*Article*

# Donor *CYP3A5* Gene Polymorphism Alone Cannot Predict Tacrolimus Intrarenal Concentration in Renal Transplant Recipients

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**Abstract:** *CYP3A5* gene polymorphism in recipients plays an important role in tacrolimus blood pharmacokinetics after renal transplantation. Even though CYP3A5 protein is expressed in renal tubular cells, little is known about the influence on the tacrolimus intrarenal exposure and hence graft outcome. The aim of our study was to investigate how the tacrolimus intrarenal concentration ($C_{tissue}$) could be predicted based on donor *CYP3A5* gene polymorphism in renal transplant recipients. A total of 52 Japanese renal transplant patients receiving tacrolimus were enrolled in this study. Seventy-four renal biopsy specimens were obtained at 3 months and 1 year after transplantation to determine the donor *CYP3A5* polymorphism and measure the $C_{tissue}$ by liquid chromatography-tandem mass spectrometry (LC-MS-MS). The tacrolimus $C_{tissue}$ ranged from 52 to 399 pg/mg tissue ($n = 74$) and was weak but significantly correlated with tacrolimus trough concentration ($C_0$) at 3 months after transplantation (Spearman, $r = 0.3560$, $p = 0.0096$). No significant relationship was observed between the donor *CYP3A5* gene polymorphism and $C_{tissue}$ or $C_{tissue}/C_0$. These data showed that the tacrolimus systemic level has an impact on tacrolimus renal accumulation after renal transplantation. However, donor *CYP3A5* gene polymorphism alone cannot be used to predict tacrolimus intrarenal exposure. This study may be valuable for exploring tacrolimus renal metabolism and toxicology mechanism in renal transplant recipients.

**Keywords:** tacrolimus; renal transplantation; intrarenal concentration; whole blood concentration; LC-MS/MS

## 1. Introduction

Tacrolimus is a commonly used calcineurin inhibitor (CNI) for immunosuppressive therapy to prevent allograft rejection after renal transplantation. With a narrow therapeutic index and high inter-patient pharmacokinetic variability, monitoring the tacrolimus trough concentration ($C_0$) in whole blood has been recommended in post-transplantation clinical routine to optimize the therapy outcome and minimize adverse effects [1,2]. However, within target therapeutic range, the risk of adverse events such as acute rejection (AR) could not be completely avoided [3]. Tacrolimus is predominantly metabolized by hepatic and intestinal cytochrome P450 (CYP) enzymes. Three desmethyl metabolites, namely, 13-O-desmethyl tacrolimus (M1), 31-O-desmethyl tacrolimus (M2), and 15-O-desmethyl tacrolimus (M3), have been identified as the major tacrolimus metabolites [4] (Figure 1) and might contribute to the tacrolimus-induced toxic effects [5–7].
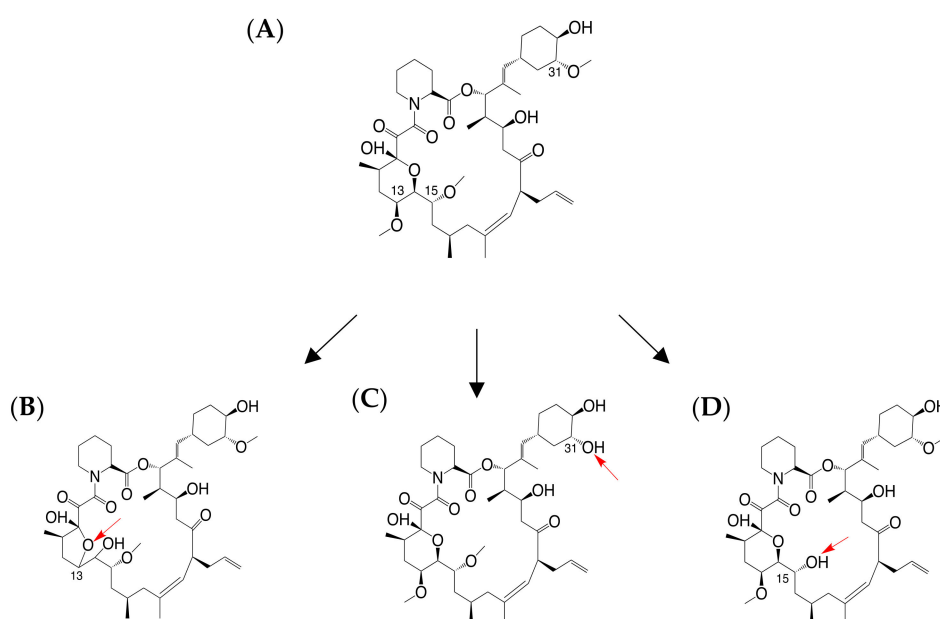


