was very high, at ~ 5000 pg/ml. Therefore, the difference in baseline iPTH level may affect the degree of influence on CYP3A activity. Especially in patients with end-stage renal failure, serum iPTH levels vary greatly depending on whether they have SHPT and whether they are treated with cinacalcet or evocalcet.[24] In fact, serum iPTH levels in our patients varied from 76.5 to 243.8 pg/ml (IQR) (median: 166.5 pg/ml), showing great variation even though all patients had end-stage renal failure. According to a previous outpatient cohort cross-sectional study,[25] the iPTH level increased with the progression of CKD, and median iPTH

TABLE 2 Multivariate analyses for factors associated with tacrolimus concentration/dose per body weight

Dependent/independent variable	Model r^2 or partial r^2	p value	Parameter	
			Estimate	95% CI
Tacrolimus concentration/dose per body weight	0.212	0.005		
Serum intact parathyroid hormone level	0.101	0.015	0.048	0.010 to 0.086
CYP3A5 expressor	0.111	0.015	−21.178	−38.110 to −4.247

Abbreviation: 95% CI, 95% confidence interval.

level in patients with glomerular filtration rate (GFR) 40 ml/min or less was higher than the reference value of 65 pg/ml; the levels at GFR 30–39, 20–29, and less than 20 ml/min were ~ 70, 90, and 140 pg/ml, respectively. Furthermore, ~ 80% of patients with end-stage renal disease with GFR less than 20 ml/min had iPTH levels higher than 65 pg/ml. Therefore, the iPTH levels in our subjects with end-stage renal disease were almost consistent with the previous study. Our results seem to contradict the finding of no association between PTH and CYP3A activity in patients with stable renal transplantation reported by Suzuki et al.,[23] which may be due to the difference in iPTH levels between stable post-transplant patients and patients with end-stage renal disease. However, the association between tacrolimus C/D and other uremic toxins, such as indoxyl sulfate, was not evaluated. Because we cannot rule out the possibility that PTH positively correlates with other molecules that accumulate in renal failure and partially mediates the reduction in CYP3A, the cause of the seemingly contradictory findings remain controversial.

Tacrolimus C/D was significantly lower in CYP3A5 expressors (CYP3A5*1*1 or *1*3) than in CYP3A5 nonexpressors (CYP3A5*3*3; Figure 1). Most tacrolimus is metabolized by CYP3A4 and CYP3A5 in hepatic tissue and small intestine, and CYP3A5 is believed to be a more efficient enzyme for tacrolimus metabolism than CYP3A4.[26] CYP3A5 polymorphism is a fundamentally important determinant of tacrolimus pharmacokinetics and dose requirement. Niioka et al.[27] indicated that CYP3A5 polymorphism (*3*3) has no influence on dose-adjusted AUC_{0-24} of tacrolimus administered by continuous intravenous infusion. Because orally administered tacrolimus is presumably predominantly metabolized by CYP3A5 or CYP3A4 in the small intestine, CYP3A5 polymorphism influences the bioavailability of oral tacrolimus formulation. Previous in vitro and in vivo studies supported that iPTH downregulated CYP3A protein expression in both the liver and small intestine.[14] Taken the above findings into consideration, the elevation of tacrolimus C/D induced by iPTH could be due to the reduced expression of CYP3A in the small intestine, but not in the liver.

Patients with a homozygous mutation of 6986A>G in CYP3A5 intron 3 cannot eliminate tacrolimus using CYP3A5.[28] Therefore, they metabolize tacrolimus only by CYP3A4. In general, it is not possible to prove to what extent the reduced CYP3A activity observed in animal studies is attributed to the relative expression levels of CYP3A4 and CYP3A5 in humans. Suzuki et al.[29] suggested that CYP3A activity may increase markedly with recovery of renal function in patients with the CYP3A5*1 allele, or that CYP3A activity may not decrease remarkably during renal failure in patients without the CYP3A5*1 allele. Thus, we speculate that CYP3A activity in CYP3A5 nonexpressors is not affected by uremic toxins accumulated in renal failure. In fact, no significant correlation between tacrolimus C/D and serum iPTH level was found in CYP3A5 nonexpressors ($r = 0.286$, $p = 0.106$). However, there was also no significant correlation between these variables in CYP3A5 expressors ($r = 0.449$, $p = 0.093$). Further analysis in a larger number of CYP3A5 expressors and nonexpressors is required to verify the associations among tacrolimus C/D, serum iPTH level, and CYP3A5 expression status.

