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Combinational effect of intestinal and hepatic *CYP3A5* genotypes on tacrolimus pharmacokinetics in recipients of living donor liver transplantation

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Abstract

Background—For living donor liver transplantation, the genetic association of *CYP3A5* genotype of recipient's native intestine and donor's liver allograft with tacrolimus pharmacokinetics has not been explained completely considering liver regeneration time. The goal of study was to investigate the longitudinal effects of recipient-donor combinational *CYP3A5* genotypes on tacrolimus dose-normalized concentration (C/D ratio) in blood.

Methods—Tacrolimus blood concentrations were measured for fifty-eight Korean adult living donor liver transplant recipients on tacrolimus-based immunosuppressants during 4 years of follow-up. *CYP3A5* was genotyped for both recipient and donor, and the recipient-donor combinational genetic effect on tacrolimus C/D ratios were evaluated as a function of time after adjusting for covariates including demographics and clinical variables.

Results—CYP3A5 expresser recipients grafted from CYP3A5 expresser donors consistently had the least C/D ratio throughout the entire study period, whereas CYP3A5 expresser recipients grafted from CYP3A5 nonexpresser donors had an intermediate, and CYP3A5 nonexpresser recipients grafted from CYP3A5 nonexpresser donors had the largest C/D ratio (all $p < 0.01$). The CYP3A5 nonexpresser recipients grafted from CYP3A5 expresser donors showed a significant decrease from the largest to the intermediate in C/D ratio for the first month.

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Conflict of interest All authors declared no conflict of interest.

Conclusions—*CYP3A5* genotypes of both recipient and donor were important factors influencing pharmacokinetic variability of tacrolimus. The recipient-donor combinational genetic effect on C/D ratio changed over time after transplantation.

Keywords

tacrolimus; living donor liver transplantation; *CYP3A5*; donor; recipient

The calcineurin inhibitor tacrolimus is an immunosuppressive drug that has a narrow therapeutic index with high interindividual variations in its pharmacokinetics, which makes it difficult to establish an empirical dosage regimen in organ transplant recipients (1). Therefore, routine therapeutic drug monitoring (TDM) of tacrolimus is recommended to optimize the therapy for prevention of allograft rejections and adverse effects (2). Among the potential causes for large variability, recent pharmacogenetic studies have suggested genetic association of *CYP3A5* genotype and tacrolimus pharmacokinetics (3, 4). Solid-organ recipients with at least one wild-type allele *CYP3A5*1*, cytochrome P450 (CYP) 3A5 expressers, exhibited the lower tacrolimus dose-normalized concentration (C/D ratio) and required a higher dose to reach the same blood concentration than the recipients with homozygous *CYP3A5*3* allele, CYP3A5 nonexpressers (5, 6). Furthermore, dose-normalized tacrolimus exposure almost doubled in CYP3A5 nonexpressers compared with CYP3A5 expressers (7).

As tacrolimus is metabolized by both intestinal and hepatic CYP3A5 enzymes, the combined contribution of CYP3A5 expressions in native intestine and liver allograft should be considered for the pharmacokinetics of tacrolimus in liver transplant recipients (4, 6). Therefore, in the case of patients receiving liver transplantation the *CYP3A5* genotypes of both the recipients' native intestine themselves and donors' liver allograft, recipient-donor combinational effect of *CYP3A5* genotype, could be essential to the marked inter-individual variation in postoperative tacrolimus pharmacokinetics.

However, the recipient-donor combinational effect of *CYP3A5* genotype on tacrolimus dose and blood C/D ratio in living donor liver transplantation (LDLT) remains to be elucidated. LDLT is becoming increasingly important strategy in reducing the waiting time mortality in liver failure patients (8, 9). For LDLT, the physiologic recovery of liver allograft should be different from a full sized liver transplantation since only a partial liver is transplanted (10). That is, in LDLT the systemic clearance of tacrolimus by the partial graft liver will gradually increase with postoperative time as the grafted liver regenerates its mass. Therefore, in order to understand inter- and intra-individual variations in tacrolimus pharmacokinetics over time in LDLT recipients, it is critically important to evaluate time-dependent tacrolimus clearance as a function of *CYP3A5* genotypes of both native intestine and the graft liver. To our knowledge, however, this time trend on tacrolimus pharmacokinetics has not been clearly demonstrated. The previous studies were limited to relatively large graft size from deceased donor liver transplantation (DDLT) (11, 12), short study period (13), or analysis without consideration of continuous time trend and repeated measurements of tacrolimus concentration (14).