**Figure 1.** Chemical structure of (**A**) tacrolimus; (**B**) 13-O-desmethyl tacrolimus (M1); (**C**) 31-O-desmethyl tacrolimus (M2); (**D**) 15-O-desmethyl tacrolimus (M3). Each site of metabolism is indicated by a red arrow in the corresponding metabolite structures, respectively.

The loss-of-function *CYP3A5* 6986A>G (*CYP3A5*\*3) polymorphism significantly affects the metabolism and pharmacokinetics of tacrolimus [8,9]. Patients with *CYP3A5*\*1 allele require a higher tacrolimus dose to reach target $C_0$ levels than those with *CYP3A5*\*3/\*3 genotype [10–12]. Even though tacrolimus is primarily metabolized by CYP3A5 protein in the liver and small intestine, it has been reported that the CYP3A5 protein is also expressed in renal tubular epithelial cells [13]. The human kidney microsomes with *CYP3A5*\*1 allele was associated with a higher metabolic activity compared to those with *CYP3A5*\*3/\*3 genotype [14]. Therefore, some studies have hypothesized that tacrolimus intrarenal concentration ($C_{tissue}$) could be affected by the graft *CYP3A5* gene polymorphism (donor genotype) and suggested that the tacrolimus intrarenal levels could be a better predictor of histological AR than blood concentrations [10,15]. However, so far, there have been only two studies that have preliminarily measured the tacrolimus concentration in human kidney tissues [16,17], and little clinical information is available about association between the donor *CYP3A5* gene polymorphism and tacrolimus renal metabolism. Therefore, in this study, we investigated the potential factors (tacrolimus blood levels and donor *CYP3A5* gene polymorphism) that determine tacrolimus intrarenal exposure and the relationship between tacrolimus intrarenal level and biopsy-proven subclinical AR (subAR) in renal transplant recipients. We focused on evaluating the capability of utilizing donor *CYP3A5* gene polymorphism to predict tacrolimus $C_{tissue}$ in renal transplant recipients.

## 2. Results

### 2.1. Patient Characteristics and CYP3A5 Polymorphism

All patients included in this study underwent at least one protocol renal biopsy ($n = 52$). A total of 74 renal biopsies were obtained: 52 biopsies and 22 biopsies at 3 months and 1 year after transplantation, respectively. The demographic data of recipients admitted in this study are shown in Table 1. The mean recipient age was observed to be 43.9 ± 13.3 years and their mean body weight was 58.15 ± 14.48 kg. The frequencies for *CYP3A5* genotypes in both donors and recipients are summarized in Table 1. Among the 52 renal transplant recipients and their corresponding donors, 23 (44.2%) recipients and 25 (48.1%) donors exhibited *1/*1 or *1/*3 genotype, while 29 (55.8%) recipients and 27 (51.9%) donors carried *3/*3 genotype. The allele frequencies for *CYP3A5*3 in donors and recipients were 71.2% and 74.0%, respectively. The results were consistent with minor allele frequency of *CYP3A5*3 in Asian population as reported in [18].

