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PLOS ONE

# Impact of the *CYP3A5*, *CYP3A4*, *COMT*, *IL-10* and *POR* Genetic Polymorphisms on Tacrolimus Metabolism in Chinese Renal Transplant Recipients

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## Abstract

Tacrolimus is a widely used immunosuppressive drug for preventing the rejection of solid organ transplants. The efficacy of tacrolimus shows considerable variability, which might be related to genetic variation among recipients. We conducted a retrospective study of 240 Chinese renal transplant recipients receiving tacrolimus as immunosuppressive drug. The retrospective data of all patients were collected for 40 days after transplantation. Seventeen SNPs of *CYP3A5*, *CYP3A4*, *COMT*, *IL-10* and *POR* were identified by the SNaPshot assay. Tacrolimus blood concentrations were obtained on days 1–3, days 6–8 and days 12–14 after transplantation, as well as during the period of the predefined therapeutic concentration range. Kruskal–Wallis test was used to examine the effect of genetic variation on the tacrolimus concentration/dose ratio ($C_0/D$) at different time points. Chi-square test was used to compare the proportions of patients who achieved the target $C_0$ range in the different genotypic groups at weeks 1, 2, 3 and 4 after transplantation. After correction for multiple testing, there was a significant association of $C_0/D$ with *CYP3A5*3*, *CYP3A4*1G* and *CYP3A4* rs4646437 T>C at different time points after transplantation. The proportion of patients in the *IL-10 rs1800871-TT* group who achieved the target $C_0$ range was greater ($p = 0.004$) compared to the *IL-10 rs1800871-CT* and *IL-10 rs1800871-CC* groups at week 3 after transplantation. *CYP3A5*3*, *CYP3A4 *1G*, *CYP3A4* rs4646437 T>C and *IL-10* rs1800871 C>T might be potential polymorphisms affecting the interindividual variability in tacrolimus metabolism among Chinese renal transplant recipients.

**Citation:** Li C-J, Li L, Lin L, Jiang H-X, Zhong Z-Y, et al. (2014) Impact of the *CYP3A5*, *CYP3A4*, *COMT*, *IL-10* and *POR* Genetic Polymorphisms on Tacrolimus Metabolism in Chinese Renal Transplant Recipients. PLoS ONE 9(1): e86206. doi:10.1371/journal.pone.0086206

**Editor:** Marie-Pierre Dubé, Universite de Montreal, Canada

**Received** May 14, 2013; **Accepted** December 9, 2013; **Published** January 21, 2014



**Funding:** This work was funded by a programme grant from the National Natural Science Fund of China to Liang Li (NSFC, No. 81101328), the Pearl River Young Talents of Science and Technology in Guangzhou to Liang Li (No. 2013J2200050), the Natural Science Fund of Guangdong to Liang Li (No. S2012040007745), the Research Fund for the President of Nanfang Hospital to Chuan-Jiang Li. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Tacrolimus is an effective immunosuppressive drug widely used in solid organ transplantation to prevent rejection [1]. It is characterized by a narrow therapeutic range and large inter- and intraindividual variability in its pharmacokinetics [2]. Therefore, daily drug monitoring and dosage adjustment of tacrolimus are widely used so that the concentrations of the drug can be adjusted to achieve the target trough blood concentration ($C_0$) range [3]. In current clinical practice, it can take several weeks to reach the target $C_0$ range and transplant recipients experience significant risk of graft rejection or toxicity during this period; so, it is very important to achieve a stable maintenance dose as quickly as possible [4]. However, dose requirement and the length of time required to reach the target $C_0$ range show significant interindividual and interethnic variability. Full understanding of this mechanism is highly desirable for the patients to improve the therapeutic efficacy and reduce the side effects.

Tacrolimus is metabolized mainly by biotransformation enzymes cytochrome P450 (CYP) 3A4 and 3A5 [3,5]. The single nucleotide polymorphism (SNP) *6986A>G* in intron 3 of the *CYP3A5* gene, referred to as *CYP3A5*3*, results in a splicing defect and the absence of protein activity, unlike the A nucleotide with normal protein activity, referred to as *CYP3A5*1*. Patients carrying at least one *CYP3A5*1* allele are named CYP3A5 expressers and those with *CYP3A5*3/*3* genotype are named CYP3A5 non-expressers [6]. It has been shown that CYP3A5 expressers require a higher maintenance tacrolimus dose and longer time to achieve the target tacrolimus $C_0$ compared to CYP3A5 nonexpressers among organ transplant recipients [7–12]. Moreover, a study revealed that the *CYP3A5* rs28365085 T>C might have functional consequence on CYP3A5 activity [13]. Besides the SNPs of *CYP3A5* gene, the functional variants of *CYP3A4* gene may also influence tacrolimus pharmacokinetics. Wang et al. reported that *CYP3A4 *22* (rs35599367, *intron 6 C>T*) markedly affects CYP3A4

