ORIGINAL ARTICLE

The tacrolimus metabolism affect post-transplant outcome mediating acute rejection and delayed graft function: analysis from Korean Organ Transplantation Registry data

Han Ro[1], Jong Cheol Jeong[2], Jin Min Kong[3], Ji Won Min[4], Sung Kwang Park[5], Joongyub Lee[6], Tai Yeon Koo[7], Jaeseok Yang[8], Myoung Soo Kim[9], Seungsik Hwang[10] & Curie Ahn[11]

1 Department of Internal Medicine, Gil Hospital, Gachon University, Incheon, Korea
2 Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Korea
3 Department of Internal Medicine, BHS Hanseo Hospital, Busan, Korea
4 Department of Internal Medicine, Bucheon St. Mary's Hospital, The Catholic University of Korea, Bucheon, Korea
5 Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Korea
6 Department of Prevention and Management, School of Medicine, Inha University Hospital, Inha University, Incheon, Korea
7 Transplantation Research Institute, Seoul National University Hospital, Seoul, Korea
8 Department of Surgery, Transplantation Center, Seoul National University Hospital, Seoul, Korea
9 Department of Surgery, Yonsei University College of Medicine, Seoul, Korea
10 Department of Public Health Sciences, Graduate School of Public Health, Seoul National University, Seoul, Korea
11 Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea

Correspondence
Jong Cheol Jeong, Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro 173 beon-gil, Bundang-gu, Seongnam, Gyeonggi-do 463-707, Korea.
Tel.: +82-31-787-7073;
fax: +82-31-787-4051;
e-mail: jcj0425@empal.com

SUMMARY

Tacrolimus is a key drug in kidney transplantation (KT) with a narrow therapeutic index. The association between the tacrolimus metabolism rate and KT outcomes have not been investigated in large-scale multi-center studies. The Korean Organ Transplantation Registry (KOTRY) datasets were used. A total of 3456 KT recipients were analyzed. The tacrolimus metabolism rate was defined as blood trough concentration of tacrolimus (C_0) divided by the daily dose (D). The patients were grouped into fast, intermediate, or slow metabolizers by the C_0/D measured 6 months after transplantation. The slow metabolism group was associated with a 2.7 ml/min/1.73 m^2 higher adjusted estimated glomerular filtration rate (eGFR) at 6 months [95% confidence interval (C.I.) 1.2–4.3, $P = 0.001$], less acute rejection (AR) within 6 months [Odds ratio (OR) 0.744, 95% C.I. 0.585–0.947, $P = 0.016$], and less interstitial fibrosis and tubular atrophy [OR 0.606, 95% C.I. 0.390–0.940, $P = 0.025$]. Fast tacrolimus metabolism affected the 6-month post-KT eGFR through mediation of AR [natural indirect effect (NIE) −0.434, 95% C.I. −0.856 to −0.012, $P = 0.044$) and delayed graft function (DGF; NIE −0.119, 95% C.I. −0.231 to −0.007, $P = 0.038$). Slow tacrolimus metabolism was associated with better post-KT eGFR. AR and DGF were found to be significant mediators.

Transplant International 2021; 34: 163–174

Key words
kidney transplantation, pharmacogenetics, tacrolimus metabolism

Received: 25 February 2020; Revision requested: 14 May 2020; Accepted: 21 October 2020;
Published online: 9 November 2020

doi:10.1111/tri.13777

Introduction

Tacrolimus is a key drug in solid organ transplantation which has a narrow therapeutic index. Since the ELITE-Symphony trial reported the best estimated glomerular filtration rate (eGFR) in the low dose tacrolimus group, use of low dose of tacrolimus has become a standard maintenance immunosuppressant strategy [1,2]. As the ELITE-Symphony trial excluded high-risk patients who had more than 20% panel reactive antibodies or positive crossmatch and were co-administered intravenous daclizumab until 2 months, a study on maintenance immunosuppressants based on more generalizable data is needed.

Exposure to immunosuppressants strongly affects drug adherence and inherent metabolism. Poor compliance itself is a significant risk factor for acute rejection, development of dnDSA and allograft failure [3–6]. Drug metabolism is another key factor for maintaining the area under the curve of tacrolimus, and a different dosing strategy should be implemented depending on the rate of drug metabolism [8,9]. The association between tacrolimus metabolism and genetic polymorphisms have been examined in many studies, which were systematically reviewed in the previous article [9]. The cytochrome P450 protein and the CYP3A4/5 subfamily are the most important enzymes in tacrolimus metabolism. Expressor of CYP3A5 protein metabolizes tacrolimus in a faster way. CYP3A5 6986A>G (rs776746, CYP3A5*3) is the main single nucleotide polymorphism that results in functional variation. This mutation is present in the intronic lesion affecting alternative splicing, which leads to synthesis of a truncated and inactive CYP3A5 enzyme [10]. The frequency of the CYP3A5 expressor allele is known to be different among people of different ethnicity but is 76.5% for 6986A>G in Koreans [11–13]. Other than the *CYP3A5* gene, various gene polymorphisms including *ABCB1*, *CYP3A4*, *CYP2C8*, *NR1I2*, *PPP3CA*, and *PPP3CB* were reported to correlate with tacrolimus metabolism trough levels [14]. However, genotyping of several polymorphic genes is not routine in current clinical practice. Therefore, development of a simple clinical index to measure the tacrolimus metabolism rate is worthwhile [15]. As the number of previous studies investigating the rate of tacrolimus metabolism in association with the clinical outcome of kidney transplantation in Asian patients is limited [16–19], we investigated the association of tacrolimus metabolism with the clinical outcomes of kidney transplant recipients using data from the Korean Organ Transplant Registry (KOTRY).

Methods

Study population

The Korean organ transplantation registry (KOTRY) is a nationwide solid organ transplant cohort launched in 2014. The design and methods of KOTRY were described in detail in the previous report [20]. In brief, pretransplant evaluation, immunologic risks, induction and maintenance immunosuppressants, kidney biopsy results, treatments of clinical or biopsy-proven acute rejection, graft function measured as eGFR, and graft and patient survival data were collected. For this study, the dataset on kidney transplant recipients who received a kidney transplantation (KT) from 2014 to 2017 was used. A total of 3484 KT recipients were prescribed tacrolimus 6-months post-transplant. Among them, 28 were excluded because tacrolimus level measurements were missing. Thus, a total of 3456 patients were analyzed.