**Table 1.** Characteristics of patients.

| Characteristics | $n = 52$ |
|---|---|
| Recipient age (years) | 43.9 ± 13.3 |
| Recipient sex (male/female) | 31/21 |
| Body weight (kg) | 58.15 ± 14.48 |
| Reasons for renal transplantation (n) | |
| IgA nephropathy | 8 |
| Diabetic gastropathy | 8 |
| Chronic glomerulonephritis | 10 |
| Polycystic kidney | 3 |
| Type 1 diabetes | 2 |
| Type 2 diabetes | 3 |
| Hypertensive nephrosclerosis | 3 |
| Others | 15 |
| Serum creatinine (mg/dL) | |
| Pre-transplant | 7.85 ± 3.38 |
| 3-month | 1.14 ± 0.28 |
| Donor *CYP3A5* genotype | |
| *1/*1 or *1/*3 | 25 (48.1%) |
| *3/*3 | 27 (51.9%) |
| Recipient *CYP3A5* genotype | |
| *1/*1 or *1/*3 | 23 (44.2%) |
| *3/*3 | 29 (55.8%) |

Data are expressed as mean ± standard deviation, number (%).

### 2.2. Influence of CYP3A5 Polymorphism on Tacrolimus Pharmacokinetics

We evaluated the influence of the donor and recipient *CYP3A5* polymorphism on tacrolimus pharmacokinetics by assessing $C_0$ and dose-adjusted $C_0$ ($C_0/D$) of tacrolimus. No significant relationship was observed between the donor *CYP3A5* polymorphism and tacrolimus pharmacokinetics. On the other hand, *CYP3A5*3 allele in recipients had a significant impact on $C_0/D$ as reported by previous studies [19–21]. Recipients with *CYP3A5*3/*3 had a significantly higher $C_0/D$ than those with *CYP3A5*3 allele at both 3 months and 1 year after renal transplantation ($p < 0.0001$ and $p = 0.0167$, respectively) (Table 2).

### 2.3. Association between Intrarenal and Whole Blood Tacrolimus Levels

Since a previous study has reported that in renal transplant recipients, changes in tacrolimus intrarenal concentrations reflect the blood concentration of tacrolimus [17], we investigated the association between the tacrolimus $C_0$ and $C_{tissue}$ at 3 months and 1 year after renal transplantation,

respectively. A correlation ($r = 0.3560$, $p = 0.0096$) between the tacrolimus $C_{tissue}$ and $C_0$ was observed only at 3 months after transplantation (Figure 2). This result implies that the tacrolimus blood levels may affect tacrolimus intrarenal accumulation.