Based on this background, we hypothesized that tacrolimus clearance would change over time by recipient-donor combinational effect of *CYP3A5* genotypes in patients after LDLT, and aimed to evaluate longitudinal effect of recipient-donor combinational *CYP 3A5* genotypes on blood tacrolimus C/D ratio in Korean adults LDLT recipients.

RESULTS

Characteristics of the Study Participants

A total of 58 *de novo* ethnically Korean adult LDLT recipients administering tacrolimus were stratified into four study groups according to the *CYP3A5* genotype of recipients and donors: CYP3A5 expresser recipient grafted from CYP3A5 expresser donor (R_ED_E, n=10); CYP3A5 expresser recipient grafted from CYP3A5 nonexpresser donor (R_ED_N, n=13); CYP3A5 nonexpresser recipient grafted from CYP3A5 expresser donor (R_ND_E, n=8); CYP3A5 nonexpresser recipient grafted from CYP3A5 nonexpresser donor (R_ND_N, n=27). The demographic and baseline clinical characteristics of the study participants were compatible among 4 genotype groups (all $p>0.05$), except for recipient's age ($p<0.05$) (Table 1). R_ED_N were youngest (44.5±8.8 years) while R_ND_N were oldest (52.6±7.0 years) ($p=0.049$). The mean length of hospital stay for transplantation was 27±18 days ranging from 13 to 127. The most prevalent primary indication of transplantation was hepatitis B viral cirrhosis, which accounted for 75.0% of all cases, followed by alcoholic cirrhosis (8.3%), hepatitis C viral cirrhosis (6.7%), biliary cirrhosis (5.0%), fulminant hepatitis (3.3%), and cryptogenic cirrhosis (1.7%). Incidental hepatocellular carcinoma in addition to the primary disease was present in 29 of the recipients (49.2%). Six patients died during the study (at 1 and 8 months, and 1.5, 2, 3, 4 years post operation). Child-Pugh scores consisted of 13.6% of grade A (5–6), 22.0% of grade B (7–9), and 64.4% of grade C (10–15). A total of 23 cases of acute cellular rejection (ACR) were observed during the study (19 cases within the first 4 weeks while the others occurred at 0.5, 0.7, 1 and 3.6 years, respectively). Severity of ACR based on the rejection activity index (RAI) (15) were 73.9% of mild (RAI 2–4), 26.1% of moderate (RAI 5–6), and none of severe (RAI 7–9).

Genotype Frequencies

CYP3A5 genotype frequencies for recipients and donors of liver grafts are shown in Table 2. As a feature of liver transplantation, there were cases in which the *CYP3A5* genotype of the recipient was different from that of donor, however, there was no difference in *CYP3A5* genotype frequencies between recipients and donors ($p=0.610$). The allele frequency of the *CYP3A5**3 variant of the whole recipient and donor was 79.7%. The observed *CYP3A5* genotype frequency distributions for recipients and donors were consistent with Hardy-Weinberg equilibrium (HWE) (all $p>0.05$).

Time Trend of the C/D Ratio Stratified by Recipient-Donor Combinational *CYP3A5* Genotype

Figure 1 presents tacrolimus C/D ratio over 48 months post transplantation stratified by four recipient-donor combinational *CYP3A5* genetic groups. The C/D ratios for all 4 groups tended to decline, though with different degree, during the first 6 months post transplantation, and remained fairly stable thereafter. Approximately after 4 months post transplantation, C/D ratios of CYP3A5 expresser liver (donor expresser) were lower than those of nonexpresser regardless of recipients' genotype ($R_E\mathbf{D_E}$, $R_N\mathbf{D_E}$ < $R_E\mathbf{D_N}$, $R_N\mathbf{D_N}$). Given the same donor genotype, C/D ratios of CYP3A5 expresser intestine (recipient expresser) were lower than those of nonexpresser ($\mathbf{R_E}D_E$ < $\mathbf{R_N}D_E$ given the same D_E and $\mathbf{R_E}D_N$ < $\mathbf{R_N}D_N$ given the same D_N). However, during the early period until about 4 months post transplantation, C/D ratios of CYP3A5 expresser intestine were lower than those of nonexpresser independent of donors' genotype ($\mathbf{R_E}D_E$, $\mathbf{R_E}D_N$ < $\mathbf{R_N}D_E$, $\mathbf{R_N}D_N$). The overall C/D ratio during the entire study period was nearly 2-folds greater in both intestinal and hepatic nonexpressers (R_ND_N) than both expressers (R_ED_E).