Study objective and design

We tested whether tacrolimus metabolism was associated with post-transplantation allograft function. Multivariable longitudinal linear regression models were used to test the study hypothesis. In addition to multilevel regression, we also used causal mediation analysis to assess whether the effect of tacrolimus metabolism for post-transplantation allograft eGFR is mediated by specific mechanisms [21].

Study outcome, exposure, mediator, and covariables

The main outcome was the eGFR 6-months and 1-year post-transplant. The secondary outcomes were allograft survival and IFTA of biopsies within 1-year post-transplant. For study exposure, tacrolimus metabolism was used, which was estimated by dividing measured trough concentration (C_0) by the dosage (D). Tacrolimus C_0/D were available at 6-month and 1-year post-transplant; we used measurement values at 6 months as representative values of each recipient's metabolism. Patients were grouped into tertiles based on tacrolimus metabolism rates, and the study outcomes were compared between groups. Acute rejection and delayed graft function were used as the mediator in each causal mediation analysis. In our study, acute rejection was defined as the composite outcome of clinical rejection (rejection treatment without kidney biopsy results) and biopsy-proven rejection. For the analysis, only acute rejection before 6 months was used. Delayed graft function was defined

Transplant International 2021; 34: 163–174

as hemodialysis within 1-week post-transplant. Banff 2007 was a formal standard for reporting pathology in KOTRY, from which we collect data on the presence of IFTA. Pathology reports were based on the readings of pathologists at a local center.

Study ethics, covariables and statistical model

The study protocol was approved by the Ajou University Hospital institutional review board (P01-201412-RS-02-02). Patient privacy was preserved in all instances, and the study methods complied with the tenets of the Declaration of Helsinki. Rates of missing covariables in the KOTRY datasets were under 0.05%, which enabled complete data analysis in most of our analysis. Continuous data are presented as a mean with standard deviation. Categorical data are presented as a count with a percentage. Concordance between two different C_0/D measurements was tested using intraclass correlation coefficients (ICC) [22]. ICC and their 95% confidence intervals (C.I.) were estimated based on a mean-rating ($k = 2$), consistency of agreement, and the two-way mixed effects model. Binary event outcomes were evaluated using multivariable logistic regression. The random effects model was applied to estimate the regression coefficient for repeated measurements of post-transplantation eGFR in the multilevel linear regression model. Multivariable adjustment was performed by predefined models based on medical knowledge, which were applied in a stepwise manner: model 1 was unadjusted; model 2 was adjusted for clinical characteristics, including age, sex, primary cause of end-stage renal disease, and recipient CMV IgG serostatus; model 3 was additionally adjusted for donor traits, including deceased donor transplantation, delayed graft function, cold ischemia time, and donor pretransplantation eGFR; model 4 was additionally adjusted for immunological variables, including number of HLA DR mismatch, desensitization, and acute rejection before C_0/D measurements; and model 5 was additionally adjusted for tacrolimus trough concentrations at 6 months post-transplantation. All statistical analyses were performed using Stata software (version 15; StataCorp LP, College Station, TX, USA). The library 'PARAMED' in Stata was used for causal mediation analysis.

Results

The distribution of C_0/D is depicted in Fig. 1. The median value of tacrolimus metabolism was 1.73 ng/ml/mg. The highest tertile of C_0/D was the slow metabolizer group, as a high trough level was maintained with low drug dosage. The lowest tertile of C_0/D was the fast metabolizer group. Breakpoints of tacrolimus metabolism tertiles were 1.3 ng/ml/mg and 2.3 ng/ml/mg. ICC analysis results showed moderate to good consistency (ICC 0.753, 95% C.I. 0.734–0.772, $P < 0.001$) between 6-month and 1-year C_0/D.

The baseline characteristics at the time of transplantation were compared according to the tacrolimus metabolism groups in Table 1. The mean age of the patients in the slow metabolizer group was higher than that of those in the other groups (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 45.9 ± 11.6 vs. 48.5 ± 11.4 vs. 51.4 ± 10.7 years, $P < 0.001$). In the slow metabolizer group, there were fewer females (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 45.6% vs. 43.5% vs. 33.4%, $P < 0.001$); the proportion of patients with diabetes as the cause of ESRD was higher (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 19.6% vs. 20.9% vs. 27.0%, $P < 0.001$); and the proportion of patients undergoing deceased donor transplantation was higher (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 36.6% vs. 38.2% vs. 43.3%, $P = 0.001$). Higher BMI was a clinical trait in the slow metabolizer group. However, cold ischemic time, desensitization rate, and donor-recipient MHC class II mismatch numbers were not statistically different. Deceased donor kidney transplantations were more common in the slow metabolizer group (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 36.1% vs. 37.5% vs. 43.2%, $P = 0.001$). The slow metabolizer group showed high trough levels of tacrolimus at 6 months post-transplantation (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 5.4 ± 2.0 vs. 6.6 ± 2.1 vs. 7.9 ± 2.7 ng/ml, $P < 0.001$). The difference in tacrolimus dosage was more prominent in daily dosages (2.4 ± 0.9 mg/day in the slow metabolizer group vs. 6.2 ± 2.5 mg/day in the fast metabolizer group). Steroid usage can reduce tacrolimus levels by inducing the CYP3A5 enzyme. Steroid dosage converted as prednisone dosage was lower in the slow metabolizer group (fast metabolizer vs. intermediate metabolizer vs. slow metabolizer, 7.2 ± 3.8 vs. 7.0 ± 3.5 vs. 6.5 ± 3.1, $P < 0.001$).