mRNA level and could serve as a biomarker for predicting response to CYP3A4-metabolized drugs [14]. The *CYP3A4* rs33972239 delT locates in exon 13 of *CYP3A4* gene. So it is a susceptible variant affecting the enzyme activity. He et al. reported that *CYP3A4*1G* (rs2242480, *20230 C>T*) allele can increase the activity of the CYP3A4 enzyme [15]. In addition, schirmer et al. reported that *CYP3A4* rs4646437 T>C can affect the hepatic CYP3A4 protein expression levels [16]. Cytochrome P450 oxidoreductase (*POR*) is required for drug metabolism by all microsomal cytochrome P450 enzymes. Zhang et al. reported that SNPs in the *POR* gene influence the rates of P450-mediated drug metabolism in patients [17]. Other studies reported that *POR* rs1057868 C>T and *POR* rs2868177 A>G are associated with CYP3A activity [17,18]. These SNPs associated with the CYP3A function might influence tacrolimus pharmacokinetics. In a multicenter study, Jacobson et al. reported that rs2239393 A>G and rs4646312 T>C of catechol-*O*-methyltransferase (*COMT*) gene are associated with variation of tacrolimus $C_0/D$ [19]. This information suggests that the genetic polymorphisms of *COMT* gene may also affect tacrolimus metabolism. Interleukin-10 (*IL-10*) can regulate CYP3A enzyme activity. It is reported that the administration of IL-10 down-regulated CYP3A activity by 12% in healthy subjects [20]. Thus, the CYP3A-dependent tacrolimus metabolism may be influenced by *IL-10* gene polymorphisms. In addition to the genetic mechanism, clinical factors associated with tacrolimus pharmacokinetics have been reported [21].

Although several factors have been confirmed to impact on tacrolimus pharmacokinetics, some factors with the potential to influence tacrolimus metabolism need to be investigated, especially in different ethnic groups. The aim of this retrospective study was to evaluate the influence of *CYP3A4*, *CYP3A5*, *COMT*, *IL-10* and *POR* SNPs on $C_0/D$ and the length of time required to reach the target $C_0$ range during the early phase after transplantation in a group of Chinese renal transplant recipients.

## Materials and Methods

### Study Design and Patient Population

The study protocol was approved by the Ethical Committee of Nanfang Hospital, an affiliate of the Southern Medical University, China. Written informed consent was obtained from all recipients before their participation in the study. The retrospective study population, from the Nanfang Hospital in Guangzhou, consisted of the renal transplant recipients who received tacrolimus as immunosuppressant between January 2007 and August 2012. Patients with conditions that could affect tacrolimus pharmacokinetics and pharmacodynamics were excluded. Exclusion criteria were hepatitis B (58 patients), hepatitis C (6), cancer (5), systemic lupus erythematosus (SLE) with long-term hormone therapy (4), liver and renal transplantation (7), second renal transplantation (10), acute rejection (5), <18 years old (2). Finally, a total of 240 patients were eligible for the retrospective study. Demographic characteristics, laboratory test results and drug administration history were extracted from electronic medical records. The retrospective data of all patients were collected for 40 days after transplantation.

### Immunosuppressant Regimens and Tacrolimus Measurement

All patients were treated with a combination of immunosuppressants consisting of tacrolimus, mycophenolate mofetil and steroids. The first oral administration of tacrolimus was given approximately 12 h after the transplantation. The initial dosage was calculated according to the weight of the patient (0.10 mg/kg body weight, twice a day) and subsequently adjusted according to the trough blood concentration ($C_0$), which was measured by the Microparticle Enzyme ImmunoAssay on an IMx analyzer (Abbott Laboratories, Chicago, IL). Patients' $C_0$ were measured every other day after transplantation during hospitalization and twice a week after they were discharged from the hospital. The predefined $C_0$ range was 10–12 ng/ml, and the stable maintenance tacrolimus dose was the dosage at which the target $C_0$ range was achieved for more than 2 consecutive days and following $C_0$ values were within the range 9–14 ng/ml. This dosage did not change and was considered to be the stable maintenance tacrolimus dose. The length of time required to reach the target $C_0$ range was the period from transplantation to the time when patients achieved the stable maintenance tacrolimus dose *D*. $C_0$ concentration was dose-corrected ($C_0/D$) using the corresponding 24 h dose on a mg/kg

