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.
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/S.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.8-13 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 immunosassay 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, χ2 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 inducer15 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.
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 non-expressors. The tacrolimus C/D was significantly lower in CYP3A5 expressors than that in non-expressors ($p = 0.029$).

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 non-expressors, 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 phenotype conversion by administration of CYP3A inhibitors.

**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) | 0.476a    |
| Age (year)                                  | 42.0 [37.8–59.8] | 49.0 [36.5–63.5] | 41.0 [38.0–39.0]   | 0.327b    |
| Body weight (kg)                            | 61.1 [53.8–70.0] | 57.3 [50.1–69.1] | 63.9 [56.9–69.8]   | 0.322b    |
| Dose/body weight (mg/kg)                    | 0.114 [0.094–0.143] | 0.133 [0.111–0.153] | 0.106 [0.092–0.141] | 0.161b    |
| Tacrolimus concentration (ng/ml)            | 9.1 [6.8–11.3]  | 8.7 [6.6–10.5]  | 9.6 [7.0–11.5]     | 0.415c    |
| Albumin (g/dl)                              | 4.28 [3.93–4.45] | 3.98 [3.66–4.45] | 4.28 [4.00–4.45]   | 0.296b    |
| Alanine aminotransferase (U/L)              | 9.8 [7.9–14.3]  | 9.8 [8.5–14.7]  | 10.1 [7.8–13.7]    | 0.903c    |
| 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 (×10$^3$/mm$^3$)           | 5.38 [4.52–6.22] | 4.78 [3.95–5.60] | 5.49 [4.59–6.60]   | 0.033b    |
| Red blood cell (×10$^6$/mm$^3$)             | 3.87 [3.56–4.28] | 3.63 [3.33–4.19] | 3.98 [3.63–4.38]   | 0.157c    |
| Hemoglobin (g/dl)                           | 11.6 [10.6–13.2] | 11.1 [10.4–12.7] | 11.9 [10.8–13.3]   | 0.075c    |
| Hematocrit (%)                              | 35.7 [32.5–38.7] | 34.3 [31.1–37.4] | 36.5 [33.6–39.9]   | 0.063c    |
| Platelet count (×10$^3$/μl)                 | 184.5 [150.3–212.8] | 182.0 [135.5–213.5] | 185.0 [162.0–211.0] | 0.416c    |
| Intact parathyroid hormone level (pg/ml)    | 166.5 [76.5–243.8] | 191.0 [79.0–256.0] | 161.0 [81.0–232.0] | 0.556b    |
| 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)           |           |
| Nephroclerosis                              | 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.

*Categorical variable was analyzed by Fisher’s exact test. Data normality was analyzed by Shapiro–Wilk test.

*Nonparametric data were analyzed by Mann–Whitney $U$ test.

*Parametric data were analyzed by Student’s $t$-test."
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 subgroup, 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
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 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

**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

**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 | 0.048 | 0.010 to 0.086 |
| Serum intact parathyroid hormone level | 0.101 | 0.015 | \(-21.178 \) | \(-38.11 \) to \(-4.247 \) |
| CYP3A5 expressor | 0.111 | 0.015 | |

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., 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 non-expressors (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. CYP3A5 polymorphism is a fundamentally important determinant of tacrolimus pharmacokinetics and dose requirement. Niikura et al. indicated that CYP3A5 polymorphism (*3*3) has no influence on dose-adjusted AUC 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. 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 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. 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 accumulate in renal failure and partially mediates elevation of tacrolimus C/D.

Tacrolimus C/D 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 (multi-drug resistance-1 [MDR1]). Thus, the bioavailability and C/D in patients with high expression of MDR1 in the small intestine are low. Goto et al. 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 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. Furthermore, African patients with CKD also have higher blood concentrations of iPTH, 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. 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.

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**SUPPORTING INFORMATION**

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

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