For the assessment of transplantation outcomes using the C_0/D, post-transplantation eGFR shows an association with the C_0/D at 6 months, which showed a 2.9 ml/min/1.73 m^2 higher eGFR in the slow metabolism group (95% C.I. 1.217–4.561, $P = 0.001$, Table 2) (unadjusted

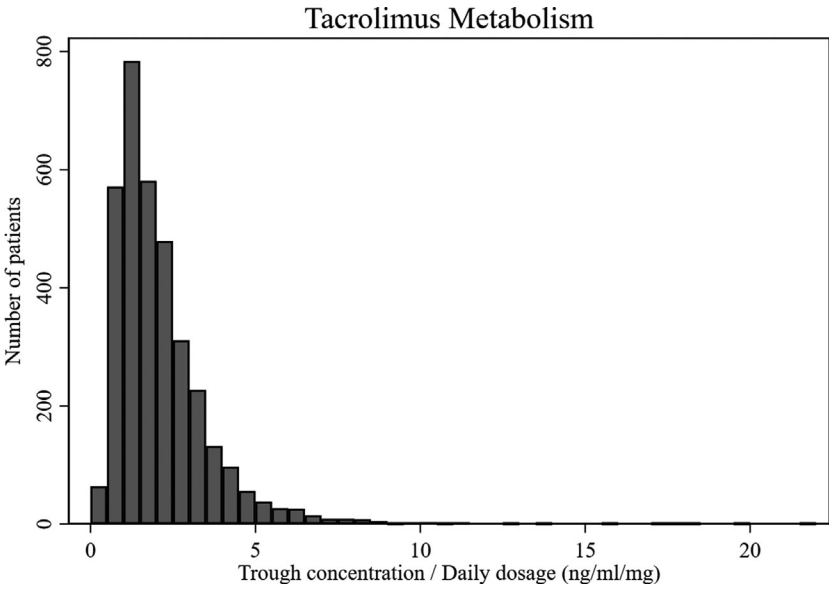

Figure 1 Distribution of tacrolimus metabolism.

model). The post-transplant 6 months and 1 year eGFR of slow metabolizer group was 68.5 ± 21.8 ml/min/1.73 m^2 and 70.4 ± 21.5 ml/min/1.73 m^2 compared to 65.6 ± 19.9 ml/min/1.73 m^2 (6 months) and 68.4 ± 20.0 ml/min/1.73 m^2 (1 year) of intermediate metabolizer group. In the fully adjusted model, the slow metabolizer group was associated with a 2.7 ml/min/1.73 m^2 higher eGFR (95% C.I. 1.163–4.317, $P = 0.001$). Figure 2 shows the regression coefficient and the 95% C.I. of the mixed-level multivariable regression model for post-transplantation eGFR. Among the baseline traits, old age, donor old age, female sex, cause of end-stage renal disease, pretransplantation donor eGFR, and cold ischemic time showed a significant association with the post-transplantation allograft eGFR.

As possible mediating mechanisms, delayed graft function and acute rejection were associated with post-transplantation allograft eGFR (delayed graft function, −6.019, 95% C.I. −9.272 to −2.766, $P < 0.001$; acute rejection, −10.017, 95% C.I. −12.231 to −7.803, $P < 0.001$). The allograft eGFR was better at 1 year post-transplantation compared to the 6-month post-transplantation eGFR (2.553, 95% C.I. 1.834–3.272, $P < 0.001$). In the fast metabolizer group, there was a negative interaction with time (−1.613, 95% C.I. −2.635 to −0.592, $P = 0.002$).

Tacrolimus metabolism was associated with the incidence of acute rejection. In the multivariable logistic regression analysis, shown in Table 3, slow tacrolimus metabolism was associated with reduced incidence of acute rejection (OR 0.744, 95% C.I. 0.585–0.947, $P = 0.016$). There was not an increased incidence of acute rejection in the fast metabolizer group. Among the patients with graft biopsies, slow metabolism was accompanied by less IFTA in allograft biopsies. In Table 4, the slow metabolizer group showed reduced odds ratios for IFTA in kidney biopsies (0.606, 95% C.I. 0.390–0.940, $P = 0.025$).

We tested whether tacrolimus metabolism was associated with post-transplantation eGFR by the mediation of acute rejection or delayed graft function by using the causal mediation model (Table 5). Compared to the slow metabolizers, the fast metabolizers showed a natural direct effect in the 6-month allograft eGFR (−3.175, 95% C.I. −4.964 to −1.385, $P = 0.001$). The natural indirect effect (NIE) of tacrolimus metabolism, which mediated acute rejection, was small but significant (−0.434, 95%. C.I. −0.856 to −0.012, $P = 0.044$; model 1 in Table 4). When the model was used to test the hypothesis that tacrolimus metabolism is mediated through delayed graft function to affect the 6-month post-transplantation eGFR (model 2), the NIE was also small but statistically significant. When we applied the delayed graft function-mediated model to assess the post-transplantation eGFR at the time of discharge, we found that the NIE of tacrolimus metabolism was significant and relatively large (−0.417, 95% C.I. −0.702 to −0.131, $P = 0.004$). Internal validation using bootstrapping resamples (Table S1) and sensitivity analysis in deceased donor kidney transplantation subgroup (Table S2) showed similar results.

Discussion

We investigated whether the simple clinical index of tacrolimus metabolism was associated with the clinical

Table 1. Baseline clinical characteristics of the study population according to tacrolimus metabolism.