**Table 1.** Demographics, clinical characteristics of the Chinese renal transplant recipients.

| | Days 1 to 3 | Days 6 to 8 | Days 12 to 14 | Stable condition |
|---|---|---|---|---|
| | n = 240 | n = 240 | n = 240 | n = 183 |
| Age (years), (mean ± SD) | 41.03±12.22 | 41.03±12.22 | 41.03±12.22 | 41.77±12.14 |
| Gender (male/female) | 161/79 | 161/79 | 161/79 | 124/59 |
| Body weight (kg) (mean ± SD) | 57.94±10.12 | 57.94±10.12 | 57.94±10.12 | 58.25±10.18 |
| Hematocrit (%) | 0.353±0.0559 | 0.339±0.0577 | 0.303±0.0496 | 0.309±0.0413 |
| Hemoglobin (g/L) | 115.2±19.8 | 112.8±18.8 | 100.8±16.4 | 101.2±14.0 |
| Albumin (g/L) | 35.3±4.5 | 35.9±4.9 | 37.0±5.3 | 38.6±4.5 |
| Alanine aminotransferase (ALT), (U/L) | 18.0±14.0 | 27.9±35.6 | 40.6±45.4 | 29.7±31.2 |
| Aspartate aminotransferase(AST), (U/L) | 19.3±11.6 | 22.2±16.4 | 22.5±14.0 | 17.7±9.0 |
| Total bilirubin (TBIL), (μmol/L) | 10.22±4.43 | 11.19±4.57 | 9.21±3.70 | 8.99±3.31 |
| Unconjugated bilirubin(IBIL), (μmol/L) | 7.12±3.15 | 7.80±3.34 | 6.24±2.69 | 6.08±2.55 |
| Tacrolimus dose (mg/day) | 6.64±1.56 | 7.02±2.16 | 7.88±2.97 | 8.30±3.15 |
| Tacrolimus concentration (ng/ml) | 10.8±5.4 | 10.3±4.0 | 10.1±3.5 | 11.0±1.3 |
| Concentration/Dose Ratio (ng/ml)/(mg/kg) | 95.5±49.1 | 95.7±57.0 | 86.3±52.7 | 90.8±47.1 |

doi:10.1371/journal.pone.0086206.t001

**Table 2.** Frequencies of allelic variants in the Chinese renal transplant recipients.

| Genotypes (n,%) | Days 1 to14 | Stable condition |
|---|---|---|
| | N = 240 | N = 183 |
| *CYP3A5* (*1/*1, *1/*3, *3/*3) | 21/103/116 | 17/81/85 |
| | (8.75%,42.92%,48.33%) | (9.29%,44.26%,46.45%) |
| *CYP3A5* rs28365085 T>C (T/T, T/C, C/C) | 240/0/0 | 183/0/0 |
| | (100%,0%,0%) | (100%,0%,0%) |
| *CYP3A4* (*1/*1, *1/*1G, *1G/*1G) | 131/90/19 | 98/73/12 |
| | (54.58%,37.50%,7.92%) | (53.55%, 39.89%, 6.56%) |
| *CYP3A4* rs4646437 T>C (T/T, T/C, C/C) | 10/80/150 | 6/63/114 |
| | (4.17%,33.33%,62.50%) | (3.28%,34.42%,62.30%) |
| *CYP3A4* (*1/*1,*1/*22, *22/*22) | 240/0/0 | 183/0/0 |
| | (100%,0%,0%) | (100%,0%,0%) |
| *CYP3A4* rs33972239 delT (−/−, −/T, T/T) | 240/0/0 | 183/0/0 |
| | (100%,0%,0%) | (100%,0%,0%) |
| *POR* rs1057868 C>T (C/C, C/T, T/T) | 101/107/32 | 67/90/26 |
| | (42.08%,44.58%,13.34%) | (36.61%,49.18%,14.21%) |
| *POR* rs2868177 A>G (A/A, A/G, G/G) | 84/104/52 | 65/85/33 |
| | (35.00%, 43.33%,21.67%) | (35.52%,46.45%,18.03%) |
| *COMT* rs4646312 T>C (T/T, T/C, C/C) | 115/98/27 | 92/73/18 |
| | (47.92%, 40.83%, 11.25%) | (50.27%,39.89%,9.84%) |
| *COMT* rs2239393 A>G (A/A, A/G, G/G) | 116/96/28 | 93/71/19 |
| | (48.33%, 40.00%, 11.67%) | (50.82%,38.80%,10.38%) |
| *COMT* rs737865 T>C (T/T, T/C, C/C) | 126/92/22 | 99/70/14 |
| | (52.50%,38.33%, 9.17%) | (54.10%,38.25%,7.65%) |
| *COMT* rs6267 G>T (G/G, G/T, T/T) | 213/26/1 | 163/19/1 |
| | (88.75%,10.83%, 0.42%) | (89.07%,10.38%,0.55%) |
| *COMT* rs4680 G>A (G/G, G/A, A/A) | 133/86/21 | 97/69/17 |
| | (55.42%, 35.83%, 8.75%) | (53.01%,37.70%,9.29%) |
| *COMT* rs165599 G>A (G/G, G/A, A/A) | 54/138/48 | 38/106/39 |
| | (22.50%, 57.50%, 20.00%) | (20.77%,57.92%,21.31%) |
| *IL-10* rs1800871 C>T (C/C, C/T, T/T) | 15/111/114 | 8/84/91 |
| | (6.25%, 46.25%, 47.50%) | (4.37%,45.90%,49.73%) |
| *IL-10* rs1800896 A>G (A/A, A/G, G/G) | 217/23/0 | 168/15/0 |
| | (90.42%, 9.58%, 0%) | (91.80%,8.20%,0%) |
| *IL-10* rs1800872 C>A (C/C, C/A, A/A) | 15/112/113 | 8/85/90 |
| | (6.25%, 46.67%, 47.08%) | (4.37%,46.45%,49.18%) |

