Original Article

IL-3 and *CTLA4* gene polymorphisms may influence the tacrolimus dose requirement in Chinese kidney transplant recipients

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Abstract

The highly variable pharmacokinetics and narrow therapeutic window of tacrolimus (TAC) has hampered its clinical use. Genetic polymorphisms may contribute to the variable response, but the evidence is not compelling, and the explanation is unclear. In this study we attempted to find previously unknown genetic factors that may influence the TAC dose requirements. The association of 105 pathway-related single nucleotide polymorphisms (SNPs) with TAC dose-adjusted concentrations (C0/D) was examined at 7, 30 and 90 d post-operation in 382 Chinese kidney transplant recipients. In *CYP3A5* non-expressers, the patients carrying the *IL-3* rs181781 AA genotype showed a significantly higher TAC logC0/D than those with the AG genotype at 30 and 90 d post-operation (AA *vs* AG, 2.21±0.06 *vs* 2.01±0.03, *P*=0.004; and 2.17±0.06 *vs* 2.03±0.03, *P*=0.033, respectively), and than those with the GG genotype at 30 d (AA *vs* GG, 2.21±0.06 *vs* 2.04±0.03, *P*=0.011). At 30 d, the TAC logC0/D in the grouped AG+GG genotypes of *CTLA4* rs4553808 was significantly lower than that in the AA genotype (*P*=0.041) in *CYP3A5* expressers, but it was higher (*P*=0.008) in the non-expressers. We further validated the influence of *CYP3A5* rs776746, *CYP3A4* rs2242480 and rs4646437 on the TAC C0/D; other candidate SNPs were not associated with the differences in TAC C0/D. In conclusion, genetic polymorphisms in the immune genes *IL-3* rs181781 and *CTLA4* rs4553808 may influence the TAC C0/D. They may, together with *CYP3A5* rs776746, *CYP3A4* rs2242480 and rs4646437, contribute to the variation in TAC dose requirements. When conducting individualized therapy with tacrolimus, these genetic factors should be taken into account.

Keywords: tacrolimus; Chinese kidney transplant recipients; individualized therapy; dose-adjusted concentration; gene polymorphism; CYP; IL-3; CTLA4

Introduction

Tacrolimus (FK506, TAC) is a calcineurin inhibitor that has been widely used in modern immunosuppressive therapy for solid organ transplantation since it was approved in 1994[1, 2]. Its efficacy has been confirmed through clinical trials and clinical prac-

tice. However, TAC is characterized by an oral bioavailability varying from 4% to 89% (mean approximately 25%) and a narrow therapeutic index[3]. In addition, TAC doses are poorly correlated with the concentrations in whole blood, and large inter-individual differences exist in the TAC dose required to achieve target blood concentrations[1, 2, 4, 5]. It was reported in a study of Chinese renal transplant recipients that the maximum area under the curve (AUC$_{max}$) was almost 4 times higher than the minimum (AUC$_{min}$) after the first oral dose[6, 7]. Therefore, it is very likely that a patient can receive a dose that is either too high or too low, resulting in an increased risk of graft rejec-

tion, graft loss, and reduced survival as well as an increased risk of infections and malignancies[8]. Although therapeutic drug monitoring (TDM) can reduce this risk, it is actually a trial-and-error approach and has no predictive value for the initial TAC dose[8]. Pharmacogenomics may be helpful in predicting the appropriate TAC dose to use after transplantation, one that will shorten the time to reach a stable drug dose.

Some clinical factors and genetic factors play critical roles in determining TAC pharmacokinetics (PK)[9–11]. Tacrolimus is known to be transported by P-glycoprotein (MDR1 or ABCB1) and metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5. TAC forms a complex with the immunophilin FK-binding protein 12 (FKBP12) and strongly inhibits calcineurin phosphatase activity, IL-2 expression, and subsequently, T-cell activation[7]. Genetic polymorphisms in TAC pharmacokinetic pathways have been widely investigated (www.pharmgkb.org), and it has been confirmed that a single-nucleotide polymorphism, 6986A>G, within intron 3 of the *CYP3A5* gene can affect the pharmacokinetics of tacrolimus[5, 12]. Recent studies have also shown that TAC pharmacokinetics can be affected by polymorphisms in *CYP3A4* (*22, *B, *1G), *ABCB1* (2677G>A/T, 1236C>T, 3435C>T), P450 (cytochrome) oxidoreductase (*POR*) *28, nuclear receptor subfamily 1, group I, member 2 (*NR1I2*), catechol-O-methyltransferase (*COMT*), Toll-like receptor 4 (*TLR4*), and *IL-18*[13–20]. However, these conclusions are controversial. There are also reports that polymorphisms in genes that participate in the pharmacodynamic (PD) pathway, including *IL-2, IL-6,* and *IL-18*, and genes involved in the immune system, for instance *TLR4*, can influence TAC pharmacokinetics[20–22]. The existing algorithms, including genetic and clinical factors, for TAC dose prediction only account for 40% to 60% of TAC dose variations[9, 23]. The rest of the variations may be caused by unknown genetic factors that are involved in TAC pharmacodynamics, the immune system, or other related pathways. Our study includes common SNPs involved in these pathways, including rs776746 (*ie*, *3 or 6986A>G) in *CYP3A5*, rs2242480 (*ie*, *1G or 20230 C>T) and rs4646437 in *CYP3A4*, rs181781 in *IL-3,* and rs4553808 in the cytotoxic T lymphocyte antigen-4 (*CTLA4*) gene. Because the C/D ratio (the ratio of the lowest concentration of the drug in the blood to the corresponding daily TAC dose) is a predictor of the TAC metabolism rate[24], we used the C/D ratio as an index of tacrolimus pharmacokinetics, with the aim of finding previously unknown genetic factors that influence the TAC dose requirement and that may be useful for individualized therapy with tacrolimus.