This retrospective research has several limitations. First, we did not investigate the influence of other uremic toxins, such as indoxyl sulfate, that accumulate in renal failure and may induce downregulation of CYP3A protein expression. Additionally, other factors, such as FGF23, ionized calcium, phosphorus, and vitamin D levels, that fluctuate in SHPT were also not evaluated. Therefore, we cannot rule out the possibility that PTH correlates positively with other molecules that accumulates in renal failure and partially mediates elevation of tacrolimus C/D. Second, tacrolimus concentration and iPTH level were measured on different days in almost all recruited patients (median interval between two measurements [IQR]: 87 [41.8–175.0] days, with a maximum interval of 535 days) because the iPTH levels were obtained retrospectively from electronic medical records. In the future, we should prospectively examine the association between tacrolimus C/D and the levels of other uremic toxins, including intact PTH using measurements at the same point. Third, the number of patients who were CYP3A5 expressors was small ($N = 15$; only 2 with CYP3A5*1*1 genotype). Moreover, the number of patients who were not co-administered CYP3A inhibitor or inducer was also small ($N = 28$), with only six patients in CYP3A5 expressors. This may account for the inability to demonstrate a significant difference in tacrolimus C/D between CYP3A5 expressors and nonexpressors in patients not on CYP3A5 inhibitor or inducer ($p = 0.054$; Table S2). The small number of CYP3A5 expressors probably also accounts for the low r^2 values ($r^2 = 0.111$) for CYP3A5 expressor as an independent factor associated with tacrolimus C/D. Fourth, the analyses were performed based on a single dose-normalized blood tacrolimus concentration, and not clearance. Hence, we cannot conclude that the fluctuation of tacrolimus blood concentration is a result of reduced metabolic capacity. Future study should calculate clearance based on multiple blood levels and evaluate whether iPTH and CYP3A5 polymorphism affect clearance of tacrolimus. Fifth, we did not evaluate the influence of iPTH on P-glycoprotein. Tacrolimus is a typical substrate for P-glycoprotein (multidrug resistance-1 [MDR1]). Thus, the bioavailability and C/D in patients with high expression of MDR1 in the small intestine are low.[30] Goto et al.[31] reported that no MDR1 polymorphism correlated with the intestinal expression of MDR1 mRNA or the tacrolimus C/D ratio in living-donor liver transplantation recipients. On the other hand, PTH has been reported to affect the activity of MDR1[20] and contribute to the fluctuations in blood levels of tacrolimus. However, the

influence of iPTH on the expression and activity of MDR1 in the small intestine is not completely elucidated. Sixth, as all the patients examined were Japanese, the results cannot be generalized to other races, such as White and African. The frequency of CYP3A5*1 carrier is ~ 50% in Japanese, and is ~ 15% and 85%, respectively, in White and African patients, with a large racial difference.[32] Furthermore, African patients with CKD also have higher blood concentrations of iPTH,[33] which may have genetic basis unrelated to CYP3A.

