# -NOTE-

## Blood concentrations of tacrolimus upon conversion from rabeprazole to vonoprazan in renal transplant recipients: correlation with cytochrome P450 gene polymorphisms

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**Abstract** (192/200 words)

We evaluated the impact of vonoprazan on blood concentrations of tacrolimus via a retrospective analysis of 52 renal transplant recipients who took tacrolimus and converted from rabeprazole to vonoprazan between August 2018 and September 2019. We compared tacrolimus trough levels upon conversion among groups that were classified based on cytochrome P450 (CYP) gene polymorphisms. CYP3A5 groups were heterozygous or homozygous for *CYP3A5*1* and *CYP3A5*3* alleles. *CYP2C19* genotypes were classified as extensive (**1/*1)*, intermediate (**1/*2* and **1/*3*) or poor metabolizers (**2/*2*, **2/*3* and **3/*3*). Tacrolimus trough levels increased only 0.3 ng/mL upon conversion in the *CYP3A5*3/*3* group: 5.8 [3.4-7.2] vs 6.1 [3.8-7.9]; $p = 0.06$. No statistically significance changes in tacrolimus levels also occurred in the *CYP3A5*1/*1* or *CYP3A5*1/*3* groups. Subgroup analyses of *CYP3A5*3/*3* demonstrated low changes for all three *CYP2C19* subgroups: 5.2 [4.3-6.5] vs 6.2 [4.3-7.9]; $p = 0.07$, 6.1 [3.4-7.2] vs 6.7 [4.6-7.9]; $p = 0.12$ and 5.4 [3.6-6.5] vs 4.7 [3.8-6.3]; $p = 1.00$, respectively. Conversion to vonoprazan thus resulted in little increase of tacrolimus trough levels, even in the group predicted to be most susceptible (*CYP3A5*3/*3* and *2C19*1/*1*), thus supporting the safety of concomitant use of vonoprazan with tacrolimus.

1 **Keywords**

2 Cytochrome P450; tacrolimus; renal transplantation; CYP2C19; vonoprazan; rabeprazole

## Abbreviations

AUC, area under the concentration-time curve

CYP, cytochrome P450

PPI, proton pump inhibitor

## Introduction

It is essential to control the concentration of tacrolimus in the blood of organ transplant recipients in order to achieve long-term graft survival [1]. It is often difficult to reach or maintain the target tacrolimus blood concentration because the bioavailability of tacrolimus varies dramatically among individuals [2]. In addition, concomitant medications can have unexpected effects on tacrolimus concentrations if they impinge on the tacrolimus metabolic system. Proton pump inhibitors (PPIs), frequently administered to prevent upper gastrointestinal complications in renal transplant recipients, are one class of medications that potentially affect the blood concentration of tacrolimus [3].

Tacrolimus is predominantly metabolized by CYP3A4 and CYP3A5. Accordingly, concomitant use of medications that affect the CYP system can result in changes in blood concentrations of tacrolimus [4]. The previous study demonstrated that the *CYP2C19* gene polymorphism was associated with the impact on the blood concentration of tacrolimus upon concomitant use with a CYP3A4 inhibitor [5]. Thus, the extent of the impact of concomitant medications on tacrolimus trough levels depends on polymorphisms in the not only *CYP3A5* but also *CYP2C19* genes [6] [7]. Dependence on both CYP3A5 and CYP2C19 occurs because the extent of competition for the CYP3A4

enzyme, which is shared by tacrolimus with several drugs with which it is often co-administered, is strongly influenced by *CYP3A5* and *CYP2C19* gene polymorphisms.

Rabeprazole has been widely used as a PPI in transplant recipients, based on a small retrospective study that suggested that it was unlikely to affect blood concentrations of tacrolimus regardless of the *CYP2C19* gene polymorphism [8]. However, vonoprazan, a novel acid suppressing agent, is increasingly being used in renal transplant recipients as an alternative to PPIs. Vonoprazan has an advantage over PPIs in that it is stable in an acidic environment and does not require acid activation, allowing it to achieve more rapid and longer-lasting inhibition of gastric acid secretion [9].