Materials and methods
Ethics statement
This study was approved by the Ethics Committee of the Institute of Clinical Pharmacology, Central South University (CTXY-120030-2), and it has been registered at http://www.chictr.org (ChiCTR-RNC-12002894). Written informed consent was obtained from all participants. All allogeneic kidney grafts were from voluntary living donors or from donors after cardiac death (DCD) obtained from 2010 to 2014.

Study population
All stable tacrolimus-treated kidney transplant recipients in the Third Xiangya Hospital and the Peking University Third Hospital from 2010 to 2014 were recruited for this retrospective study. We collected routine TDM (whole-blood C0), demographic and clinical data from the Chinese Scientific Registry of Kidney Transplantation (http://www.csrkt.org/). We selected eligible patients using the following inclusion criteria: (1) patients experiencing kidney transplantation for the first time, (2) patients taking TAC as the main immunosuppressive drug for a period of 3 months or longer post operation, and (3) patients volunteering to participate in this study. The exclusion criteria were (1) patients who changed TAC for cyclosporine or other immunosuppressive agent within 3 months post operation, (2) patients for whom important clinical data, *eg*, weight, TAC doses or blood concentrations, were missing, (3) patients who experienced graft failure or death after the operation, (4) patients for whom the quality of extracted DNA samples did not meet the MassARRAY sequence requirements. In total, 382 Chinese kidney transplant patients were included (for patient selection see Supplementary Figure S1), and all of them were followed for more than 3 months. The characteristics of the patients are given in Table 1. According to the therapeutic regimen, all patients were treated with tacrolimus combined with mycophenolic acid administered as its prodrug, mycophenolate mofetil, and a low dose of methylprednisolone. All patients received induction therapy by either antithymocyte globulin or daclizumab based on the patient risk for rejection. A tacrolimus loading dose of 0.10

Table 1. Characteristics of patients.

Variable	Value
Age (year), range (mean±SD)	10–70 (36.4±10.3)
Gender, *n* (%)	
Male	247 (64.8%)
Female	134 (35.2)
Weight (kg), range (mean±SD)	33–92 (58.4±10.0)
BMI, (kg/m²), range (mean±SD)	14.8–38.3 (21.2±3.6)
Hypertension, *n* (%)	
Yes	269 (70.8%)
No	111 (29.2)
TAC initial dose (mg/d), range (mean±SD)	1.8–16.4 (5.2±1.8)
TAC initial C (ng/mL), range (mean±SD)	0.9–21.1 (7.2±13.7)
GC dose (mg/d), range (mean±SD)	2.0–300.0 (21.8±24.3)
MMF dose (mg/d), range (mean±SD)	250–2500 (1272.9±332.6)
GLU (mmol/L), range (mean±SD)	3.5–12.6 (5.6±1.1)
BUN (mmol/L), range (mean±SD)	2.5–33.7 (7.2±4.3)
ALT (IU/L), range (mean±SD)	1.0–98 (22.5±14.9)
AST (IU/L), range (mean±SD)	5.0–54 (18.6±8.5)
Hb (g/L), range (mean±SD)	38.2–196 (127.1±22.0)
TP (g/L), range (mean±SD)	50.7–107.0 (71.2±8.5)

Abbreviations: ALT, alanine aminotransferase; AST, Aspartate transaminase; BMI, body mass index; BUN, urea nitrogen; C, concentration; GC, Glucocorticoid; GLU, glucose; MMF, mycophenolate mofetil; Hb, hemoglobin; SD, standard deviation; TAC, Tacrolimus; TP, total protein

mg/kg every 12 h was begun on the day of transplantation, and the tacrolimus dose was subsequently adjusted to achieve low predose blood concentrations of 6–12 ng/mL for the first 3 months. Tacrolimus doses were adjusted according to TDM.

Study design

We conducted a retrospective study to investigate the impact of pathway related SNPs on tacrolimus dose requirements during a 3 month post-transplantation period. The C/D ratio (ie, dose-adjusted drug C0) as an index of tacrolimus pharmacokinetics was used as an outcome variable. This ratio, defined as the drug C0 concentration per daily weight-adjusted drug dose $[(ng/mL)/(mg \cdot kg^{-1} \cdot d^{-1})]$, has commonly been used in other studies[25]. From 64 candidate genes, 105 SNPs (Supplementary SNP distributions) selected by a review of the published literature were genotyped in a cohort of 382 Chinese kidney transplant recipients who received similar therapy regimens. Doses and C0 values at 7, 30, and 90 d were collected and analyzed. Other clinical data, including gender, age, and body mass index, were also analyzed.