doi:10.1371/journal.pone.0086206.t002

basis. $C_0/D$ on days 1–3, 6–8 and 12–14 after transplantation, as well as the period of the predefined tacrolimus therapeutic range were selected as the representative ratio parameters of the early phase after transplantation. The corresponding laboratory parameters including hemoglobin, hematocrit, albumin, alanine aminotransferase, aspartate aminotransferase, total bilirubin and unconjugated bilirubin were obtained. The relationships between representative ratio parameters and the genetic variants were analyzed in this study.

## SNP Genotyping and Linkage Disequilibrium Measurement

Human DNA was extracted from leukocytes in peripheral blood using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China). The SNPs of the *CYP3A5*, *CYP3A4*, *COMT*, *IL-10* and *POR* genes meeting the following two criteria were selected for our study. (1) It has been reported that the SNPs might affect the corresponding gene activity, or the SNPs are located in the coding region of the gene. (2) The minor allele frequency (MAF) is >5% in the CHB population (data from HapMap). Finally, the *CYP3A5* rs776746 A>G (*CYP3A5*3* allele), *CYP3A5* rs28365085T>C, *CYP3A4* rs2242480 C>T (*CYP3A4*1G* allele), *CYP3A4* rs35599367 C>T (*CYP3A4*22* allele), *CYP3A4* rs4646437 T>C, *CYP3A4* rs33972239 delT, *POR* rs1057868 C>T, *POR* rs2868177 A>G, *COMT* rs4646312 T>C, *COMT* rs2239393 A>G, *COMT* rs737865 T>C, *COMT* rs6267 G>T, *COMT* rs4680 G>A, *COMT* rs165599 G>A, *IL-10* rs1800871 C>T, *IL-10* rs1800872 C>A and *IL-10* rs1800896 A>G were analyzed in this study. The genotypes of the 17 SNPs were determined by the