Since C/D ratio of R_ND_E changed dramatically over time, we further analyzed the difference between R_ND_E and R_ED_N over time as illustrated in Figure 2. As the 95% confidence band

(shaded area) suggested (i.e., zero excluded), C/D ratio between the two groups was significantly different until approximately 1 month of transplantation, but the difference was diminished thereafter. This motivated performing separate analyses for comparison of C/D ratios among 4 genotype groups by two different time periods, until one month and after one month.

Effects of *CYP3A5* Genotype on the Tacrolimus C/D Ratio

Table 3 presents the results for fixed effects in the final model for examining the association between log-transformed tacrolimus C/D ratio and the recipient-donor combinational *CYP3A5* genotype groups after adjusting for covariates. After adjustment of the covariates, *CYP3A5* genotypes remained as the most important factor affecting tacrolimus C/D ratio. The nonexpression of CYP3A5 was significantly associated with higher tacrolimus C/D ratio. For example, both intestinal and hepatic nonexpressers, R_ND_N, was associated with an increase of 0.89 and 0.8 in log-transformed tacrolimus C/D ratio compared to both expressers, R_ED_E, until and after one month, respectively ($p<0.001$). If either intestine or liver was CYP3A5 nonexpresser (R_ND_E or R_ED_N), C/D ratio increased by a range of ~0.5 when compared with both expresseres (R_ED_E) ($p\leq0.01$) whereas it decreased by approximately 0.3 in comparison with both nonexpressers (R_ND_N) (all $p<0.01$ except $p=0.178$ for R_ND_E vs. R_ND_N until 1 month). Among covariates in the model, after adjusting for the genotypes, albumin was negatively associated with the log-transformed tacrolimus C/D ratio during the entire study period ($p<0.001$). Time after transplantation is an important variable in the changing tacrolimus C/D ratio.

DISCUSSION

To the best of our knowledge, this prospective study is the first study to examine time-dependent effect of combinational *CYP3A5* genotype in graft liver and native intestine on the C/D ratio of tacrolimus in adult LDLT recipients. Our major finding was that recipients' native intestinal *CYP3A5* genotype played more important role than donors' hepatic genotype in early stage after transplantation, and the roles would be gradually changed to be switched in later stage. Thus, C/D ratio of R_ND_E was significantly higher than that of R_ED_N shortly after transplantation, but decreased rapidly during the first month of post-transplantation, so that there was no difference between two groups after 1 month. In addition, as expected, our study confirmed that C/D ratio was consistently the greatest in R_ND_N whereas it was the lowest in R_ED_E throughout the entire study period.

As hepatic metabolism by CYP3A enzymes is considered a major eliminating process of tacrolimus, liver regeneration from the ischemia reperfusion injury as well as enlarging the liver mass after transplantation in LDLT may have influence on the total clearance of tacrolimus. In LDLT, tacrolimus metabolism is reduced during the liver regeneration period (16) due to the recovery of liver mass to the standard liver volume (17, 18) as well as the down-regulation of the CYP3A enzyme system (19). In the present study, the significant difference in C/D ratio between the R_ND_E and R_ED_N groups was not distinctive after the first month of transplantation indicating the recovery of hepatic metabolism along with the regeneration of partial liver allograft takes about one month. Meanwhile, this recovery time in DDLT can be conjectured to be shorter due to receipt of a full-sized liver graft instead of partial grafted liver such as in LDLT (11, 12).