The impact of concomitant vonoprazan treatment on tacrolimus metabolism is incompletely understood. In particular, the relevance of gene polymorphisms on blood concentrations of tacrolimus in patients taking concomitant vonoprazan or switching to vonoprazan from any conventional PPIs is unknown. Vonoprazan is known to inhibit CYP3A4 in a time-dependent manner, and thus this valuable drug could impact tacrolimus concentrations. It is therefore important to evaluate the effect of concomitant vonoprazan on tacrolimus pharmacokinetics, in order to determine whether kidney transplant recipients can take advantage of the novel benefits provided by vonoprazan.

In this study, we investigated the safety of converting from rabeprazole to vonoprazan by renal transplant recipients taking tacrolimus. The tacrolimus trough levels before and after conversion were evaluated for groups that were classified based on *CYP3A5* and *CYP2C19* gene polymorphisms.

## Material and Methods

### *Patients*

The study initially enrolled 70 Asian renal transplant recipients who received once-daily prolonged-release tacrolimus (Graceptor®, Astellas Pharm Inc., Japan) and who converted from rabeprazole to vonoprazan between August 2018 and September 2019. The following exclusion criteria were used: (i) patients who had changed their dose of tacrolimus or concomitant medications that are likely to affect the CYP3A or CYP2C19 systems (eg, $Ca^{2+}$ channel blockers) at the time of conversion; (ii) patients for whom tacrolimus trough levels were not measured or were measured under inappropriate circumstances (eg, the patient by chance was taking tacrolimus before the measurement period); (iii) patients whose liver or renal function was not stable; and (iv) patients under 18 years of age. This study was approved by the ethics board of our institution (research ID; 2001-024). The potential participants were given the opportunity to opt-out. The study procedures were carried out in accordance with the Declaration of Helsinki.

### *Measurements*

The age, sex, dose of tacrolimus (mg/day) and duration between transplantation and the date on conversion (month) were obtained for each patient retrospectively. Body weight (kg), tacrolimus trough level (ng/mL) and serum creatinine (mg/dL) at the first day of

conversion and at the next outpatient visit were obtained for comparison. Tacrolimus trough levels were monitored via an affinity column-mediated immunoassay (Dimension EXL 200; SIEMENS Healthcare, Tokyo, Japan).

***Regimen and protocol of tacrolimus***

The tacrolimus dose was determined by calculating area under the concentration-time curve ($AUC_{0-24}$) at the point of discharge after surgery. The $AUC_{0-24}$ target was 100-250 ng•h/mL. Thereafter, the dose of tacrolimus was adjusted up or down by 0.5-1.0 mg/body to reach a trough value of 3.0-5.0 ng/mL measured at the outpatient visit. One year later, the $AUC_{0-24}$ was measured again. All patients took tacrolimus at 10:00 a.m. followed by vonoprazan or rabeprazole at 9:00 a.m.

***Genotyping***

Genomic DNA was isolated from cryopreserved serum or buffy coat collected at the time of renal transplantation with a NucleoSpin® Blood kit (Takara Bio Inc., Shiga, Japan). The following alleles were evaluated: *CYP3A5*1*, *CYP3A5*3*, *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3*. *CYP3A5* and *CYP2C19* genotyping were determined by a polymerase chain reaction and restriction fragment length polymorphism method using SspI, SmaI and BamHI as previously described [10] [11] [12]. All patients were classified into three genotype categories for both loci according to the genetic polymorphism results. The

classifications for CYP3A5 included *CYP3A5*1/*1*, *CYP3A5*1/*3* and *CYP3A5*3/*3*. *CYP2C19* genetic polymorphism classifications included extensive metabolizers (*CYP2C19*1/*1*), intermediate metabolizers (*CYP2C19*1/*2* and **1/*3*) and poor metabolizers (*CYP2C19*2/*2*, **2/*3* and **3/*3*).

***Statistical analyses***

Data are presented as median [range]. Mann-Whitney *U* test and Fisher's exact test were used for comparison of patient's characteristics. The Wilcoxon signed-rank test was used to evaluate changes in tacrolimus trough levels before and after conversion for each genotype group. All tests were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13]. A $p$-value $< 0.05$ was considered to indicate statistical significance.