Genotyping analysis

Qualified 2 mL blood samples were collected from each patient. Genomic DNA was isolated from whole blood samples using Wizard Genomic DNA Purification Kits (Promega) and stored at -80 °C for analysis. The DNA concentration, purity, and degree of degradation were detected by agarose gel electrophoresis, and the OD260/OD280 values were verified by nucleic acid and protein assays. Participants were genotyped for rs776746 (CYP3A5 *3, 6986A>G), rs2242480 (CYP3A4 *1G, 20230C>T), CYP3A4 rs4646437, IL-3 rs181781, CTLA4 rs4553808 and other candidate SNPs by using the MassARRAY Compact System.

Statistical analysis

The data were analyzed using the IBM SPSS 20.0 statistical software. The data are expressed as the mean±SE (standard error) except when stated otherwise. The outcome variable C0/D at 7, 30, and 90 d post-transplantation were normalized by a logarithmic transformation and were confirmed to be not significantly different from a normal distribution by the Kolmogorov-Smirnov test. The general linear model was used to assess the statistical significance of differences in the logC0/D among different genotypes. Multiple comparison correction by age and gender was appropriately applied. The Hardy-Weinberg equilibrium test was performed using the appropriate 2 test. Pairwise r^2 and D' values for linkage disequilibrium were calculated using the online web tool SHEsis software (http://analysis2.biox.cn/myAnalysis.php). Groups were compared using the general linear model ANOVA or the Wilcoxon-Mann-Whitney test for continuous variables and X^2 or Fisher's exact test for categorical variables. The null hypothesis was rejected with a P value <0.05.

Results

Clinical characteristics of recipients

A total of 382 kidney transplant recipients (135 from Peking and 247 from Xiangya) were included in our retrospective study (Supplementary Figure S1). The collected clinical characteristics of all the recipients are listed in Table 1; 64.8% of the recipients were males. The average age was 36.4±10.3 years, and the average weight was 58.4±10.0 kg. The mean TAC initial dose among patients was 5.2±1.8 mg/d (range 3–16 mg/d).

Genotypes

Among the 105 candidate SNPs, we found only 6 SNPs (CYP3A5 rs776746, CYP3A4 rs2242480 and rs4646437, IL-3 rs181781, CTLA4 rs4553808 and ATP1B2 rs1641536) that were significantly associated with TAC logC0/D after the data were adjusted by age and gender. All the 6 SNPs were in Hardy-Weinberg equilibrium (Table 2). There were no significant clinical data variations among the different genotypes of CYP3A4 rs2242480, rs4646437, IL-3 rs181781 and ATP1B2 rs1641536 (Supplementary Table S1–S4). Although the daily doses of mycophenolate mofetil (MMF) at 1 week and 1 month showed differences, they were not significant (Supplementary Table S5). The glucose concentrations at 1 week in the different genotypes of CYP3A5 rs776746 and the urea nitrogen (BUN) in the different genotypes of CTLA4 rs4553808 were significantly different (Supplementary Tables S5, S6). To exclude the influence of the clinical variations, we adjusted them as the covariates in the following analysis. No significant differences were observed in the clinical characteristics of CYP3A5 expressers (rs776746 AA+AG) and non-expressers (rs776746 GG) (Supplementary Table S7).

Table 2. Allelic frequency of recipients according to genotypes.

Gene	SNP	Genotype			Allelic frequency (%)	P value*
CYP3A5	Rs776746	AA (n=24)	AG (n=155)	GG (n=192)	A/G (27.4/72.6)	0.325
ATP1B2	Rs1641536	AA (n=42)	AG (n=173)	GG (n=165)	A/G (33.8/66.2)	0.739
CYP3A4	Rs2242480	TT (n=23)	CT (n=160)	CC (n=196)	T/C (27.2/72.8)	0.730
CYP3A4	Rs4646437	TT (n=8)	CT (n=102)	CC (n=270)	T/C (15.5/84.5)	0.650
IL-3	Rs181781	GG (n=155)	AG (n=177)	AA (n=47)	G/A (64.2/35.8)	0.747
CTLA4	Rs4553808	AA (n=236)	AG (n=106)	GG (n=9)	A/G (82.3/17.7)	0.473

*Hardy-Weinberg equilibrium (HWE) Chi-squared test.

The association of *IL-3* rs181781, *CTLA4* rs4553808 and *ATP1B2* rs1641536 with the TAC logC0/D