**Table 2.** Tacrolimus pharmacokinetics (PK) parameter according to *CYP3A5* genotype.

| PK-Parameter | CYP3A5 Genotype | 3 Months after Renal Transplantation ($n = 52$) | | | 1 Year after Renal Transplantation ($n = 22$) | | |
|---|---|---|---|---|---|---|---|
| | | *n* | Mean ± SD | *p* | *n* | Mean ± SD | *p* |
| $C_0$ (ng/mL) | Recipient *CYP3A5*\*1 | 23 | 5.30 ± 1.32 | 0.5368 | 8 | 5.01 ± 1.45 | 0.7002 |
| | Recipient *CYP3A5*\*3/\*3 | 29 | 5.08 ± 1.35 | | 14 | 5.37 ± 1.00 | |
| $C_0/D$ (ng/mL/mg) | Recipient *CYP3A5*\*1 | 23 | 0.95 ± 0.37 | <0.0001 | 8 | 0.97 ± 0.37 | 0.0167 |
| | Recipient *CYP3A5*\*3/\*3 | 29 | 1.56 ± 0.66 | | 14 | 1.58 ± 0.68 | |
| $C_0$ (ng/mL) | Donor *CYP3A5*\*1 | 25 | 5.03 ± 1.11 | 0.5760 | 10 | 5.32 ± 1.24 | 0.7339 |
| | Donor *CYP3A5*\*3/\*3 | 27 | 5.31 ± 1.51 | | 12 | 5.18 ± 1.14 | |
| $C_0/D$ (ng/mL/mg) | Donor *CYP3A5*\*1 | 25 | 1.31 ± 0.69 | 0.9964 | 10 | 1.43 ± 0.60 | 0.4078 |
| | Donor *CYP3A5*\*3/\*3 | 27 | 1.27 ± 0.57 | | 12 | 1.30 ± 0.71 | |
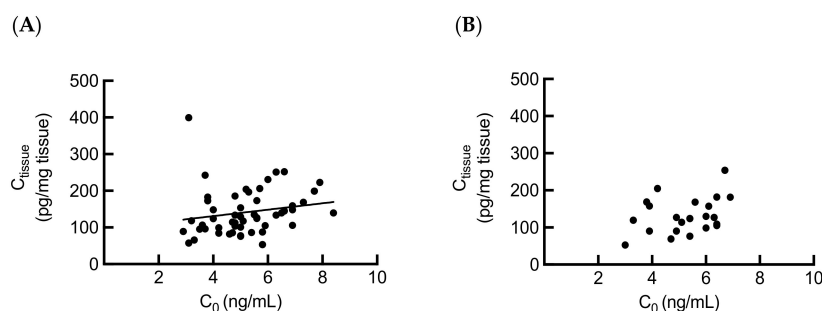


**Figure 2.** Correlation between the tacrolimus $C_0$ and $C_{tissue}$ at (**A**) 3 months ($n = 52$, $r = 0.3560$, $p = 0.0096$) and (**B**) 1 year ($n = 22$, $r = 0.3368$, $p = 0.1253$) after transplantation. Statistical analyses were performed using Spearman's correlation.