In this study, a multiple logistic regression analysis by stepwise selection identified CYP3A5 polymorphism and serum iPTH level as significant factors associated with tacrolimus C/D (Table 2). In a previous report, Hirata et al.[15] revealed that blood concentration of tacrolimus correlated significantly and positively with iPTH levels, but the association with CYP3A5 polymorphism was not evaluated. Furthermore, their report showed high tacrolimus concentrations even after transplantation, suggesting that iPTH-induced inhibitory activity persisted. However, this phenomenon may also be associated with the phenotype of CYP3A5 polymorphism, which was not evaluated in their study. To the best of our knowledge, this study is the first report of the effects of both CYP3A5 polymorphism and iPTH on blood tacrolimus concentrations in kidney transplantation recipients. Controlling blood concentrations of tacrolimus is essential to prevent rejection and graft-versus-host disease in patients who undergo renal transplantation and to prolong the post-transplant survival of the transplanted kidney. These results may suggest the importance of designing the dose regimen considering not only the CYP3A5 phenotype but also serum iPTH level when using tacrolimus in patients who undergo renal transplantation. However, this retrospective research has several limitations, including small sample size. Large-scale, prospective research will need to be conducted in the future to prove the effects of CYP3A5 phenotype and serum iPTH level on tacrolimus blood concentration.

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CONFLICT OF INTEREST
The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
R.T., Y.S., T.M., and H.I. wrote the manuscript. R.T., H.W., K.H., T.M., and H.I. designed the research. R.T., Y.S., T.F., T.A., H.O., R.T., T.S., and H.M. performed the research. R.T., Y.S., and H.I. analyzed the data.

REFERENCES
1. Japanese Society for Clinical Renal Transplantation/The Japan Society for Transplantation. Annual Progress Report from the Japanese Renal Transplant Registry: number of renal transplantations in 2017 and a follow-up survey. *JPN J Transplant*. 2018;53:2-3.
2. de Jonge H, Naesens M, Kuypers DRJ. New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible consequences for therapeutic drug monitoring in solid organ transplantation. *Ther Drug Monit* 2009;31:416-435.
3. Andrews LM, Li Y, De Winter BCM, et al. Pharmacokinetic considerations related to therapeutic drug monitoring of tacrolimus in kidney transplant patients. *Expert Opin Drug Metab Toxicol*. 2017;13:1225-1236.
4. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet*. 2004;43:623-653.
5. Staatz CE, Tett SE. Clinical pharmacokinetics of once-daily tacrolimus in solid-organ transplant patients. *Clin Pharmacokinet*. 2015;54:993-1025.
6. Hustert E, Haberl M, Burk O, et al. The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics*. 2001;11:773-779.
7. Birdwell KA, Decker B, Barbarino JM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 genotype and tacrolimus dosing. *Clin Pharmacol Ther*. 2015;98:19-24.
8. Klein K, Zanger UM. Pharmacogenomics of cytochrome P450 3A4: recent progress toward the "Missing Heritability" problem. *Front Genet*. 2013;4:12.
9. Rege B, Krieg R, Gao N, Sarkar MA. Down-regulation of hepatic CYP3A in chronic renal insufficiency. *Pharm Res*. 2003;20:1600-1606.
10. Kusaba J, Kajikawa N, Kawasaki H, Kurosaki Y, Aiba T. Comparative study on altered hepatic metabolism of CYP3A substrates in rats with glycerol-induced acute renal failure. *Biopharm Drug Dispos*. 2012;33:22-29.
11. Velenosi TJ, Fu AY, Luo S, Wang H, Urquhart BL. Down-regulation of hepatic CYP3A and CYP2C mediated metabolism in rats with moderate chronic kidney disease. *Drug Metab Dispos*. 2012;40:1508-1514.
12. Dowling TC, Briglia AE, Fink JC, et al. Characterization of hepatic cytochrome p450 3A activity in patients with end-stage renal disease. *Clin Pharmacol Ther*. 2003;73:427-434.
13. Kirwan CJ, MacPhee IA, Lee T, Holt DW, Philips BJ. Acute kidney injury reduces the hepatic metabolism of midazolam in critically ill patients. *Intensive Care Med*. 2012;38:76-84.
14. Watanabe H, Sugimoto R, Ikegami K, et al. Parathyroid hormone contributes to the down-regulation of cytochrome P450 3A through the cAMP/PI3K/PKC/PKA/NF-κB signaling pathway in secondary hyperparathyroidism. *Biochem Pharmacol*. 2017;145:192-201.
15. Hirata K, Watanabe H, Toyoda M, et al. Effect of serum parathyroid hormone on tacrolimus therapy in kidney transplant patients: A possible biomarker for a tacrolimus dosage schedule. *Biol Pharm Bull*. 2019;42:786-791.
16. Cotreau MM, von Moltke LL, Greenblatt DJ. The influence of age and sex on the clearance of cytochrome P450 3A substrates. *Clin Pharmacokinet*. 2005;44:33-60.
17. Kolwankar D, Vuppalanchi R, Ethell B, et al. Association between nonalcoholic hepatic steatosis and hepatic cytochrome P-450 3A activity. *Clin Gastroenterol Hepatol*. 2007;5:388-393.
18. Lalande L, Charpiat B, Leboucher G, Tod M. Consequences of renal failure on non-renal clearance of drugs. *Clin Pharmacokinet*. 2014;53:521-532.