The *IL-3* and *CTLA4* genes are two major components of the immune system. Our results showed that both *IL-3* rs181781 and *CTLA4* rs4553808 had significant influences on the TAC logC0/D at 3 months post-transplantation with adjusted *P* values of 0.033 and 0.012, respectively (Supplementary Figure S2). Considering that *CYP3A5* rs776746 is one of the most influential genetic factors and that it showed a strong influence (*P* value <0.001) at different time points (Figure 1) in our study, we then conducted an analysis stratified by *CYP3A5*

rs776746 mutations of the influence of *IL-3* rs181781 and *CTLA4* rs4553808. We classified the patients into *CYP3A5* expressers (rs776746 AA+AG genotypes) and *CYP3A5* non-expressers (rs776746 GG genotype). No significant influence of *IL-3* rs181781 was observed on the TAC logC0/D in the *CYP3A5* expression group, whereas among the *CYP3A5* non-expressers, the *IL-3* rs181781 AA genotype showed a higher TAC logC0/D than the AG genotype at 30 and 90 d (AA *vs* AG, 2.21±0.06 *vs* 2.01±0.03, *P*=0.004; and 2.17±0.06 *vs* 2.03±0.03, *P*=0.033, respectively), and the GG genotype at 30 d (AA *vs* GG, 2.21±0.06 *vs* 2.04±0.03, *P*=0.011) post-transplantation
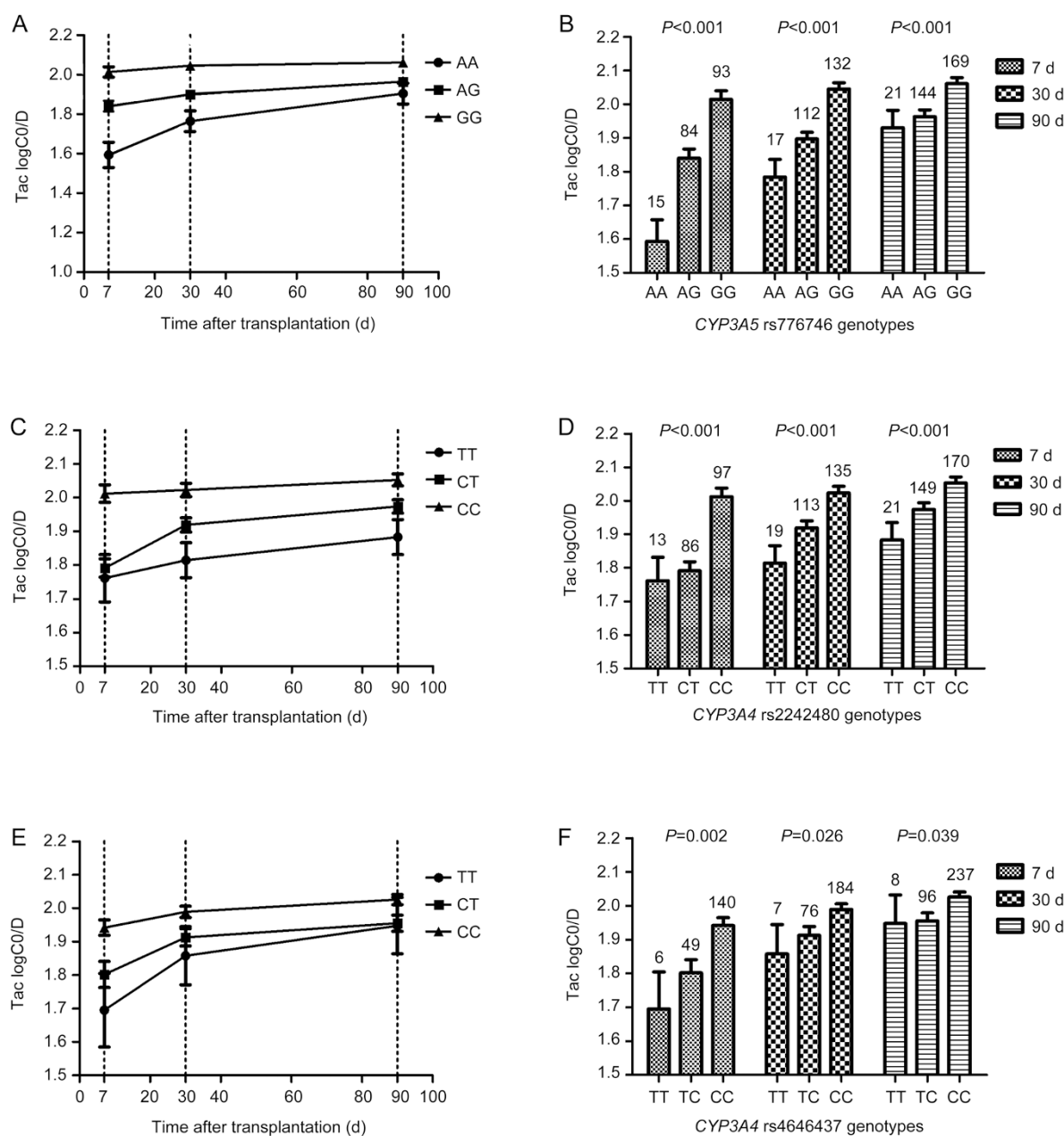

Figure 1. Effects of *CYP3A5* rs776746, *CYP3A4* rs2242480 and rs4646437 genetic polymorphisms on the TAC C0/D. Different genotypes of *CYP3A5* rs776746 (A, B), *CYP3A4* rs2242480 (C, D), and *CYP3A4* rs4646437 (E, F) were found to be associated with TAC logC0/D variations at 7, 30, 90 d post transplantation after the data were adjusted for age, gender, glucose concentrations, and BUN.