Variables	Total (n = 3456)	Low tertile (fast metabolizer; n = 1155)	Mid tertile (intermediate metabolizer; n = 1153)	High tertile (slow metabolizer; n = 1148)	P
Age	48.6 ± 11.5	45.9 ± 11.6	48.5 ± 11.6	51.4 ± 10.7	<0.001
Female sex	1412 (40.9)	527 (45.6)	502 (43.5)	383 (33.4)	<0.001
Cause of ESRD					
Diabetes	777 (22.5)	226 (19.6)	241 (20.9)	310 (27.0)	<0.001
Hypertensive	563 (16.3)	190 (16.5)	175 (15.2)	198 (17.2)	
Glomerulonephritis	1186 (34.3)	418 (36.2)	404 (35.0)	364 (31.7)	
Others	275 (8.0)	97 (8.4)	80 (7.7)	89 (7.8)	
Unknown	655 (19.0)	224 (19.4)	244 (21.2)	187 (16.3)	
Body mass index (kg/m^2)	23.0 ± 3.5	22.9 ± 3.6	22.8 ± 3.5	23.4 ± 3.4	<0.001
Desensitization	753 (21.8)	260 (22.5)	254 (22.0)	239 (20.8)	0.599
Recipient CMV IgG positivity	3166 (91.6)	1048 (90.7)	1074 (93.1)	1044 (90.9)	0.465
MHC class I mismatch no.	2.3 ± 1.2	2.2 ± 1.2	2.3 ± 1.2	2.3 ± 1.3	0.197
MHC class II mismatch no.	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	0.242
Donor age	46.6 ± 12.7	46.3 ± 12.2	46.9 ± 12.9	46.8 ± 13.1	0.452
Female donor	1589 (45.8)	544 (47.1)	560 (48.4)	485 (41.9)	0.005
Deceased donor	1345 (38.9)	417 (36.1)	432 (37.5)	496 (43.2)	0.001
Donor pretransplant eGFR (ml/min/1.73 m^2)	99.3 ± 74.5	100.9 ± 76.9	100.9 ± 93.8	96.0 ± 44.4	0.200
Cold ischemic time (h)	3.5 ± 2.8	3.6 ± 2.9	3.4 ± 2.7	3.5 ± 2.8	0.390
Delayed graft function	136 (3.9)	54 (4.7)	50 (4.4)	32 (2.8)	0.046
Tacrolimus trough level (ng/ml)	6.7 ± 2.5	5.4 ± 2.0	6.6 ± 2.1	7.9 ± 2.7	<0.001
Tacrolimus dosage per day (mg/day)	4.1 ± 2.3	6.2 ± 2.5	3.9 ± 1.3	2.4 ± 0.9	<0.001
Prednisone dosage per day at 6 month	6.9 ± 3.5	7.2 ± 3.8	7.0 ± 3.5	6.5 ± 3.1	<0.001
Prednisone dosage per day at 1 year	6.0 ± 3.1	6.0 ± 2.6	6.1 ± 3.2	5.8 ± 3.3	0.068
Tacrolimus C_0/D at 6 month	2.1 ± 1.6	0.9 ± 0.2	1.8 ± 0.3	3.7 ± 1.8	<0.001
Tacrolimus C_0/D at 1 year	2.2 ± 1.5	1.2 ± 0.6	2.1 ± 1.1	3.4 ± 1.8	<0.001
6 month eGFR (ml/min/1.73 m^2)	66.8 ± 20.4	66.1 ± 19.4	65.6 ± 19.9	68.5 ± 21.8	0.001
1 year eGFR (ml/min/1.73 m^2)	68.7 ± 20.4	67.3 ± 19.6	68.4 ± 20.0	70.4 ± 21.5	0.002
Acute rejection within 6 month	545 (15.8)	187 (16.2)	200 (17.4)	158 (13.8)	0.055
Biopsy-proven acute rejection within 6 month	276 (7.9)	107 (9.3)	87 (7.5)	82 (7.1)	0.137

CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate.

Tacrolimus concentration/dosage is defined as the drug metabolizing activity of a patient. High C_0/D means slow metabolizer (= high drug concentration on relatively low drug dosage). Continuous variables are expressed as mean ± standard deviation, and categorical variables are expressed as number (percentage). Continuous variables were compared using t-test, ANOVA and categorical variables were compared by using Chi-square test, as appropriate.

outcome of kidney transplantation by analyzing the data from 3456 of the kidney transplant recipients registered in KOTRY. We grouped the kidney transplant recipients into tertiles by the tacrolimus metabolism rates. Among them, the slow metabolizer group showed the best post-transplantation eGFR, although the slow metabolizer group also had more traditional risk factors, such as elderly males and higher incidence of deceased donor kidney transplantation. Acute rejection and delayed graft function were significant mediating pathways for the 6-month post-transplantation eGFR, and the mediating effect of delayed graft function was greater for the eGFR at the post-transplantation discharge time.

Our study used the same simple clinical indicator, the tacrolimus trough concentration adjusted by the daily dose (C_0/D), used in the previous studies. Our study results are consistent with those of our previous study showing an association between eGFR with the tacrolimus metabolism rate, therefore, validating the findings of our previous study on 248 kidney transplant recipients [15], wherein better post-transplantation eGFR was noted in the slow tacrolimus metabolism group. Fast metabolizers underwent a higher number of indication renal biopsies, which showed a higher incidence of calcineurin inhibitor nephrotoxicity and BK nephropathy. Post-transplantation eGFR differences

Table 2. Analysis of tacrolimus metabolism with post-transplant estimated GFR.

Tacrolimus concentration/dosage	Low tertile (*n* = 1155)	Mid tertile (*n* = 1153)	High tertile (*n* = 1147)
Model 1			
Regression coefficient (95% CI)	0.445 (−1.219 to 2.118)	Reference	2.889 (1.217–4.561)
P-value	0.597		0.001
Model 2			
Regression coefficient (95% CI)	0.122 (−1.580 to 1.823)	Reference	3.781 (2.069–5.494)
P-value	0.889		<0.001
Model 3			
Regression coefficient (95% CI)	−0.182 (−1.735 to 1.372)	Reference	3.066 (1.497–4.635)
P-value	0.819		<0.001
Model 4			
Regression coefficient (95% CI)	−0.239 (−1.764 to 1.285)	Reference	2.797 (1.257–4.337)
P-value	0.758		<0.001
Model 5			
Regression coefficient (95% CI)	−0.188 (−1.743 to 1.367)	Reference	2.740 (1.163–4.317)
P-value	0.813		0.001

Model 1: tacrolimus metabolism, post-transplant month, interaction with time. Model 2: model 1 + Age, sex, cause of ESRD, CMV IgG. Model 3: model 2 + donor age, deceased donor kidney, delayed graft function, cold ischemia time, donor estimated glomerular filtration rate. Model 4: model 3 + HLA DR mismatch numbers, desensitization, acute rejection before 6 months. Model 5: Model 4 + tacrolimus trough concentration. Multivariable adjusted multilevel linear mixed regression model were applied to estimate the regression coefficient of tacrolimus metabolism group to the post-transplant eGFR. Acute rejection included both clinical rejection and biopsy-proven acute rejection.

between the intermediate and slow metabolizers were between 6.2 and 6.9 ml/min/1.73 m^2, which are twofold higher than the results of the present study. Relatively moderate differences in our study could be explained by the heterogeneity of the study population because we included patients at higher immunologic risks, such as patients who received ATG induction therapy or ABO-incompatible kidney transplantation.