Donor's *CYP3A5* genotype not recipient's exhibited genetic effect on tacrolimus pharmacokinetics in the studies of DDLT subjects (11, 12, 20), using one point tacrolimus concentration(21) and excluding recipient's *CYP3A5* genotype (22, 23). During the first month of our study in those CYP3A5 nonexpresser recipients, donor's *CYP3A5* genotype had minimal influence on tacrolimus metabolism similar to what was previously reported

(24). Uesugi *et al.* compared the C/D ratio of tacrolimus according to the *CYP3A5* genotype combination of the donor and recipient for the first 35 days after LDLT (13). They reported a gradual decline of the C/D ratio, no hepatic influence in intestinal non-expressers, and significant hepatic influence in intestinal expressers; however, they observed no difference between R_ND_E and R_ED_N, and no prolonged difference between R_ED_E and R_ND_E after 1 month. This may be attributable to the characteristics of their study population, which was comprised of both pediatric and adult transplant recipients. As previously described for DDLT, it may be difficult to detect the influence of intestinal metabolism in pediatric LDLT patients receiving relatively larger graft size than adults. Fukudo *et al.* found a significant impact of intestinal CYP3A5 expression on tacrolimus oral clearance only during the first 4 weeks after LDLT, whereas hepatic CYP3A5 expression significantly influenced tacrolimus dose and C/D ratio after the first month until one year post transplantation (14). The different results of these two studies might be derived from the present study design for time as a continuous variable and tacrolimus concentration as repeated measurement, which is reported for the first time in this study.

Tacrolimus is extensively distributed to red blood cells and bound to plasma protein, and the physiological status of the liver transplanted is expected to influence the pharmacokinetics of tacrolimus. Thus, covariates that have been known to affect tacrolimus disposition such as time since transplantation, hematocrit, albumin, total bilirubin, alanine transaminase (ALT), body weight, and graft-to-recipient body weight ratio (GRWR) as well as demographics (1) were adjusted to analyze the association of *CYP3A5* genetic variants with the pharmacokinetics of tacrolimus over time in the current study. The overall C/D ratio, which had stabilized at 6 months post-transplantation, gradually increased until 2–3 years after transplantation (Figure 1). This trend of increasing C/D ratio might be explained mostly by less intensive and frequent sampling of the tacrolimus trough blood levels due to prolonged or delayed outpatient follow-ups, poor adherence to tacrolimus treatment and blood sampling, and relatively low dose of tacrolimus as well as inevitable gradual deterioration of liver function over time.

The allele frequency of the *CYP3A5**3 variant was 79.7% in our study, which is consistent with the reported frequencies of 76.5 to 81.4% for this variant in the Korean population (21, 25–27). The *CYP3A5* allele was speculated to be the wild type (*1) when the *CYP3A5* 6986G variant was not detected, as the *CYP3A5**6 allele is not expressed to any significant degree among the Korean population (26, 27). Even though the allele frequencies are different across the different ethnic groups, similar contributions from *CYP3A5* to hepatic drug metabolism have been reported (4, 6).

In conclusion, *CYP3A5* genotypes of both recipient and donor were independently important factors influencing inter-individual pharmacokinetic variability of tacrolimus. And the recipient-donor combinational genetic effect on tacrolimus C/D ratio changed over time since transplantation. To individualize tacrolimus therapy, however, quantitative guidelines should be developed for tacrolimus dosage regimens according to *CYP3A5* genotype of donor and recipient pairs considering time after transplantation in adult LDLT.

METHODS

Subjects

Between 9 July 2004 and 7 August 2006, a total of 103 adult patients received LDLT at a tertiary hospital affiliated with a university in Seoul, South Korea. Fifty-nine of these patients were recruited into this prospective 4-year follow-up study. One patient was excluded because of severe hepatic impairment, which was never resolved. Inclusion criteria were: (1) patients on tacrolimus-based immunosuppressive therapy; (2) corresponding donor

participation; and (3) patients older than 18 years of age. Exclusion criteria were: patients with (1) multi-organ transplantation; (2) retransplantation; and (3) administration of any investigational or immunosuppressive agent not included as part of the study regimen during the study period. The study protocol was approved by the Institutional Review Board (C-0505-148-005). All procedures were performed in accordance with the recommendations of the Declaration of Helsinki on Biomedical Research Involving Human Subjects, as well as the International Conference on the Harmonization of the Technical Requirements for the Registration of Pharmaceuticals for Human Use–Good Clinical Practice guidelines. All participants provided written informed consent before enrollment in the study.