## Results

Our study ultimately included 52 patients. Patient background characteristics and the results of genotyping are shown in **Table 1**. The allele frequencies of *CYP3A5*3*, *CYP2C19*2* and *CYP2C19*3* were 70.2%, 38.5% and 10.6%, respectively. None of the patients had notable changes in weight or serum creatinine between the day of the conversion and the day of the next outpatient visit (data not shown). Patients with *CYP3A5*3/*3* took smaller doses of tacrolimus compared to patients with *CYP3A5*1/*3 + *1/*1*. There were no complaints of adverse effects possibly triggered by vonoprazan during the period from the date of conversion to the next visit, and no patients discontinued vonoprazan or tacrolimus treatment.

The median tacrolimus trough level among all recipients slightly increased following conversion from rabeprazole to vonoprazan; 5.8 [3.3-8.0] vs 6.0 [3.2-7.9]; $p = 0.09$, but the increase was not statistically significant. We evaluated tacrolimus trough levels within groups as classified according to *CYP3A5* gene polymorphism (**Fig. 1A**). A Wilcoxon signed-rank test on this set of data indicated a statistically insignificant increase in tacrolimus trough levels for the *CYP3A5*3/*3* group before and after conversion to vonoprazan; 5.8 [3.4-7.2] vs 6.1 [3.8-7.9]; $p = 0.06$. No statistically significant differences between tacrolimus trough levels upon conversion were also noted for *CYP3A5*1/*1* (*p*

= 0.38) and *CYP3A5*1/*3* ($p$ = 0.73) groups. The percent change rate of tacrolimus blood concentrations by the change from rabeprazole to vonoprazan between *CYP*1/*1 + *1/*3* group and *CYP*3/*3* genotype was 101% [69-146] vs 105% [74-164]; $p$ = 0.35.

We also evaluated a potential association between changes in tacrolimus trough levels and *CYP2C19* gene polymorphism among both the 27 *CYP3A5*1/*1 + *1/*3* and 25 *CYP3A5*3/*3* patients. The median [range] and individual tacrolimus trough levels before and after conversion in each *CYP2C19* gene group are shown in **Figs 1B and 1C**, respectively. The changes in tacrolimus trough levels for CYP2C19 extensive, intermediate and poor metabolizer groups were not statistically significantly different: 6.8 [4.3-8.0] vs 6.1 [4.1-7.4]; $p$ = 0.18, 5.6 [3.3-6.7] vs 5.7 [3.2-7.6]; $p$ = 0.23 and 5.2 [4.1-5.9] vs 5.2 [4.4-6.3]; $p$ = 0.67 among *CYP3A5*1/*1 + *1/*3* group, 5.2 [4.3-6.5] vs 6.2 [4.3-7.9]; $p$ = 0.07, 6.1 [3.4-7.2] vs 6.7 [4.6-7.9]; $p$ = 0.12 and 5.4 [3.6-6.5] vs 4.7 [3.8-6.3]; $p$ = 1.00 among *CYP3A5*3/*3* group, respectively.

## Discussion

We evaluated the impact of conversion from rabeprazole to vonoprazan on blood concentrations of tacrolimus in renal transplant recipients. Following conversion, tacrolimus trough levels did not undergo a statistically significant increase regardless of *CYP3A5* gene polymorphism. Patients in *CYP3A5*1/*1+*1/*3* group and *CYP3A5*3/*3* group were further classified according to their *CYP2C19* gene polymorphisms. There was not statistically significant in all *CYP2C19* subgroup analyses.

A recently published retrospective cohort study in a single center (n = 52) showed that blood concentrations of tacrolimus increased with a statistical significance following conversion from rabeprazole to vonoprazan, but the change was ignorable [14]. They used tacrolimus trough concentration/tacrolimus dose (ng/mL)/(mg/day). Mean ± standard deviation upon conversion was 1.98 ± 1.02 vs 2.19 ± 1.15 ($p < 0.001$) and the change was only 0.21. Our results clearly demonstrated that conversion from rabeprazole to vonoprazan did not increase tacrolimus trough levels with statistical significance. Moreover, our study newly revealed an understanding to previous findings by analyzing subgroups of patients classified based on *CYP* genetic polymorphisms.