(Figure 2). For *CTLA4* rs4553808 (-1661 A>G), however, the frequency of the G allele was lower, and it has been reported that the functional effect of *CTLA4* can be attributed to the -1661G allele[26]. In our study, considering the restriction of the small number (*n*=9) of GG genotypes among the patients, we grouped the G allele carriers (GG+AG genotypes) when conducting the stratified analysis with the *CYP3A5* rs776746 mutations. The results of our study showed that the TAC logC0/D among the AG+GG genotypes was lower in *CYP3A5* expressers (AG+GG *vs* AA, 1.83±0.04 *vs* 1.91±0.02, *P*=0.041) at 30 d, whereas in non-expressers, it was higher (AG+GG *vs* AA, 2.11±0.03 *vs* 2.01±0.02, *P*=0.008). However, the TAC logC0/D showed no difference at 7 or 90 d post-operation (Figure 2). When we analyzed the influence of *ATP1B2* rs1641536, however, although it showed an influence on the TAC logC0/D at 7 d post-operation (Supplementary Figure S2), after validation by the *CYP3A5* rs776746 stratified analysis, the effect did not exist in the *CYP3A5* expressers or in the non-expressers.

The association of *CYP3A5* rs776746, *CYP3A4* rs2242480 and rs4646437 with the TAC logC0/D

CYP3A5 and CYP3A4 are the two main metabolic enzymes

of tacrolimus. We found that *CYP3A5* rs776746 and *CYP3A4* rs2242480 and rs4646437 were associated with the TAC logC0/D after being adjusted by clinical data (including age and gender) at 7, 30, and 90 d post-transplantation. *CYP3A5* rs776746 showed the strongest influence (*P* value <0.001) at different time points (Figure 1). In the stratified analysis for the influence of *CYP3A4* rs2242480 and rs4646437, we found that in the *CYP3A5* expression group, a significant difference was observed in the TAC logC0/D of different genotypes of rs2242480 at 7 d post-transplantation. The TAC logC0/D of patients carrying the CC genotype was significantly higher than that of patients with the CT genotype (CC *vs* CT, 1.95±0.06 *vs* 1.77±0.03; *P*=0.021) or the TT genotype (CC *vs* TT, 1.95±0.06 *vs* 1.73±0.07; *P*=0.029), whereas it was not significantly different in patients with different rs4646437 genotypes at different time points (Figure 3). For the *CYP3A5* non-expression group, however, because of the limited number of cases of rs2242480 TT (*n*=3) and rs4646437 TT (*n*=1), we grouped them together with the rs2242480 CT and rs4646437 CT genotype patients when performing the analysis. The TAC logC0/D was significantly influenced by both rs2242480 (CC *vs* CT+TT, 2.08±0.02 *vs* 1.96±0.04; *P*=0.01) and rs4646437 (CC
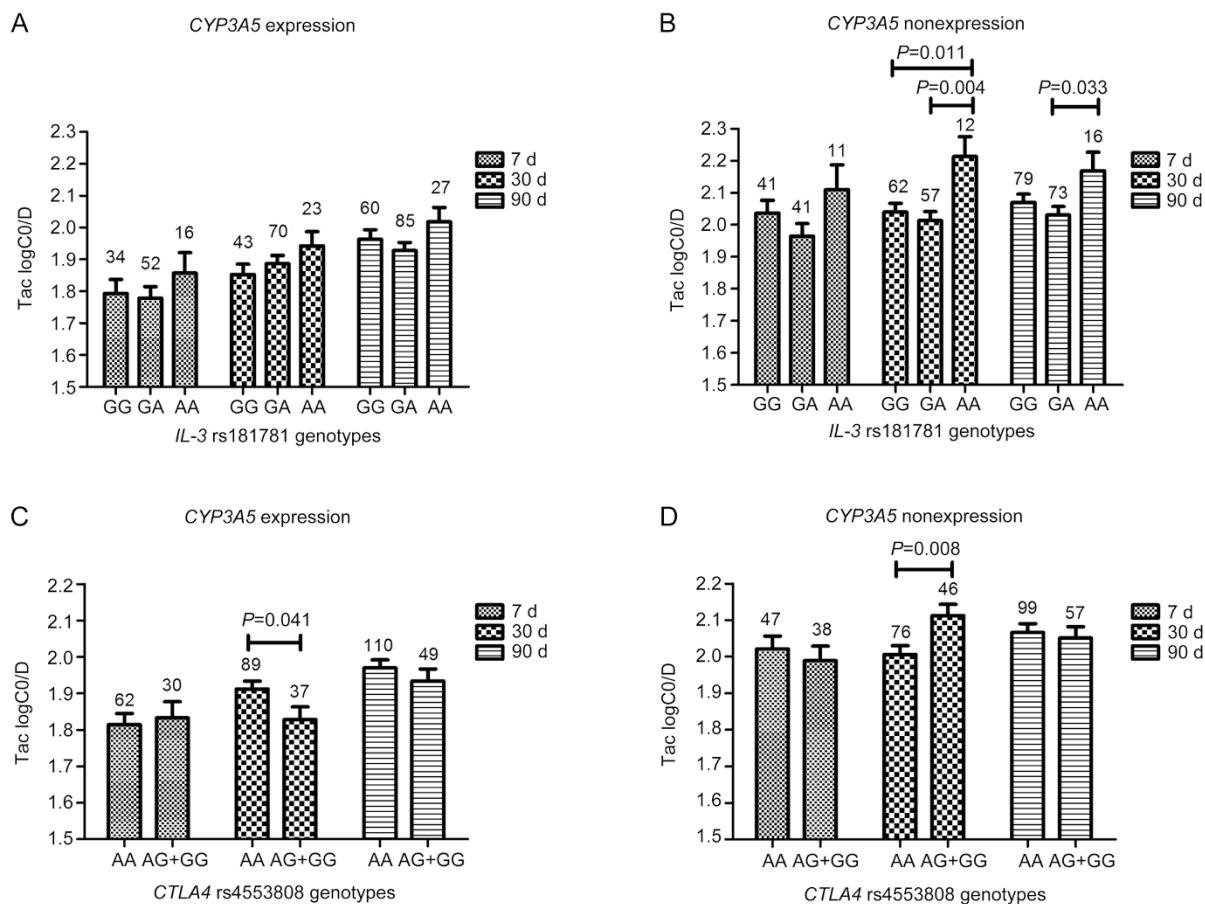