Genotype Identification

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral whole-blood samples of each subject using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The presence of the *CYP3A5*3* allele (6986A>G, rs 776746) was identified by mismatch polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis, as reported previously (25). The oligonucleotides used for PCR were synthesized commercially (Bioneer Co. Ltd., Daejeon, Korea). PCR was carried out in a GeneAmp PCR system 2400 (Perkin Elmer, Boston, MA). Digested PCR products were analyzed by electrophoretic separation on agarose gels containing ethidium bromide. Ten percent of the DNA samples were directly sequenced for validation, which confirmed the results. Laboratory staffs were blinded to the patients' clinical data.

Immunosuppression Protocol

Our institutional immunosuppression protocol, a triple or double regimen including tacrolimus and corticosteroids with or without mycophenolate mofetil (MMF), was applied to all study participants. The initial tacrolimus dosage was 0.03 mg/kg for the triple regimen or 0.05 mg/kg for the double regimen, administered twice daily at 10:00 and 22:00 on an empty stomach starting on the post operational day (POD) 1. The following doses were adjusted based on the measured blood concentration in order to achieve the following target trough concentrations: 8–13 ng/mL until 2 weeks, 7–10 ng/mL between 2 weeks and 3 months, 5–7 ng/mL between 3 and 6 months, and approximately 5 ng/mL thereafter for the triple regimen; and 13–17 ng/mL, 8–13 ng/mL, 5–10 ng/mL, and approximately 5 ng/mL during the respective periods for the double regimen. Clinical staffs adjusting tacrolimus dosage were blinded to the individual patient's genotyping results. Methylprednisolone was administered intravenously at a dose of 500 mg before and after reperfusion, then tapered gradually, switched to oral prednisolone at 20 mg on POD 7, and then tapered to discontinuation approximately 6 months after transplantation. MMF was administered at 0.5 g with white blood cells (WBC) count of >3500/L or 0.25 g with WBC count of 2500–3500/L twice a day with tacrolimus. Patients did not receive any other medications known to interact significantly with tacrolimus. Prophylactic fluconazole of 50 mg daily was allowed due to its small amount and application to all participants.

Data Collection and TDM

Tacrolimus C/D ratio was calculated as tacrolimus trough blood concentration in ng/mL divided by a dose administered prior to blood sampling in mg. Tacrolimus trough concentrations from the routine TDM were adopted to calculate C/D ratio. Blood samples for tacrolimus routine TDM were collected daily at about 09:00 in the morning, beginning the day after the first dose and continuing until the day of discharge. Subsequent samples were obtained at each outpatient visit. Whole-blood tacrolimus concentrations were determined by microparticle enzyme immunoassay using an IMx analyzer and tacrolimus II reagent (Abbott Laboratories Diagnostic Division, Abbott Park, IL). Clinical laboratory test markers were measured simultaneously.

The written and electronic medical records of each subject were reviewed for abstraction of basic demographic information, concomitant medications and laboratory test results associated with tacrolimus concentrantion with corresponding dose and body weight, hematocrit, albumin, total bilirubin, ALT and pathological data. The amount of methylprednisolone was converted to prednisolone by multiplying by 1.25.

Statistics

We performed an exploratory analysis to examine the time trends of C/D ratio changes for the four groups stratified by the *CYP3A5* genotype of recipients and donors. To further examine the differences in the time trends of C/D ratio between $R_N D_E$ and $R_E D_N$, cluster bootstrap analysis was performed with 5000 bootstraps, and the median and 95% confidence band were constructed for the difference. The distribution of C/D ratio was skewed to the right, and therefore logarithmically transformed C/D ratio values were used in all analyses to reduce the skewness of the distribution.