Several factors might affect post-transplant eGFR in terms of tacrolimus metabolism. A previous study showed an association between acute rejection and fast tacrolimus metabolism [23]. A total of 683 patients were studied, and the biopsy-proven acute rejection rate was 13.3%. Compared to the slow metabolizer group, the fast metabolizer group showed a hazard ratio of 2.39 (95%. C.I. 1.70–2.99) for acute rejection within 90-days post-transplantation. In our study, the rate of biopsy-proven acute rejection was low (overall 7.9% within 6 months) compared to that in the previous study, and the rate of biopsy-proven acute rejection was not statistically different between the different tacrolimus metabolism groups (data not shown). However, when clinical rejection was included as an outcome variable, the tacrolimus slow metabolizer group was associated with a decreased acute rejection rate. The association between acute rejection rates and tacrolimus trough levels, which are more direct measurements of the tacrolimus AUC than the inherent features, such as tacrolimus metabolism rate, is debatable. A classic study clearly reported that a low tacrolimus trough level was associated with an increased acute rejection rate [24]; however, that finding has not been replicated in the modern immunosuppressant era. Bouamar et al. [25], studied 1304 patients using pooled data from three large trials and failed to show any significant correlation between tacrolimus trough levels and incidences of biopsy-proven acute rejection. However, another study group reported that tacrolimus trough levels were associated with acute rejection [26,27]. It is not clear why does the association between acute rejection and the level of tacrolimus, a key maintenance immunosuppressant, show disparate results. As Israni et al pointed out [26], the level of the tacrolimus trough in the modern immunosuppressant era is much narrower than in the classic report from Kershner, wherein the level of tacrolimus was above 25 ng/ml in the high dosage group [24]. In addition, a combination of more advanced immunosuppressants, such as mycophenolic acid, could compensate for the low level of tacrolimus compared to the weaker immunosuppressant azathioprine. In our study, acute rejection was shown to be a significant mediator in the association of tacrolimus metabolism with post-transplantation eGFR; however, the impact was very small. We explain this phenomenon by the

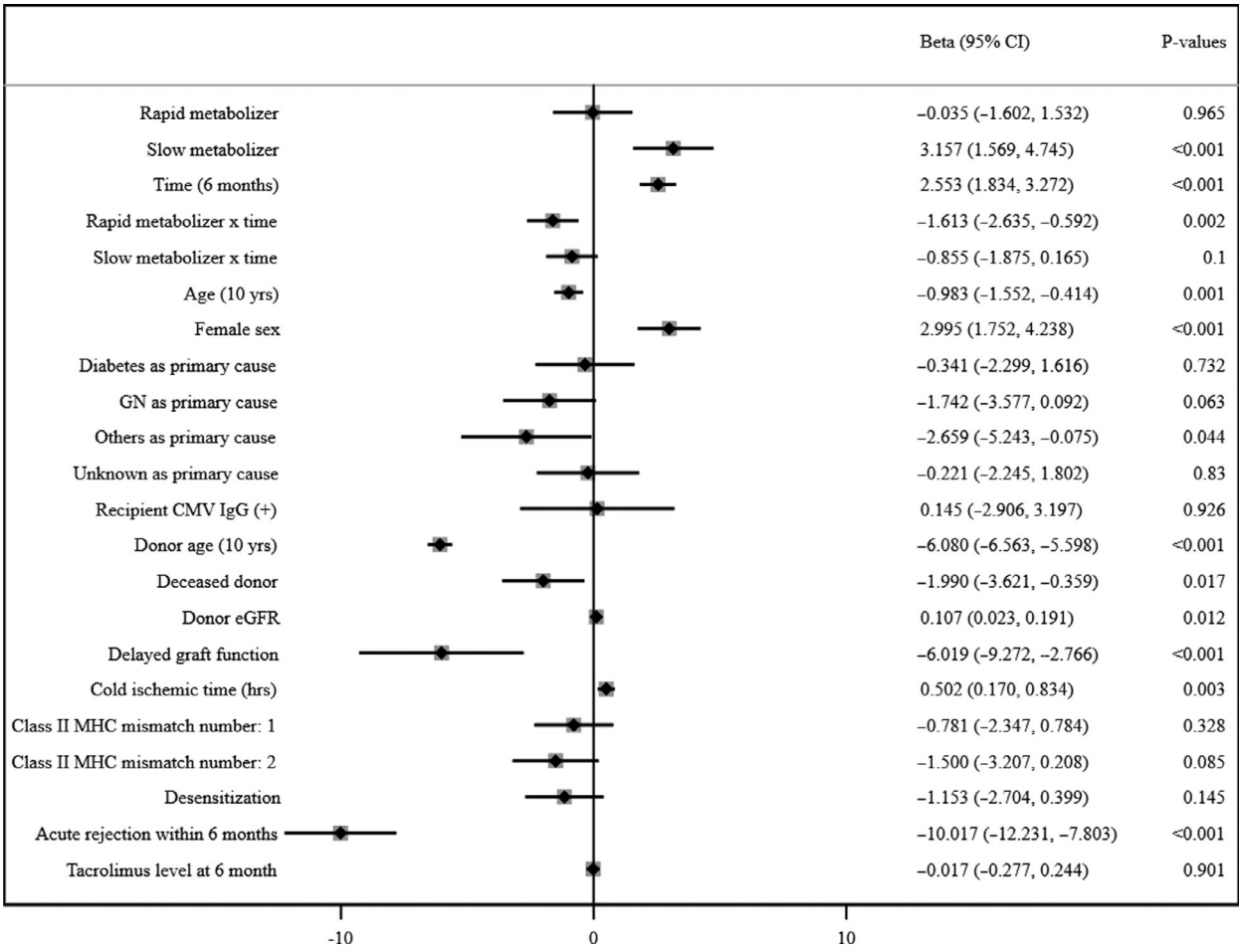

Figure 2 Regression coefficients of multivariable linear regression.

difference in susceptibility and treatment response to acute rejection, which might not be mediated by tacrolimus metabolism because steroid pulse treatment or anti-thymoglobulin is generally used for the treatment of acute rejection.

Delayed graft function was shown to be a significant mediator in the association of tacrolimus metabolism with post-transplantation eGFR, especially in the early post-transplantation period. This finding is consistent with that of a previous report that slow tacrolimus metabolizers showed better eGFR in liver transplant recipients [28]. As there is no kidney-oriented rejection mechanism in liver transplant recipients, tacrolimus metabolism affects the kidney function by the mechanism of calcineurin inhibitor toxicity, which can be presented as delayed graft function in a kidney transplant setting. Hence, our study reports the importance of the tacrolimus metabolism rate during early post-transplantation care, especially with respect to calcineurin inhibitor toxicity.