*CYP3A5* gene polymorphisms is correlated with blood concentrations of tacrolimus [6]. In patients with the *CYP3A5*3/*3* genotype, who is poor metabolizer of CYP3A5, the

CYP3A4 enzyme would be the major route for the metabolism of tacrolimus. In our study, the median tacrolimus dose in the *CYP3A5*3/*3* group was significantly smaller than *CYP3A5*1/*3* + **1/*1*, suggesting a lower metabolic capacity. Accordingly, patients with *CYP3A5*3/*3* are likely to more susceptible to antagonism of CYP3A4 metabolism by vonoprazan, which is mainly metabolized by this enzyme [15]. However, the change of tacrolimus trough upon conversion was not statistical significance in *CYP3A5*3/*3* group.

Rabeprazole is slightly metabolized by CYP2C19, whereas vonoprazan is hardly metabolized by CYP2C19 [14]. Patients in the CYP2C19 extensive and intermediate metabolizer subgroups are predicted to partially metabolize rabeprazole by CYP2C19 and thus should not have experienced significant antagonism on the CYP3A4 enzyme prior to conversion to vonoprazan. Thus, these patients were likely to experience relatively large increases in tacrolimus concentrations when they switched to vonoprazan, as a result of new CYP3A4 antagonism. In particular, CYP2C19 extensive metabolizer subgroup was expected to be sensitive to the conversion. Our results suggested that conversion induced little increase of tacrolimus trough levels, even in the CYP2C19 extensive metabolizer subgroup of the *CYP3A5*3/*3* group, which was expected to be the most sensitive to the conversion.

This study had several limitations. First, it was a small retrospective cohort study

performed in a single institution. However, participants who would potentially bias results were excluded. In particular, only patients with no change in tacrolimus dose or concomitant medication were included. Therefore, although the number of subjects was small, we believe that the study design effectively supported reliability of the results. Secondly, the clinical cohort data made it difficult to accurately isolate the effect of vonoprazan on tacrolimus concentrations. Transplant recipients are typically started on any acid suppressant immediately after the procedure, and they are instructed to take these medications consistently. It was impossible, therefore, to evaluate the impact of acid suppressants alone on tacrolimus levels in the early post-transplant period, when other medications were being changed dramatically. To offset this potential complicating factor, we assessed tacrolimus levels during a period following transplantation when renal function and drug regimen were stable.

In conclusion, this study suggests that the conversion from rabeprazole to vonoprazan resulted in no changes in blood concentrations of tacrolimus, even in the *CYP* polymorphism groups predicted to be most susceptible. Vonoprazan is considered safe for concomitant use in renal transplant recipients taking tacrolimus.

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**Disclosure Statement**

The authors have no conflicts of interest.

**Authors' contributions:**

S.W.: Protocol development, Data collection, Data analysis, Manuscript writing. M.A.: Protocol and project development, Data management, Data analysis, Manuscript editing. J.M.: Project development, Data collection, Data management, Data analysis, Manuscript editing. K.Y.: Project development, Data management, Manuscript editing. T.S.: Project development, Data collection. Y.M.: Project development, Manuscript editing. Y.M.: Project development, Data analysis, Manuscript editing. T.S.: Data collection, Data

analysis. R.K.: Data management, Manuscript writing. S.N.: Data management, Manuscript editing. K.W.: Data analysis, Manuscript editing. Y.K., H.T., K.T., M.K., H.M., S.K., H.S., N.A., J.W., M.W., T.W. and Y.N.: Project development, Manuscript editing. All authors have read and agreed to the final version of the manuscript.

## References


[1] Shapiro R, Jordan ML, Scantlebury VP, Vivas C, Fung JJ, McCauley J, et al. A prospective randomized trial of FK506-based immunosuppression after renal transplantation. Transplantation 1995;59(4):485-90.

[2] Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 1995;29(6):404-30.

[3] Hosohata K, Masuda S, Ogura Y, Oike F, Takada Y, Katsura T, et al. Interaction between tacrolimus and lansoprazole, but not rabeprazole in living-donor liver transplant patients with defects of CYP2C19 and CYP3A5. Drug Metab Pharmacokinet 2008;23(2):134-8.

[4] Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug Metab Pharmacokinet 2007;22(5):328-35.

[5] Imamura CK, Furihata K, Okamoto S, Tanigawara Y. Impact of cytochrome P450 2C19 polymorphisms on the pharmacokinetics of tacrolimus when coadministered with voriconazole. J Clin Pharmacol 2016;56(4):408-13.