Figure 2. The influence of *IL-3* rs181781 and *CTLA4* rs4553808 on TAC logC0/D in *CYP3A5* expressers and non-expressers. (A and B) represent the association of *IL3* rs181781 polymorphisms with TAC logC0/D in *CYP3A5* expressers and non-expressers, respectively; (C and D) represent the association of *CTLA4* rs4553808 polymorphisms with TAC logC0/D in *CYP3A5* expressers and non-expressers, respectively.
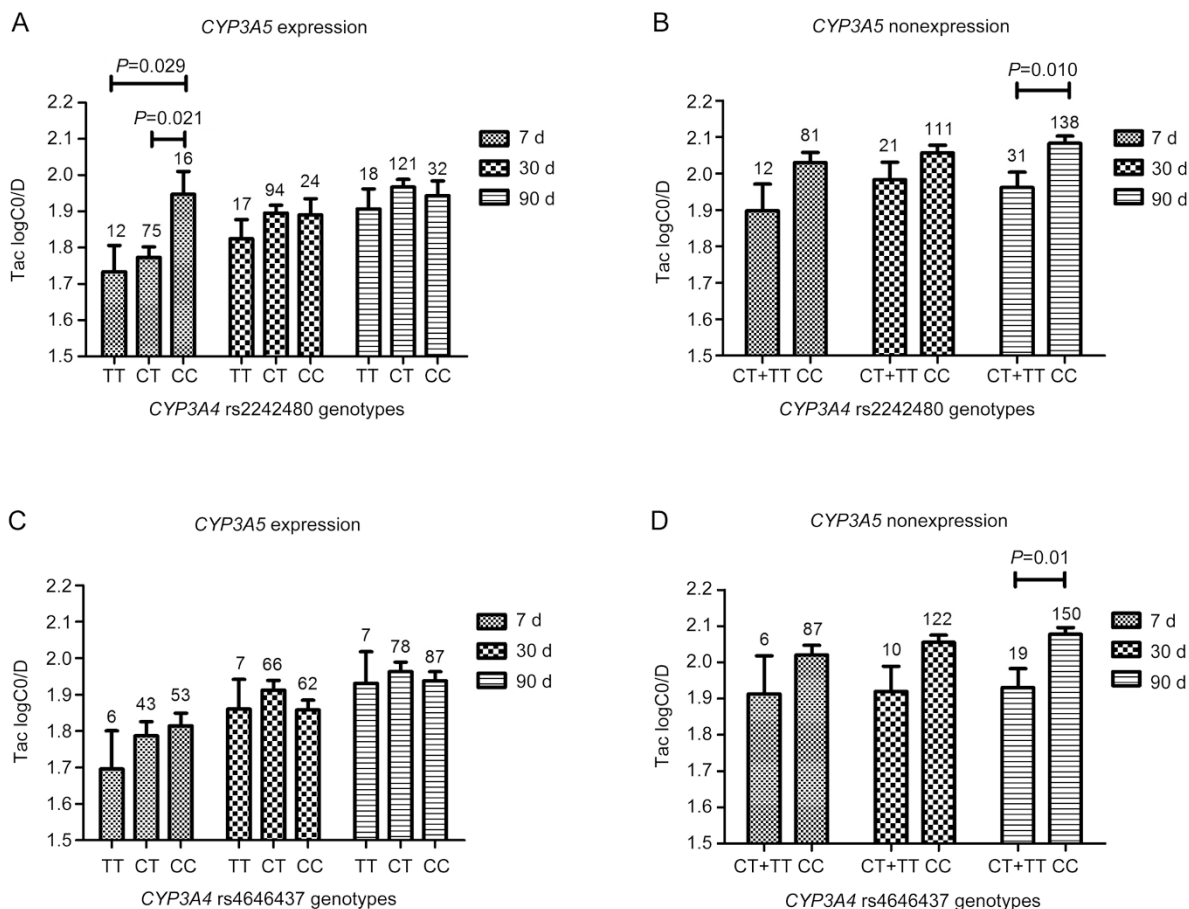
Figure 3. The influence of *CYP3A4* rs2242480 and rs4646437 on TAC logC0/D in *CYP3A5* expressers and non-expressers. (A and B) represent the association of *CYP3A4* rs2242480 polymorphisms with TAC logC0/D in *CYP3A5* expressers and non-expressers, respectively; (C and D) represent the association of *CYP3A4* rs4646437 polymorphisms with TAC logC0/D in *CYP3A5* expressers and non-expressers, respectively.