The exploratory analyses motivated performing separate analyses for two periods, from the first day until one month and thereafter. The differences in C/D ratio among 4 groups were examined separately in these two periods, using mixed effects models to take into account the correlation due to repeated measurements. The following covariates were selected *a priori* based on their potential effects on C/D ratio and adjusted in the analyses: age, body weight at each tacrolimus measurement, GRWR, hematocrit, albumin, total bilirubin, and ALT. To model linear and nonlinear time trends, we included a continuous time variable as well as its quadratic function in the models. The GRWR values for two subjects were missing, which were imputed using the median of GRWR values from 56 subjects. HWE was tested using Pearson's χ^2 test for HWE in the R package genetics and our own R program. Data were presented as means, standard deviations or ranges.

All analyses were performed using STATA 11.0 (Stata Corp., College Station, TX) and the statistical programming language R version 2.10.0.(28)

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ABBREVIATIONS

ACR	acute cellular rejection
ALT	alanine transaminase
C/D ratio	dose-normalized concentration
CYP	cytochrome P450
DDLT	deceased donor liver transplantation
GRWR	graft-to-recipient body weight ratio

HWE	Hardy-Weinberg equilibrium
LDLT	living donor liver transplantation
MMF	mycophenolate mofetil
PCR	polymerase chain reaction
POD	post operational day
RAI	rejection activity index
R_ED_E	CYP3A5 expresser recipient grafted from CYP3A5 expresser donor
R_ED_N	CYP3A5 expresser recipient grafted from CYP3A5 nonexpresser donor
R_ND_E	CYP3A5 nonexpresser recipient grafted from CYP3A5 expresser donor
R_ND_N	CYP3A5 nonexpresser recipient grafted from CYP3A5 nonexpresser donor
TDM	therapeutic drug monitoring
WBC	white blood cells

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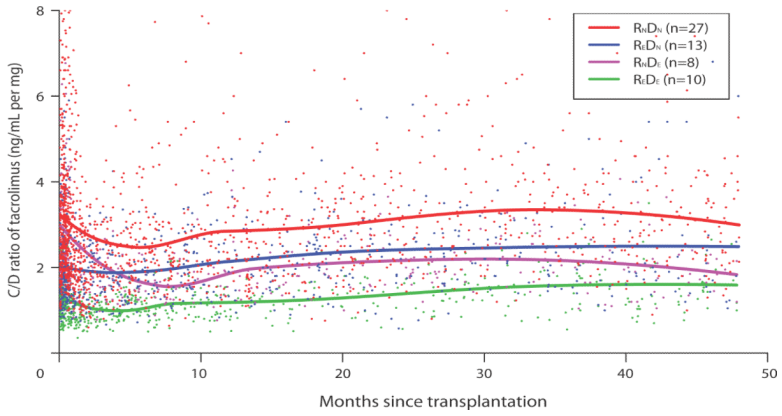

FIGURE 1.

Tacrolimus dose-normalized concentration (C/D ratio, ng/mL per mg) stratified by the four donor-recipient combinational *CYP3A5* genotype groups over 48 months after transplantation. R_ED_E, CYP3A5 expresser recipient grafted from CYP3A5 expresser donor; R_ED_N, CYP3A5 expresser recipient grafted from CYP3A5 nonexpresser donor; R_ND_E, CYP3A5 nonexpresser recipient grafted from CYP3A5 expresser donor; R_ND_N, CYP3A5 nonexpresser recipient grafted from CYP3A5 nonexpresser donor

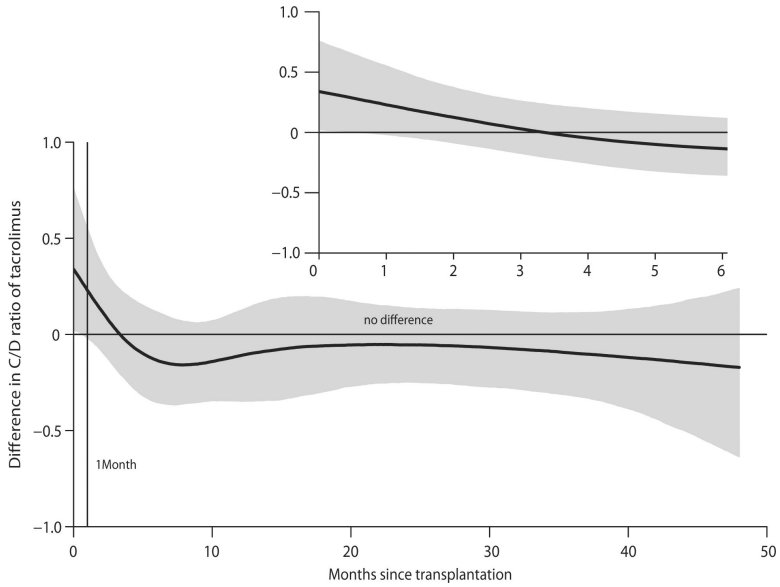

FIGURE 2.