IFTA in the allograft biopsy was decreased in the slow metabolizer group, which was consistent with the findings of previous studies [28,29]. IFTA is a strong determinant of eGFR and could be interpreted as a sequela of subclinical rejection or the histologic manifestation of calcineurin inhibitor toxicity [24,30]. We cannot comment on whether the rapid washout of tacrolimus leads to under-immunosuppression and consecutive subclinical rejection because it is impossible to detect subclinical rejection without a protocol biopsy. Therefore, it was not feasible to test whether subclinical rejection mediates the effects of the tacrolimus metabolism rate on the post-transplantation eGFR. In addition, delayed graft function is often mixed or accompanied by acute rejection [31]; however, in our dataset, acute rejection was not associated with delayed graft function (data not shown). In addition, there was a recent report that fast metabolism is noticeably associated with higher C2 tacrolimus levels and calcineurin inhibitor-induced

Table 3. Tacrolimus metabolism with acute rejection within 6 months.

Tacrolimus concentration/dosage	Low tertile (fast metabolizer; *n* = 1155)	Mid tertile (intermediate metabolizer; *n* = 1153)	High tertile (slow metabolizer; *n* = 1148)
Model 1			
Hazard ratio (95% CI)	0.921 (0.740–1.145)	Reference	0.760 (0.606–0.954)
P-value	0.458		0.018
Model 2			
Hazard ratio (95% CI)	0.922 (0.737–1.154)	Reference	0.764 (0.606–0.964)
P-value	0.478		0.023
Model 3			
Hazard ratio (95% CI)	0.932 (0.744–1.168)	Reference	0.779 (0.617–0.984)
P-value	0.542		0.036
Model 4			
Hazard ratio (95% CI)	0.925 (0.737–1.160)	Reference	0.773 (0.612–0.978)
P-value	0.500		0.032
Model 5			
Hazard ratio (95% CI)	0.958 (0.760–1.207)	Reference	0.744 (0.585–0.947)
P-value	0.714		0.016

Model 1: unadjusted. Model 2: adjusted for age, sex, cause of ESRD, CMV IgG. Model 3: model 2 + deceased donor kidney, delayed graft function, cold ischemia time. Model 4: model 3 + HLA mismatch numbers, desensitization. Model 5: model 4 + tacrolimus trough concentration. Multivariable adjusted logistic regression models were applied. Acute rejection included both clinical rejection and biopsy-proven acute rejection.

nephrotoxicity [32]. Our study finding of reduced IFTA and better eGFR could also be interpreted as the effect of reduced calcineurin inhibitor nephrotoxicity, although the statistical testing of calcineurin inhibitor toxicity as mediator was not feasible.

Similar clinical outcomes were reported when investigations were performed using either C_0/D or *CYP3A5* genotyping. In the fast metabolizer group or equivalent *CYP3A5* expressor group, chronic nephrotoxicity and acute rejection were observed more frequently. In the present study, the impact of tacrolimus metabolism on the allograft eGFR was partly mediated by delayed graft function in the early post-transplantation period. As the C_0/D can be estimated after tacrolimus concentration stabilized, the implementation of the C_0/D clinical index in the early post-transplantation period is limited. We, therefore, think that pretransplantation genotyping is important. Therefore, one can imagine the intervention (pretransplantation cardiac management and choice of a less nephrotoxic initial immunosuppressant choice) to reduce delayed graft function aiming at improving post-transplantation eGFR in the genotype-predicted fast metabolizer. A recent study reported that peak concentration of tacrolimus in fast metabolizers is markedly different between immediate-release tacrolimus and once-daily extended-release tacrolimus, when the equivalent AUC is maintained [33]. Therefore, it would be very interesting to determine whether usage of continuous release tacrolimus in the fast metabolizer group could alleviate delayed graft function and lead to better eGFR in future.

There are limitations in the present study. Direct measurements of drug compliance were not feasible in this study; hence, the authors could not argue the complete exclusion of the effect of drug compliance. However, there is an additive risk in the fast metabolizer group because under-immunosuppression could occur more seriously in the fast metabolizer group, and intraindividual variability is another important issue in the maintenance of immunosuppression [7,17,34]. The association of acute rejection was not based on biopsy-proven rejection. However, the biopsy-proven acute rejection rate was low compared to that in previous studies [23], which could be due to more favorable immunologic traits, such as decreased incidence of deceased donor kidney transplantation or more frequent desensitization due to ABO-incompatible kidney transplantation. We could not adjust the tacrolimus trough levels measured prior to 6 months for the assessment of tacrolimus metabolism with acute rejection. We assumed that tacrolimus metabolism is an inherent trait, and we used the 6-month C_0/D to the involvement of the DGF mechanism in the post-transplantation discharge eGFR. There was a fair association between the 6-month C_0/D and 1-year C_0/D, and these indirect measurements might explain the lesser effect of tacrolimus

Table 4. Logistic regression of tacrolimus metabolism with IFTA at allograft biopsy.

Tacrolimus concentration/dosage	Low tertile (n = 413)	Mid tertile (n = 406)	High tertile (n = 398)
Model 1			
Odds ratio (95% CI)	0.852 (0.591–1.230)	Reference	0.640 (0.433–0.948)
P-value	0.392		0.026
Model 2			
Odds ratio (95% CI)	0.870 (0.595–1.272)	Reference	0.630 (0.417–0.952)
P-value	0.473		0.028
Model 3			
Odds ratio (95% CI)	0.879 (0.596–1.295)	Reference	0.651 (0.427–0.992)
P-value	0.513		0.046
Model 4			
Odds ratio (95% CI)	0.896 (0.606–1.325)	Reference	0.672 (0.440–1.026)
P-value	0.582		0.066
Model 5			
Odds ratio (95% CI)	0.966 (0.648–1.440)	Reference	0.606 (0.390–0.940)
P-value	0.866		0.025

Model 1: unadjusted. Model 2: adjusted for age, sex, cause of ESRD, CMV IgG. Model 3: model 2 + donor age, deceased donor kidney, delayed graft function, cold ischemia time, donor estimated glomerular filtration rate. Model 4: model 3 + HLA mismatch numbers, desensitization, acute rejection before 6 months. Model 5: model 4 + tacrolimus trough concentration. Multivariable adjusted logistic regression models were applied. Acute rejection included both clinical rejection and biopsy-proven acute rejection.

Table 5. Results of causal mediation analysis.