vs CT+TT, 2.08±0.02 *vs* 1.93±0.05; *P*=0.01) mutations at 90 d post-transplantation (Figure 3). The rs2242480 and rs4646437 mutations showed a similar trend. Considering that these 2 SNPs and *CYP3A5* rs776746 are closely spaced on chromosome 7, we conducted a linkage disequilibrium (LD) analysis. Our results showed that an LD block exists between rs2242480 and rs4646437 (*D'*=0.96, *r²*=0.45), whereas the LD of these with *CYP3A5* rs776746 is not significant, with a *D'*=0.68 for both (Supplementary Figure S3).

Discussion

IL-3 is a secreted glycoprotein that participates in the immune response. Previous studies have demonstrated that a promoter SNP of *IL-3*, rs181781, is associated with acute rejection (AR) after kidney transplantation[27]. Our study showed that in *CYP3A5* non-expressers, the *IL-3* rs181781 AA genotype showed higher TAC logC0/Ds than the AG genotypes at 30 and 90 d (*P*=0.004 and *P*=0.033, respectively), and the GG genotypes at 30 d (*P*=0.011) post-transplantation (Figure 2). As reported by Lee *et al*, in the promoter SNP rs181781 site, 3 transcription factors (Sp1, SRF, and HNF2C) bind to the A-containing sequence, whereas only 1 (SP1) binds to the

G-containing sequence; therefore, it is likely that rs181781 may influence the expression of *IL-3* by influencing transcription factor binding[27]. Considering the role of IL-3 in the immune system, differences in the expression of IL-3 may influence the tacrolimus dose requirements.

The human *CTLA-4* gene, which is approximately 6.2 kb in size, is located on human chromosome 2[28]. The gene behaves as a critical negative regulator of the T cell-mediated immune response that can inhibit T Helper (TH) 1 and TH2 cytokine production and cell cycle progression in antigen presenting cells (APC)[29]. Numerous studies have shown the association of *CTLA-4* polymorphisms with allograft rejection, end-stage renal disease, delayed graft function and infection after renal transplantation[29-32], and the expression pattern of the protein encoded by CTLA4 was found to be altered by the polymorphisms rs4553808 (-1661A/G) in the CTLA4 gene promoter[33]. It is reported that a carry of G allele variation will increase CTLA4 mRNA level and expression, thereby promoting consensus sequence in the regulatory region of the CTLA4 promoter in humans[26, 34]. The result of our study revealed that polymorphism of rs4553808 (-1661A/G) in CTLA4 gene promoter may influence TAC dose requirement. Compared

with AA genotypes, the TAC logC0/D at 30 d post-transplantation in the grouped AG+GG genotypes is significant lower in CYP3A5 expression group patients (*P*=0.041; Figure 2); however, when compared with the different genotypes in the *CYP3A5* non-expression patients, the result was reversed. The TAC logC0/D at 30 d post-transplantation in the grouped AG+GG genotypes was significantly higher than that of the AA genotypes (*P*=0.008, Figure 2). The results observed in the *CYP3A5* expression group may be related to the expression state of *CYP3A5*, which is the polymorphism that has the greatest influence on TAC pharmacokinetics[5]. In the *CYP3A5* non-expression patients, the results may be more convincing because they exclude the effect of *CYP3A5* expression. However, our results showed no difference at 7 or 90 d post-operation. This result is credible considering the fact that it takes 2 or more weeks to reach a stable maintenance dose of TAC[23].

To our knowledge, our study is the first to report that the *IL-3* rs181781 and *CTLA4* rs4553808 genetic polymorphisms can affect the TAC dose requirement. As key elements in the immune system, changes in the expression of *IL-3* and *CTLA4* can lead to alterations of the immune response, which in turn can influence the TAC dose required to cause immune suppression. It is difficult to understand the result of a study showing that after TAC treatment for 24 h, the expression level of IL-3 was significantly down-regulated[35]. In fact, other cytokines, including IL-2, IL-4, IL-6 and IL-18, can be selectively inhibited by tacrolimus[36]. Previous studies have reported genetic associations of *IL-2, IL-4* and *IL-6* with blood concentrations of calcineurin inhibitors[21]. Genetic polymorphisms in *IL-18* (*eg*, rs5744247) and *IL-10* (*eg*, -819 C/T and -592 C/A) can also affect TAC dose requirements[37, 38]. However, the exact mechanism by which these polymorphisms affect TAC dose requirements is unknown. There is a hypothesis that these immune related genes may influence the expression of genes that play important roles in TAC pharmacokinetics, and a study reported that increased serum IL-1beta, IL-6, and IL-10 could lead to a significant decrease in cytochrome P450 (CYP)-mediated drug metabolism[39].