### 2.4. Influence of Donor CYP3A5 Gene Polymorphism on Tacrolimus Metabolism in Kidney

*CYP3A5* polymorphism in kidney has been reported to influence local metabolism of tacrolimus in vitro [14]. Therefore, to verify the role of *CYP3A5* polymorphism in tacrolimus renal metabolism in vivo, the association between the donor *CYP3A5* polymorphism and tacrolimus $C_{tissue}$ or $C_{tissue}/C_0$ was estimated. No significant relationship was observed between tacrolimus $C_{tissue}$ or $C_{tissue}/C_0$ and donor *CYP3A5* polymorphism at both 3 months and 1 year after transplantation (Figure 3). To further investigate the metabolism of tacrolimus in kidney, we measured the intrarenal concentrations of three major tacrolimus metabolites (M1, M2, and M3) in 74 biopsy samples, and of these 74 samples, 66 (89.2%), 15 (20.3%), and 3 (4.1%) samples had M1, M2, and M3 concentrations above the lower limit of quantification (LLOQ, 0.01 ng/mL), respectively. We found that the mean intrarenal concentrations of M1, M2, and M3 were 29.1%, 8.43%, and 5.18% of the tacrolimus intrarenal concentrations, respectively (data not shown). We also observed that M1 intrarenal concentration ($C_{M1}$) was significantly associated with the tacrolimus $C_{tissue}$ in patients both at 3 months and 1 year after transplantation (Figure 4).
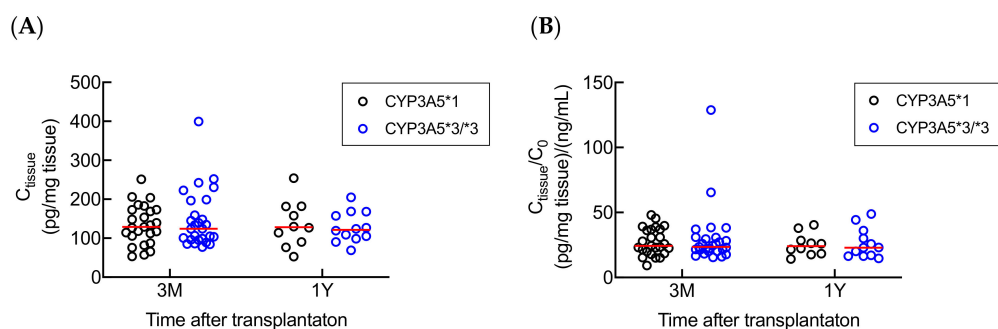
**Figure 3.** Effects of donor-*CYP3A5* genotype on tacrolimus (**A**) $C_{tissue}$ (3 months: $p = 0.8845$; 1 year: $p = 0.6873$) and (**B**) $C_{tissue}/C_0$ (3 months: $p = 0.7575$; 1 year: $p = 0.9229$) at 3 months ($n = 52$) and 1 year ($n = 22$) after transplantation. Statistical analyses were performed using Mann–Whitney U test. Bar shows the median value in each group. (Abbreviations: 3M—3 months; 1Y—1 year).
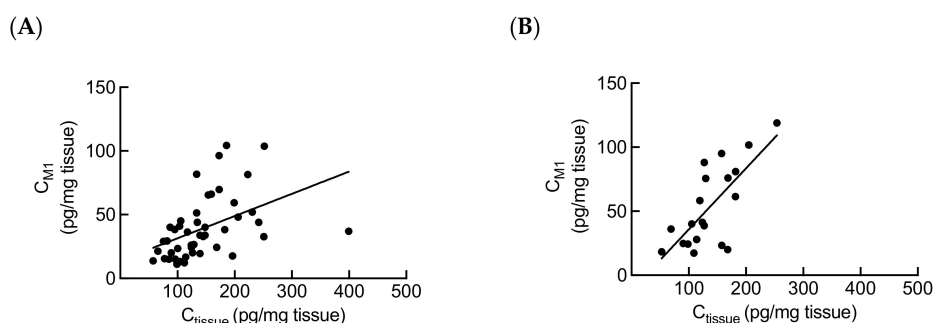


**Figure 4.** Correlation between the intrarenal concentrations of tacrolimus and M1 at (**A**) 3 months ($n = 52$, $r = 0.6008$, $p < 0.0001$) and (**B**) 1 year ($n = 22$, $r = 0.6632$, $p = 0.0014$) after transplantation. Statistical analyses were performed using Spearman's correlation.

*2.5. Associations between Subclinical Acute Rejection (subAR) and Tacrolimus $C_{tissue}$ or $C_{tissue}/C_0$.*

A total of 7 (13.5%) and 4 (18.2%) patients were diagnosed with biopsy-proven subclinical acute rejection (subAR) at 3 months and 1 year after renal transplantation, respectively. By comparing the tacrolimus $C_{tissue}$ and $C_{tissue}/C_0$ between no-subAR and subAR group of patients, no significant difference was found either at 3 months or 1 year after renal transplantation (Figure 5).
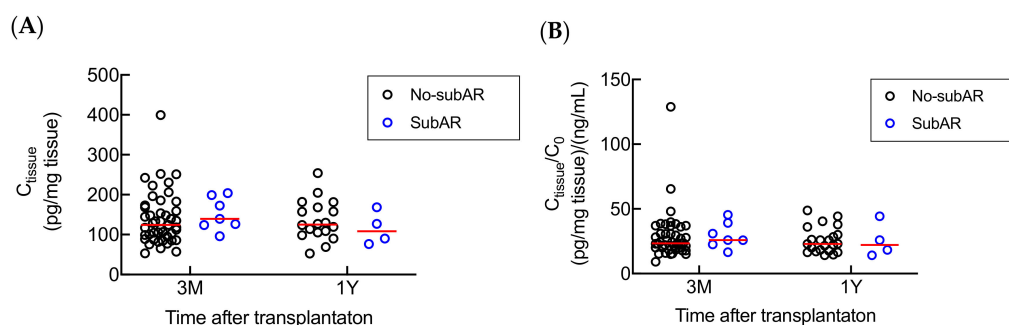