Differences in log-transformed tacrolimus dose-normalized concentration (C/D ratio, mg/mL per mg) between R_ND_E and R_ED_N over 48 months after transplantation. The C/D ratios were logarithmically transformed as the distribution was skewed to the right. R_ND_E, CYP3A5 nonexpresser recipient grafted from CYP3A5 expresser donor; R_ED_N, CYP3A5 expresser recipient grafted from CYP3A5 nonexpresser donor.

TABLE 1

Demographic and baseline characteristics of study participants

Characteristics	Overall	R_ED_E	R_ED_N	R_ND_E	R_ND_N	*p*
n (%)	58(100)	10 (17.2)	13 (22.4)	8 (13.8)	27 (46.6)	
Recipient						
Male, *n* (%)	46 (79.3)	8 (80)	9 (69.2)	8 (100)	21 (77.8)	
Age (years)	49.2±8.7 (19 to 65)	49.4±6.7 (38 to 57)	44.5±8.8 (32 to 62)	45.1±12.1 (19 to 58)	52.6±7.0 (35 to 65)	0.018[a]
Body weight (kg)	66.4±10.2 (45.3 to 86.3)	63.4±10.1 (50.2 to 80.3)	68.3±11.9 (46.7 to 86.3)	67.1±8.2 (51.8 to 76.3)	66.4±10.1 (45.3 to 86.0)	0.719
Donor						
Male, *n* (%)	41 (70.7)	8 (80)	9 (69.2)	5 (62.5)	19 (70.4)	
Age (years)	30.0±9.1 (17 to 52)	28.8±8.6 (17 to 44)	31.9±9.3 (18 to 47)	33.9±11.5 (17 to 52)	28.4±8.5 (18 to 50)	0.398
Body weight (kg)	67.1±11.3 (46.4 to 98.1)	60.4±7.8 (46.4 to 71.3)	69.7±10.7 (54.0 to 91.0)	64.0±10.4 (52.5 to 82.6)	69.2±12.3 (50.5 to 98.1)	0.128
GRWR (%)	1.07±.24 (.59 to 1.60)	1.13±.21 (.84 to 1.39)	1.17±.23 (.82 to 1.48)	.95±.20 (.59 to 1.19)	1.04±.25 (.59 to 1.60)	0.137
Hematocrit (%)	24.1±3.9 (17.8 to 36.3)	23.9±4.8 (19.5 to 36.3)	24.0±3.7 (19.6 to 30.7)	24.5±4.3 (20.9 to 34.4)	24.1±3.7 (17.8 to 33.7)	0.991
Albumin (g/dL)	3.1±.3 (2.4 to 3.6)	3.0±.3 (2.7 to 3.5)	3.1±.2 (2.8 to 3.6)	3.0±.4 (2.4 to 3.6)	3.1±.3 (2.5 to 3.6)	0.945
T.Bil (mg/dL)	3.2±2.8 (.7 to 12.0)	3.4±3.6 (.7 to 11.0)	2.7±2.0 (.8 to 7.3)	4.1±3.2 (.9 to 10.6)	3.1±2.7 (1.0 to 12.0)	0.706
ALT (IU/L)	161±117 (28 to 570)	163±111 (48 to 396)	231±164 (83 to 570)	141±60 (77 to 274)	133±96 (28 to 502)	0.205

Data are presented as mean ± SD (range).

SD, standard deviation; R_ED_E, CYP3A5 expresser recipient grafted from CYP3A5 expresser donor; R_ED_N, CYP3A5 expresser recipient grafted from CYP3A5 nonexpresser donor; R_ND_E, CYP3A5 nonexpresser recipient grafted from CYP3A5 expresser donor; R_ND_N, CYP3A5 nonexpresser recipient grafted from CYP3A5 nonexpresser donor; GRWR, graft to recipient body weight ratio; T. Bil, total bilirubin; ALT, alanine transaminase.