Our results also validate with a large sample size (334 cases) that an rs776746 mutation in intron 3 of the *CYP3A5* gene is significantly (*P*<0.001) associated with tacrolimus pharmacokinetics at different times post-transplantation (Figure 1). The *CYP3A5* gene codes for the main metabolic enzyme for tacrolimus, and in the main functional variant, *CYP3A5* 6986A>G (rs776746), a guanine is substituted for adenine. Carriers of the A allele (AA or AG, *CYP3A5* expressers) can produce very high levels of functional *CYP3A5* compared with homozygous GG carriers (*CYP3A5* non-expressers)[12]. Thus, GG genotype patients need less TAC (high C0/D ratio) to reach the target blood concentration than the A allele carriers. In accordance with this explanation, our data showed a significantly higher level of the logarithmic transformed TAC C0/D in GG than in AG or AA genotype patients at 7, 30 and 90 d post-transplantation. Interestingly, the differences in the logC0/D decreased with time but were still significant at 3 months post transplantation (Figure 1). Our study was restricted by the follow-up

time (3 months in all cases); therefore, the effect of the *CYP3A5* rs776746 polymorphism on the TAC logC0/D at longer times requires validation.

Tacrolimus can also be metabolized by CYP3A4, which is second to CYP3A5 in importance. Our results show that *CYP3A4* rs2242480 (*1G) and rs4646437 are significantly associated with the TAC logC0/D at different times after adjusting for age and gender. The results are consistent with the research results of Li *et al*[19]. A previous study suggested that the rs4646437-T allele was in strong LD (r^2=0.82) with the *CYP3A5*1 allele in Caucasian renal transplant recipients[40]. A study of Chinese groups showed a moderate degree (r^2=0.502) of LD between rs2242480 and rs776746, and a low degree (r^2=0.244) of LD between rs4646437 and rs776746[19]. In our study (r^2=0.46 and r^2=0.22, respectively), an LD block was observed between rs2242480 and rs4646437 (Supplementary Figure S3). These two SNPs should be analyzed together. However, the results of our stratified analysis showed that both rs2242480 and rs4646437 affected the TAC logC0/D at 3 months in *CYP3A5* non-expressers (GG). In *CYP3A5* expressers (AA+AG), rs2242480 also showed an influence on the TAC logC0/D at 7 d (Figure 3). The discrepancies in the relationships between the rs2242480 and rs4646437 polymorphisms and the logC0/D at d 7 and d 90 may be due to the small sample size analyzed at d 7. No influence on the TAC logC0/D was observed for rs4646437 at different time points in *CYP3A5* expressers. This is consistent with another study of *CYP3A4* rs2242480[41]. Actually, *CYP3A4* rs2242480 is a functional SNP that could increase the activity of CYP3A4, and it resembles rs4646437 that can affect the hepatic CYP3A4 protein expression[19, 42]. We conclude that *CYP3A4* rs2242480 and rs4646437 are independent factors that can influence the TAC logC0/D.

We have also found that for the ATPase beta 2 (*ATP1B2*) SNP rs1641536, the homozygous GG and AA patients showed a trend toward higher logC0/D than the heterozygous patients. The difference was significant at d 7 (*P*=0.021, Supplementary Figure S2). However, in an analysis stratified for *CYP3A5* expression, there was no difference at different times. It is likely that the significance may be caused by the effect of the *CYP3A5* rs776746 variation because of its unbalanced distribution in different genotypes of rs1641536. The effect of *ATP1B2* rs1641536 should be validated in further studies.

Our study has several limitations. This is a retrospective study, and some personal donor information was not available, although the genotypes of donors have been reported to be important factors influencing TAC dose requirements. In addition, some important clinical data, including the TAC C0 and drug dose, were not always completely recorded at 7, 30 and 90 d after renal transplantation. Consequently, it was difficult to integrate all the samples and the clinical data. Furthermore, we did not include drug combinations, *eg*, calcium channel blockers, proton pump inhibitors, and anti-fungal drugs, because in some cases, this information was missing; however, some studies have indicated that drug combinations can affect TAC blood concentrations.

Conclusions

The findings of our retrospective study suggest that among the candidate 105 SNPs that function in different pathways, the *CYP3A5* rs776746, *CYP3A4* rs2242480 and rs4646437, *IL-3* rs181781 and *CTLA4* rs4553808 genetic polymorphisms probably influence the TAC dose requirements in Chinese kidney transplant recipients.

Our findings may contribute to understanding the genetic basis for individual variations in tacrolimus pharmacokinetics, and may provide information for further studies of the clinical implications of tacrolimus for individualized medicine.

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Author contribution

Mou-ze LIU contributed to the study design, research performance, data analysis and manuscript writing; Hua-wen XIN, Ying-zi MING, and Wei ZHANG as the co-corresponding authors were involved in study design, data collection and final approval of the version to be published; Hai-yan HE, Yue-li ZHANG, Fa-zhong HE, Jian-quan LUO, Zhi-ying LUO, and Ming-jie SHAO participated in sample collection, acquisition of data, experiments and revision of the draft; Yong-fang HU, Xiao-ping CHEN, Zhao-qian LIU, and Hong-hao ZHOU contributed to the study design and data analysis.

Supplementary information

Supplementary information is available at website of Acta Pharmacologica Sinica.

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