**Figure 5.** Differences in tacrolimus (**A**) $C_{tissue}$ (3 months: $p = 0.3287$; 1 year: $p = 0.5468$) and (**B**) $C_{tissue}/C_0$ (3 months: $p = 0.3699$, 1 year: $p = 0.7743$) among the no subclinical acute rejection (no-subAR) group and subAR group at 3 months ($n = 52$) and 1 year ($n = 22$) after transplantation respectively. Statistical analyses were performed using Mann–Whitney U test. Bar shows the median value in each group. (Abbreviations: 3M—3 months; 1Y—1 year).

## 3. Discussion

The pharmacogenetics studies of tacrolimus have focused on the impact of recipient *CYP3A5* genotype on the tacrolimus pharmacokinetics; however, the role of donor *CYP3A5* polymorphism in tacrolimus systemic or local metabolism is little to know. In this study, we investigated the influence of donor *CYP3A5* polymorphism on tacrolimus pharmacokinetics at 3 months and 1 year after renal transplantation. There was no effect of donor *CYP3A5* polymorphism on the tacrolimus $C_0$ or $C_0/D$, as predicted. As the CYP3A5 metabolic capability in the kidney is not comparable to that in liver and intestine of recipients, the donor *CYP3A5* polymorphism is unlikely to contribute significantly to the systemic levels of tacrolimus [14]. Next, we investigated the relationship between the tacrolimus $C_0$ and $C_{tissue}$. Interestingly, we found a significant correlation between the tacrolimus $C_0$ and $C_{tissue}$ at 3 months after transplantation. This result was contrary to that in the study by Noll et al. [17], and this could be due to the differences in post-transplantation stage and sample size.

Previous reports have shown that the intrarenal concentration of tacrolimus or its metabolites could be affected by *CYP3A5* genotype, and further predict the clinical outcome and side effects in immunosuppressive therapy [14,15,22]. On the other hand, it has been reported that renal *CYP3A5* polymorphism may influence the development of tacrolimus-related nephrotoxicity in liver transplant patients [23]. In the present study, the donor (graft kidney) *CYP3A5* polymorphism was examined as to whether it was effective on tacrolimus local metabolism in renal transplant recipients. However, we found no significant relationship between the donor *CYP3A5*\*3 polymorphism and $C_{tissue}$ or $C_{tissue}/C_0$ after transplantation. There are several possible reasons for this result. Firstly, the expression levels and protein distribution of CYP3A5 could be variable in different areas of the kidney. Despite the fact that *CYP3A5*\*1 allele was shown to be associated with a higher CYP3A5 expression compared to *CYP3A5*\*3/\*3, the expression difference reported was limited to the proximal tubule [24]. In the present study, we were unable to ensure that all biopsies were sampled from the same position of kidney, and the data of CYP3A5 expression levels and tacrolimus intrarenal distribution were not available. Secondly, we could not exclude the possibility that ischemia reperfusion injury occurred after renal transplantation, which might have had an impact on the expression levels of CYP3A5 in the allograft organ [25,26]. Moreover, it is likely that the effect of donor *CYP3A5* polymorphism on local renal metabolism could be counterbalanced by the influence of tacrolimus systemic concentration. This result is consistent with the findings of Kuypers et al. [5]. They demonstrated that the recipient *CYP3A5*\*1 variant is associated with tacrolimus-related nephrotoxicity and suggested that this is possibly due to higher concentrations of toxic metabolites in kidney tissue. Consequently, recipient (liver and intestine) *CYP3A5* polymorphism plays an important role in the renal accumulation of tacrolimus compared to donor (graft kidney) *CYP3A5* polymorphism.