[a]Age of R_ED_N was younger than that of R_ND_N (p=0.049) by Dunnett's T3 post hoc tests.

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TABLE 2

Genotype frequencies of *CYP3A5*

			Donor (graft liver)			
				Expresser	Nonexpresser	
			*1/*1	*1/*3	*3/*3	Total
Recipient (Native intestine)	Expresser	*1/*1	1 (1.7)	1 (1.7)	2 (3.4)	4 (6.9)
		*1/*3	1 (1.7)	7 (12.1)	11 (19.0)	19 (32.8)
	Nonexpresser	*3/*3	-	8 (13.8)	27 (46.6)	35 (60.3)
	Total		2 (3.4)	16 (27.6)	40 (69.0)	58 (100)

Data are expressed as *n* (%).

TABLE 3

Results for fixed effects in the mixed-effects model for examining the association between log-transformed tacrolimus C/D ratio and the recipient-donor combinational *CYP3A5* genotype groups

	Until 1 Month		After 1 Month	
Covariate[a]	**Coefficient**[b] **(95% CI)**	***p***	**Coefficient (95% CI)**	***p***
Genotype (R_ND_N vs. R_ED_E)	.89 (.62,1.16)	< 0.001	.80 (.60,1.00)	< 0.001
Genotype (R_ED_N vs. R_ED_E)	.40 (.10, .71)	0.010	.52 (.29, .74)	< 0.001
Genotype (R_ND_E vs. R_ED_E)	.68 (32,1.03)	< 0.001	.47 (.21, .73)	< 0.001
Genotype (R_ED_N vs. R_ND_N)[c]	−.48 (−.75, −.22)	< 0.001	−.28 (−0.48, −.09)	0.005
Genotype (R_ND_E vs. R_ND_N)[c]	−.21 (−.52, .10)	0.178	−.33 (−.55, −.10)	0.004
Time[d]	.001 (.0007, .002)	< 0.001	.00004 (.00003, .00005)	< 0.001
$Time^2$	-2.03×10^{-6} (-2.62×10^{-6},-1.44×10^{-0})	< 0.001	-8.48×10^{-10} (-1.01×10^{-9},-6.88×10^{-10})	< 0.001
Albumin (g/dL)	−.13 (−.21,−.05)	.001	−.09 (−.13, −.04)	< 0.001
Hematocrit (%)	.01 (.003, .02)	.010	−.001 (−.006, .003)	.556
Body weight (kg)	.007 (−.0002, .01)	.058	−.005 (−.01, .001)	.081
T. Bil (mg/dL)	.02 (−.002, .04)	.084	−.006 (−.03, .04)	.766
ALT (IU/L)	.00003 (−.0002, .0003)	.836	.0004 (.0001, .0006)	0.002
GRWR (%)[e]	.22 (−.22, .66)	.324	−.07 (−.39, .25)	.686
Age (years)	.0008 (−.01, .01)	.894	.001 (−.008, .01)	.810

C/D ratio, dose-normalized concentration; CI, confidence interval; R_ED_E, CYP3A5 expresser recipient grafted from CYP3A5 expresser donor; R_ED_N, CYP3A5 expresser recipient grafted from CYP3A5 nonexpresser donor; R_ND_E, CYP3A5 nonexpresser recipient grafted from CYP3A5 expresser donor; R_ND_N, CYP3A5 nonexpresser recipient grafted from CYP3A5 nonexpresser donor; T. Bil, total bilirubin; ALT, alanine transaminase; GRWR, graft to recipient body weight ratio.

[a] All covariates except genotype and GRWR were measured at the same time when tacrolimus concentrations were measured.

[b] For coefficients with a positive value, the covariate results in an increase in log-transformed tacrolimus C/D ratio, whereas a negative values results in a decrease in log-transformed tacrolimus C/D ratio.

[c] Obtained using R_ND_N as a reference group.

[d] Time since transplant

[e] Missing values of GRWR for two subjects were imputed using the median of GRWR from fifty-six subjects.