[6] van Gelder T, Meziyerh S, Swen JJ, de Vries APJ, Moes D. The Clinical Impact of the C(0)/D Ratio and the CYP3A5 Genotype on Outcome in Tacrolimus Treated Kidney Transplant Recipients. Front Pharmacol 2020;11:1142.

[7] Itagaki F, Homma M, Yuzawa K, Nishimura M, Naito S, Ueda N, et al. Effect of lansoprazole and rabeprazole on tacrolimus pharmacokinetics in healthy volunteers with CYP2C19 mutations. J Pharm Pharmacol 2004;56(8):1055-9.

[8] Isoda K, Takeuchi T, Kotani T, Hirano-Kuwata S, Shoda T, Hata K, et al. The proton pump inhibitor lansoprazole, but not rabeprazole, the increased blood concentrations of calcineurin inhibitors in Japanese patients with connective tissue diseases. Intern Med 2014;53(13):1413-8.

[9] Scott DR, Munson KB, Marcus EA, Lambrecht NW, Sachs G. The binding selectivity of vonoprazan (TAK-438) to the gastric H+, K+ -ATPase. Aliment Pharmacol Ther 2015;42(11-12):1315-26.

[10] Sugimoto M, Hira D, Murata M, Kawai T, Terada T. Effect of Antibiotic Susceptibility and CYP3A4/5 and CYP2C19 Genotype on the Outcome of Vonoprazan-Containing Helicobacter pylori Eradication Therapy. Antibiotics (Basel) 2020;9(10).

[11] van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. Clin Chem 2002;48(10):1668-71.

[12] Goldstein JA, Blaisdell J. Genetic tests which identify the principal defects in CYP2C19 responsible for the polymorphism in mephenytoin metabolism. Methods Enzymol 1996;272:210-8.

[13] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48(3):452-8.

[14] Mei T, Noguchi H, Suetsugu K, Hisadome Y, Kaku K, Okabe Y, et al. Effects of Concomitant Administration of Vonoprazan Fumarate on the Tacrolimus Blood Concentration in Kidney Transplant Recipients. Biol Pharm Bull 2020;43(10):1600-3.

[15] Yamasaki H, Kawaguchi N, Nonaka M, Takahashi J, Morohashi A, Hirabayashi H, et al. In vitro metabolism of TAK-438, vonoprazan fumarate, a novel potassium-competitive acid blocker. Xenobiotica 2017;47(12):1027-34.

**Figure legend**

**Figure 1: Changes in tacrolimus trough levels during conversion from rabeprazole to vonoprazan**

(A) Tacrolimus trough levels (ng/mL) in groups classified based on *CYP3A5* gene polymorphism were measured before and after conversion to vonoprazan. (B) Patients in the *CYP3A5*1/*1 + *1/*3* group were further classified according to *CYP2C19* gene polymorphisms. (C) Patients in the *CYP3A5*3/*3* group classified according to *CYP2C19* gene polymorphisms. Group concentrations are shown as median [range]. Abbreviation: CYP, cytochrome P450.

**Table 1: Patient background characteristics**

| Parameter | *CYP3A5* genotype **1/*1 + *1/*3* (n = 27) | *CYP3A5* genotype **3/*3* (n = 25) | p |
|---|---|---|---|
| Age (years) | 47 [27-68] | 56 [18-71] | 0.26 |
| Sex (male), n [%] | 17 [63] | 17 [68] | 0.78 |
| Body weight (kg) | 65 [32-98] | 58 [33-76] | 0.07 |
| The duration between transplantation and conversion (month) | 42 [2-105] | 23 [6-99] | 0.14 |
| Tacrolimus dose (mg) | 5 [2.5-9] | 3.5 [1-5] | < 0.001 |
| Serum creatinine (mg/dl) | 1.27 [0.68-1.99] | 1.37 [0.82-2.04] | 0.78 |
| *CYP2C19* genotype, n; EM/IM/PM | 8/13/6 | 7/10/8 | - |

Data are presented as median [range]. Abbreviation: CYP, cytochrome P450.