In addition to CYP3A5, other genetic and clinical factors could be potential biomarkers to predict the tacrolimus $C_{tissue}$. Especially, the drug efflux transporter *ABCB1* is also closely related to the metabolism of tacrolimus. The activity and expression levels of *ABCB1* could contribute to the variability in tacrolimus absorption and excretion [23,27,28]. Even though *ABCB1* polymorphisms' impact on tacrolimus pharmacokinetics is still unclear [10,29–31], the *ABCB1* polymorphism has been identified as a critical factor in intracellular tacrolimus exposure [32,33]. It is widely accepted that *ABCB1* polymorphism and expression levels are more likely be associated with tacrolimus tissue distribution and drug effect or toxicity in the allograft [18,34–36]. Moreover, Knops et al. demonstrated that the *ABCB1* 3435T variant in human proximal tubule cells has a significant impact on tacrolimus metabolism in vitro [37]. Moreover, *ABCB1*, but not *CYP3A5*, polymorphisms in the liver have been reported to significantly influence tacrolimus hepatic concentrations in liver transplant recipients [38]. Therefore, it is reasonable to expect that *ABCB1* polymorphisms might contribute to the regulation of tacrolimus intrarenal metabolism.

In this study, we also measured simultaneously the concentrations of three major tacrolimus metabolites M1, M2, and M3 (Figure 1) in kidney tissues. We found M1 as the most abundant metabolite of tacrolimus in kidney, as is the case with human liver microsomes and blood reported by previous

studies [39–42]. As M1 retains around 10% of tacrolimus immunosuppressant activity [4], the clinical value and potential toxic property of M1 intrarenal exposure need to be further studied. Although we could not detect the three metabolites in all biopsy samples in this study, these results may be useful for explaining the mechanism of tacrolimus-induced nephrotoxicity.

There is an ongoing debate on whether tacrolimus $C_0$ could accurately predict or reflect acute rejection. Alternate matrices such as intracellular or allograft tissue, are recommended to improve tacrolimus treatment monitoring in recent years [43]. In fact, we indeed observed several patients who showed relatively low tacrolimus $C_{tissue}$ despite their tacrolimus $C_0$ in the normal therapeutic range. Nevertheless, we found no significant association between the tacrolimus $C_{tissue}$ or $C_{tissue}/C_0$ and the incidence of biopsy-proven subAR in our study. However, since the sample size was relatively small, whether the tacrolimus intrarenal levels could better reflect the incidence of AR or subAR than $C_0$ requires further investigation. Moreover, new biomarkers predictive of tacrolimus intrarenal level would be explored in future mechanistic and clinical monitoring studies to identify patients who are at high risk of adverse events but with a target tacrolimus therapeutic concentration in blood.

## 4. Materials and Methods

### 4.1. Subjects

A total of 59 Japanese adult renal transplant patients were enrolled in this study. All patients underwent renal transplantation between August 2014 and August 2016 at Kyushu University Hospital in Japan. The study excluded 7 patients who received everolimus in immunosuppressive therapy after transplantation. Ultimately, our study comprised of 52 renal transplant recipients. All 52 recipients received a triple-drug regimen comprising of tacrolimus, mycophenolate mofetil, and prednisolone. This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Institutional Review Board of Kyushu University Graduate School and Faculty of Medicine (approval number: 588-00, 28 July 2014). All patients enrolled in this study gave written informed consent for participation in the study and for the use of their sample.

### 4.2. Diagnostic Criteria for Subclinical Acute Rejection (SubAR)

Protocol biopsies were performed 3 months and 1 year after kidney transplantation. Each biopsy was graded according to Banff 2009 classification and diagnosed for subclinical acute rejection (SubAR). SubAR was identified by the presence of tubulointerstitial mononuclear infiltration with a requirement of <10% rise in serum creatinine in 2 weeks before the protocol biopsy and no absence of clinical functional deterioration [44].