19. Zhang Y, Zhang L, Abraham S, et al. Assessment of the impact of renal impairment on systemic exposure of new molecular entities: evaluation of recent new drug applications. *Clin Pharmacol Ther*. 2009;85:305-311.

20. Nolin TD, Naud J, Leblond FA, Pichette V. Emerging evidence of the impact of kidney disease on drug metabolism and transport. *Clin Pharmacol Ther*. 2008;83:898-903.

21. Tsujimoto M, Higuchi K, Shima D, et al. Inhibitory effects of uraemic toxins 3-indoxyl sulfate and p-cresol on losartan metabolism in vitro. *J Pharm Pharmacol*. 2010;62:133-138.

22. Sun H, Frassetto LA, Huang Y, Benet LZ. Hepatic clearance, but not gut availability, of erythromycin is altered in patients with end-stage renal disease. *Clin Pharmacol Ther*. 2010;87:465-472.

23. Suzuki Y, Muraya N, Fujioka T, et al. Factors involved in pheno-conversion of CYP3A using 4β-hydroxycholesterol in stable kidney transplant recipients. *Pharmacol Rep*. 2019;71:276-281.

24. Fukagawa M, Shimazaki R, Akizawa T. Head-to-head comparison of the new calcimimetic agent evocalcet with cinacalcet in Japanese hemodialysis patients with secondary hyperparathyroidism. *Kidney Int*. 2018;94:818-825.

25. Levin A, Bakris GL, Molitch M, et al. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int*. 2007;71:31-38.

26. Picard N, Rouguieg-Malki K, Kamar N, Rostaing L, Marquet P. CYP3A5 genotype does not influence everolimus in vitro metabolism and clinical pharmacokinetics in renal transplant recipients. *Transplantation*. 2011;91:65w2-656.

27. Niioka T, Kagaya H, Miura M, et al. Pharmaceutical and genetic determinants for interindividual differences of tacrolimus bio-availability in renal transplant recipients. *Eur J Clin Pharmacol*. 2013;69:1659-1665.

28. Goto M, Masuda S, Kiuchi T, et al. CYP3A5*1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. *Pharmacogenetics*. 2004;14:471-478.

29. Suzuki Y, Fujioka T, Sato F, et al. CYP3A5 polymorphism affects the increase in CYP3A activity after living kidney transplantation in patients with end stage renal disease. *Br J Clin Pharmacol*. 2015;80:1421-1428.

30. Masuda S, Goto M, Fukatsu S, et al. Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients. *Clin Pharmacol Ther*. 2006;79:90-102.

31. Goto M, Masuda S, Saito H, et al. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. *Pharmacogenetics*. 2002;12:451-457.

32. Barry A, Levine M. A systematic review of the effect of CYP3A5 genotype on the apparent oral clearance of tacrolimus in renal transplant recipients. *Ther Drug Monit*. 2010;32:708-714.

33. Gutiérrez OM, Parsa A, Isakova T, et al. Genetic African ancestry and markers of mineral metabolism in CKD. *Clin J Am Soc Nephrol*. 2016;11:653-662.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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