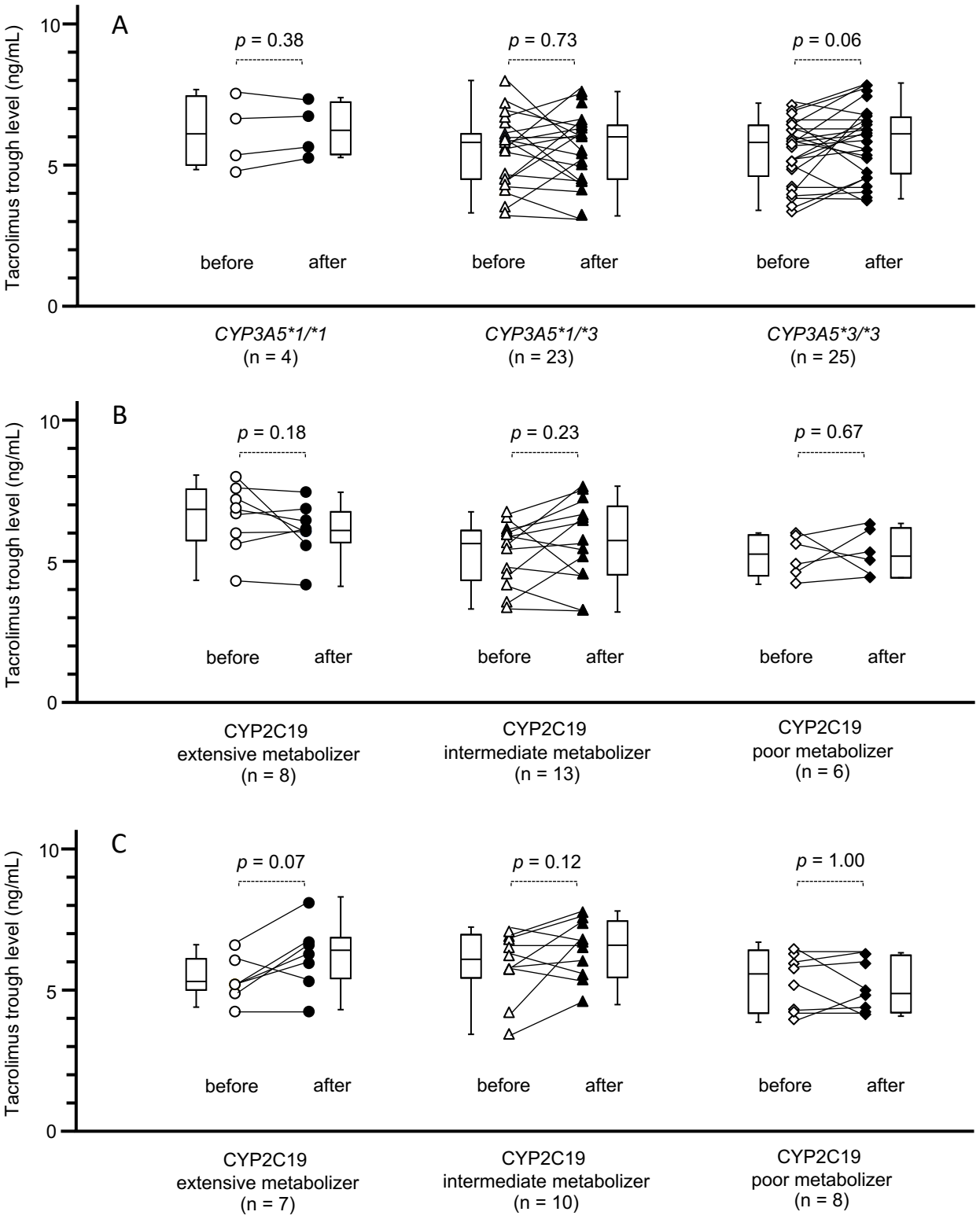
A
p = 0.38
p = 0.73
p = 0.06
Tacrolimus trough level (ng/mL)
10
5
0
before
after
CYP3A5*1/*1
(n = 4)
CYP3A5*1/*3
(n = 23)
CYP3A5*3/*3
(n = 25)
B
p = 0.18
p = 0.23
p = 0.67
CYP2C19
extensive metabolizer
(n = 8)
CYP2C19
intermediate metabolizer
(n = 13)
CYP2C19
poor metabolizer
(n = 6)
C
p = 0.07
p = 0.12
p = 1.00
CYP2C19
extensive metabolizer
(n = 7)
CYP2C19
intermediate metabolizer
(n = 10)
CYP2C19
poor metabolizer
(n = 8)

Figure. 1