### 4.3. Measurement of Tacrolimus Trough Concentration

To determine tacrolimus $C_0$, whole venous blood was obtained from patients before they received the morning dose of tacrolimus. Tacrolimus $C_0$ was measured using a Chemiluminescent Immunoassay (Architect; Abbott Park, Illinois, USA).

### 4.4. Measurement of Tacrolimus Renal Concentration

A fraction of kidney biopsies (1–3 mg of wet tissue) was used for intrarenal tacrolimus quantification, which was performed on a Shimadzu LCMS-8050 liquid triple quadrupole tandem mass spectrometer (Shimadzu, Kyoto, Japan). Samples were prepared using a procedure described by Krogstad et al. with slight modification [16]. The frozen renal biopsy was dried on filter paper for 90 min at room temperature. Once dry, the biopsy was weighed and homogenized in 100 µL ultrapure water by syringe and needle. Aliquot 50 µL of tissue homogenate was transferred to a 1.5 mL microcentrifuge tube, and 20 µL methanol was added then vortexed for 30 s. Then, aliquot 80 µL of protein precipitation solution (1 ng/mL ascomycin as internal standard in 70/30 methanol/zinc sulphate 0.1 mol/L) was added to the tube, then vortexed at 1500 rpm for 15 min. After centrifugation at 9400× *g*

for 10 min, the supernatant was transferred to vials and injected to a LC-MS system. Quantitation was performed with a GL Sciences Inertsil-ODS-3 (3 μm; 2.1 mm × 150 mm) column. Mobile phase A consisted of 2 mmol/L ammonium acetate with 0.1% formic acid (*v/v*) in water, and mobile phase B consisted of 2 mmol/L ammonium acetate with 0.1% formic acid (*v/v*) in methanol. The gradient was started at 60% B, increased to 85% B at 3 min, increased to 95% B at 6 min, increased to 100% B at 6.5 min, switched back to the starting conditions at 60% B from 6.5 min to 6.6 min, and re-equilibration for 1.4 min. The total analysis time was 8 min. The flow rate was 0.25 mL/min, and the column temperature was maintained at 60 °C. Electrospray ionization was performed in a positive mode. Analysis was based on multiple reaction monitoring (MRM) of m/z 821.40→768.35 for tacrolimus, 807.20→754.25 for M1/M2/M3, and m/z 809.3→756.3 for internal standard. The calibration curve ranges were 0.02–2 ng/mL and 0.01–1 ng/mL for tacrolimus and tacrolimus metabolites, respectively.

## 4.5. Statistical Analysis

Statistical analysis was performed using Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA). Mann–Whitney U test was used to compare the differences between tacrolimus concentrations in *CYP3A5* genotype groups and in patients with/without subAR. Correlations between tacrolimus $C_{tissue}$ and $C_0$ or M1 intrarenal concentration were analyzed by Spearman's correlation. A *p*-value < 0.05 was considered to be statistically significant.

## 5. Conclusions

This study, for the first time, demonstrated a correlation between tacrolimus $C_{tissue}$ and $C_0$ and that donor *CYP3A5* gene polymorphism alone was not sufficient to predict the renal concentration of tacrolimus at 3 months and 1 year after renal transplantation. Future studies will need to investigate the intrarenal concentration of tacrolimus or its metabolites and the adverse events in a large clinical study.

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## Abbreviations

| | |
|---|---|
| $C_0$ | Tacrolimus trough concentration |
| $C_{tissue}$ | Tacrolimus intrarenal concentration |
| CNI | Calcineurin inhibitor |
| AR | Acute rejection |
| SubAR | Subclinical acute rejection |
| $C_0/D$ | Dose-adjusted $C_0$ |
| $C_{M1}$ | Intrarenal concentration of 13-O-desmethyl tacrolimus |

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