Variables	Estimate	95% Confidence interval	*P*-value
Model 1			
Natural direct effect	−3.175	−4.964 to −1.385	0.001
Natural indirect effect	−0.434	−0.856 to −0.012	0.044
Model 2			
Natural direct effect	−3.147	−4.949 to −1.345	0.001
Natural indirect effect	−0.119	−0.231 to −0.007	0.038
Model 3			
Natural direct effect	−1.629	−3.776 to 0.519	0.137
Natural indirect effect	−0.417	−0.702 to −0.131	0.004

Model 1: Y: allograft 6 months estimated glomerular filtration rate, X: slow metabolizer vs. intermediate metabolizer, M: acute rejection within post-transplant 6 months, C: Age, donor estimated glomerular filtration rate, delayed graft function, MHC class II mismatch number, tacrolimus trough level at 6 months. Model 2: Y: allograft 6 months estimated glomerular filtration rate, X: slow metabolizer vs. intermediate metabolizer, M: delayed graft function, C: Age, donor estimated glomerular filtration rate, acute rejection within post-transplant 6 months, MHC class II mismatch number, tacrolimus trough level at 6 months. Model 3: Y: allograft estimated glomerular filtration rate at discharge, X: slow metabolizer vs. intermediate metabolizer, M: delayed graft function, C: Age, donor estimated glomerular filtration rate, MHC class II mismatch number. Y denotes outcome variable, X denotes exposure variable, M denotes mediating variable, C denote covariables.

metabolism on the eGFR compared to that reported in previous studies. The lack of data of tacrolimus formulation is another important limitation. Tacrolimus formulation may directly affect the C_0/D.

In conclusion, patients with slow tacrolimus metabolism rates showed a better eGFR in the post-transplantation course. Reduced rates of acute rejection and decreased delayed graft function rates were significant mediators in the association of tacrolimus metabolism with post-transplantation eGFR. IFTA was also reduced in the slow tacrolimus metabolism group.

Authorship

HR: conceptualization, data collection and curation, analysis, original draft preparation, review and

editing. JCJ: conceptualization, data collection and curation, analysis, original draft preparation, review and editing. JMK: data collection and curation, review and editing. JWM: data collection and curation, review and editing. SKP: data collection and curation, review and editing. JL: data collection and curation, analysis, review and editing. TYK: data collection and curation, analysis, review and editing. JY: data collection and curation, review and editing. MSK: data collection and curation, review and editing. SH: Statistical consultation, analysis, review and editing. CA: conceptualization, data collection and curation, review and editing.

Funding

The authors have declared no funding.

Conflict of interest

The authors of this manuscript have conflicts of interest to disclose as described by the Transplant International [Sponsorship for research from Astellas Pharma Korea, Inc (FY18-2070)].

Acknowledgements

This research was supported by a fund by Research of Korea Centers for Disease Control and Prevention (code: 2014-ER6301-00, 2014-ER6301-01, 2014-ER6301-02, 2017-ER6301-00, 2017-ER6301-01, 2017-ER6301-02). The KOTRY Participating Centers & Group Members: Department of Nephrology, BHS Hanseo Hospital: JinMin Kong. Department of Surgery, College of Medicine, Hanyang University: Oh Jung Kwon. Department of Internal Medicine, Korea University Anam Hospital: Myung-Gyu Kim. Department of Surgery, Yonsei University Wonju College of Medicine, Wonju Severance Christian Hospital: Sung Hoon Kim. Department of Internal Medicine, Inje University Busan Paik Hospital: Yeong Hoon Kim. Department of Internal Medicine, Bongseng Memorial Hospital, Busan, Korea: Joong Kyung Kim. Department of Internal Medicine, School of Medicine, Kyungpook National University, Kyungpook National University Hospital, Daegu, South Korea: Chan-Duck Kim. Division of Nephrology, Department of Internal Medicine, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea: Ji Won Min. Department of Internal Medicine, Chonbuk National University Medical School: Sung Kwang Park. Department of Surgery, Gil Medical Center, Gachon University College of Medicine: Yeon Ho Park. Department of Nephrology, Ajou University School of Medicine: Inwhee Park. Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine: Jae Berm Park. Department of Nephrology, Konkuk University School of Medicine: Jung Hwan Park. Department of Nephrology, Yeungnam University Hospital: Jong-Won Park. Division of Nephrology, Department of Internal Medicine, Eunpyeong St. Mary's hospital, The Catholic University of Korea, College of Medicine: Tae Hyun Ban. Organ Transplantation Center and Department of Internal Medicine, Pusan National University Hospital: Sang Heon Song. Department of Surgery, Ewha Womans University Medical Center: Seung Hwan Song. Kosin University College of Medicine, Department of Internal Medicine, Division of Nephrology: Ho Sik Shin. Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's hospital. The Catholic University of Korea, College of Medicine: Chul Woo Yang. Department of Internal Medicine, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea College of Medicine: Hye Eun Yoon. Department of Nephrology, Chungnam National University Hospital: Kang Wook Lee. Division of Nephrology, Department of Internal Medicine, Maryknoll Medical Center: Dong Ryeol Lee. Division of Nephrology, Department of Internal Medicine, Pusan National University School of Medicine: Dong Won Lee. Department of Surgery, Kangdong Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea: Samuel Lee. Department of Nephrology, Kyung Hee University Hospital at Gangdong: Sang-Ho Lee. Department of Surgery, CHA Bundang Medical Center: Jung Jun Lee. Department of Nephrology, SNU Boramae Medical Center: Jung Pyo Lee. Department of Surgery, Myongji Hospital: Jeong-Hoon Lee. Department of Internal Medicine, Soonchunhyang University Seoul Hospital: Jin Seok Jeon. Department of Surgery, Inje University Ilsan Paik Hospital: Heungman Jun. Department of Internal Medicine, Kyung Hee University College of Medicine: Kyunghwan Jeong. Department of Surgery, Ewha Womans University Mokdong Hospital: Ku Yong Chung. Department of Surgery, Ulsan University Hospital: Hong Rae Cho. Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea: Man Ki Ju. Division of Nephrology, Seoul National University Bundang Hospital: Dong-Wan Chae. Department of Surgery, Chonnam National University Medical School: Soo Jin

Na Choi. Department of Surgery, Asan Medical Center: Duck Jong Han. Department of Internal Medicine, Keimyung University School of Medicine, Daegu, Korea: Seungyeup Han. Department of Transplantation Surgery, Severance Hospital, Yonsei University College of Medicine: Kyu Ha Huh. Department of Surgery, Transplantation center, Seoul National University Hospital: Jaeseok Yang. Department of Internal Medicine, Seoul National University College of Medicine: Curie Ahn. Statistical Collaborating Centers. Medical Research Collaborating Center (MRCC): Myoungjin Jan, Heejung Ahn. The Korean National Research Institute of Health, Centers for Disease Control and Prevention: Hyun-Young Park, Hae-Rim Choi.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Results of causal mediation analysis from bootstrapping.

Table S2 Results of causal mediation analysis in deceased donor kidney transplantation subgroup.

REFERENCES

1. Ekberg H, Tedesco-Silva H, Demirbas A, *et al*. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
2. Chapman JR. The KDIGO clinical practice guidelines for the care of kidney transplant recipients. *Transplantation* 2010; **89**: 644.
3. Nevins TE, Kruse L, Skeans MA, Thomas W. The natural history of azathioprine compliance after renal transplantation. *Kidney Int* 2001; **60**: 1565.
4. Nevins TE, Thomas W. Quantitative patterns of azathioprine adherence after renal transplantation. *Transplantation* 2009; **87**: 711.
5. Nevins TE, Robiner WN, Thomas W. Predictive patterns of early medication adherence in renal transplantation. *Transplantation* 2014; **98**: 878.
6. Wiebe C, Nevins TE, Robiner WN, Thomas W, Matas AJ, Nickerson PW. The synergistic effect of class II HLA epitope-mismatch and nonadherence on acute rejection and graft survival. *Am J Transplant* 2015; **15**: 2197.
7. Shuker N, Van Gelder T, Hesselink DA. Intra-patient variability in tacrolimus exposure: causes, consequences for clinical management. *Transplant Rev* 2015; **29**: 78.
8. Shuker N, Bouamar R, van Schaik RHN, *et al*. A Randomized controlled trial comparing the efficacy of Cyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. *Am J Transplant* 2016; **16**: 2085.
9. Rojas L, Neumann I, Herrero MJ, *et al*. Effect of CYP3A5*3 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. *Pharmacogenomics J* 2015; **15**: 38.
10. Kuehl P, Zhang J, Lin Y, *et al*. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; **27**: 383.
11. Roy J-N. CYP3A5 genetic polymorphisms in different ethnic populations. *Drug Metab Dispos* 2005; **33**: 884.
12. Park SY, Kang YS, Jeong MS, Yoon HK, Han KO. Frequencies of CYP3A5 genotypes and haplotypes in a Korean population. *J Clin Pharm Ther* 2008; **33**: 61.
13. Park J-Y, Cha Y-J, Kim K-A. CYP3A5 * 3 polymorphism and its clinical implications and pharmacokinetic role. *Transl Clin Pharmacol* 2014; **22**: 3.
14. Moes DJAR, Press RR, Ackaert O, *et al*. Exploring genetic and non-genetic risk factors for delayed graft function, acute and subclinical rejection in renal transplant recipients. *Br J Clin Pharmacol* 2016; 227.
15. Thölking G, Fortmann C, Koch R, *et al*. The tacrolimus metabolism rate influences renal function after kidney transplantation. *PLoS One* 2014; **9**: 1.
16. Il Min S, Kim SY, Ahn SH, *et al*. CYP3A5 *1 allele: Impacts on early acute rejection and graft function in tacrolimus-based renal transplant recipients. *Transplantation* 2010; **90**: 1394.
17. Ro H, Il Min S, Yang J, *et al*. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. *Ther Drug Monit* 2012; **34**: 680.
18. Cho JH, Yoon YD, Park JY, *et al*. Impact of cytochrome P450 3A and ATP-binding cassette subfamily B member 1 polymorphisms on tacrolimus dose-adjusted trough concentrations among Korean renal transplant recipients. *Transplant Proc* 2012; **44**: 109.
19. Chen JS, Li LS, Cheng DR, *et al*. Effect of CYP3A5 genotype on renal allograft recipients treated with tacrolimus. *Transplant Proc* 2009; **41**: 1557.
20. Yang J, Jeong JC, Lee J, *et al*. Design and methods of the korean organ transplantation registry. *Transplant Direct* 2017; **3**: e191.
21. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods* 2013; **18**: 137.
22. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 2016; **15**: 155.
23. Egeland EJ, Robertsen I, Hermann M, *et al*. High tacrolimus clearance is a risk factor for acute rejection in the early phase after renal transplantation. *Transplantation* 2017; **101**: e273.
24. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 1996; **62**: 920.
25. Bouamar R, Shuker N, Hesselink DA, *et al*. Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials†. *Am J Transplant* 2013; **13**: 1253.
26. Israni AK, Riad SM, Leduc R, *et al*. Tacrolimus trough levels after month 3

as a predictor of acute rejection following kidney transplantation: a lesson learned from DeKAF genomics. *Transpl Int* 2013; **26**: 982.
27. Gaynor JJ, Ciancio G, Guerra G, *et al*. Lower tacrolimus trough levels are associated with subsequently higher acute rejection risk during the first 12 months after kidney transplantation. *Transpl Int* 2016; **29**: 216.
28. Thölking G, Siats L, Fortmann C, *et al*. Tacrolimus concentration/dose ratio is associated with renal function after liver transplantation. *Ann Transplant* 2016; **21**: 167.
29. Egeland EJ, Reisaeter AV, Robertsen I, *et al*. High tacrolimus clearance – a risk factor for development of interstitial fibrosis and tubular atrophy in the transplanted kidney: a retrospective single-center cohort study. *Transpl Int* 2019; **32**: 257.
30. Naesens M, Kuypers DRJ, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* 2009; **4**: 481.
31. Wu WK, Famure O, Li Y, Kim SJ. Delayed graft function and the risk of acute rejection in the modern era of kidney transplantation. *Kidney Int* 2015; **88**: 851.
32. Thölking G, Schütte-Nütgen K, Schmitz J, *et al*. A low tacrolimus concentration/dose ratio increases the risk for the development of acute calcineurin inhibitor-induced nephrotoxicity. *J Clin Med* 2019; **8**: 1586.
33. West-Thielke P, Maldonado A, Patel SJ, Stevens DR, Meier-Kriesche U, Brennan D. What's in a high dose? An analysis of tacrolimus pharmacokinetic parameters in rapid metabolizers [abstract]. *Am J Transpl* 2019; **19** (suppl 3). https://atcmeetingabstracts.com/abstract/whats-in-a-high-dose-an-analysis-of-tacrolimus-pharmacokinetic-parameters-in-rapid-metabolizers/. Accessed Feb 26, 2020.
34. Borra LCP, Roodnat JI, Kal JA, Mathot RAA, Weimar W, Van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. *Nephrol Dial Transplant* 2010; **25**: 2757.