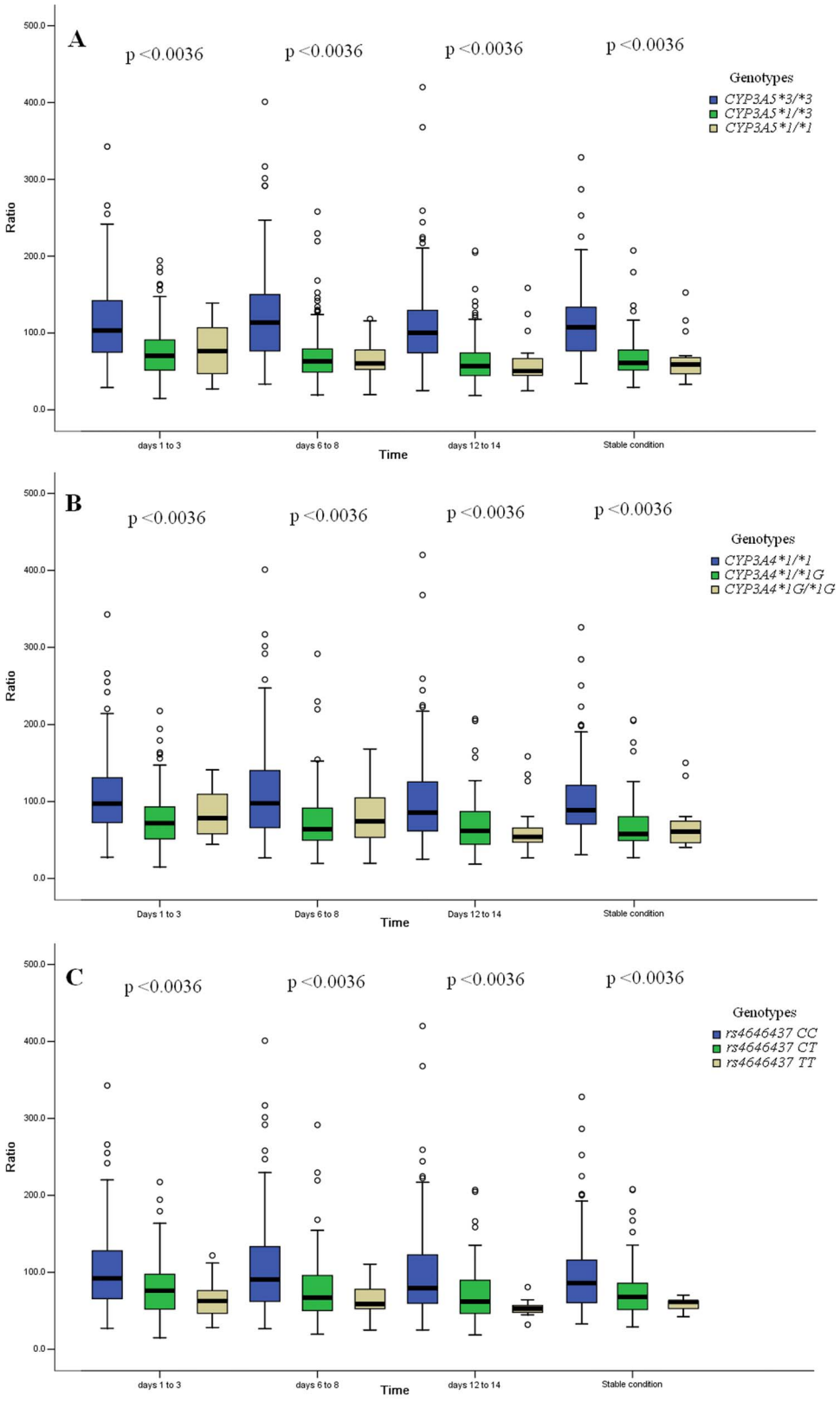


**Figure 1. Box-and-whisker plot of tacrolimus $C_0/D$ for different genotypic groups.** The boxes represent the median, 25th and 75th percentiles of the data. The circles represent deviant cases. The *X*-axis gives the times (days 1–3, 6–8 and 12–14, and the period of stable conditions after transplantation). The *Y*-axis gives the $C_0/D$. Genetic variants are: A, *CYP3A5*3*; B, *CYP3A4*1G*; and C, *CYP3A4* rs4646437 T>C. The *p* values among the genetic groups are given above the box-and-whisker plot.
doi:10.1371/journal.pone.0086206.g001

**Table 3.** Comparison of the tacrolimus $C_0/D$ in the different groups classified by genetic variant genotypes.

| Genotype | n = 240 | (days1 to 3)$C_0/D$ | p | (days6 to 8)$C_0/D$ | p | (days 12 to 14) $C_0/D$ | p |
|---|---|---|---|---|---|---|---|
| *CYP3A5*1/*1* | 21 | 78.4±35.1 | | 68.5±27.1 | | 60.7±32.7 | |
| *CYP3A5*1/*3* | 103 | 76.9±37.1 | $2.93\times10^{-9}$ | 72.9±41.2 | $3.87\times10^{-13}$ | 65.2±33.7 | $2.79\times10^{-14}$ |
| *CYP3A5*3/*3* | 116 | 115.0±53.2 | | 120.9±62.4 | | 109.6±59.3 | |
| *CYP3A4*1/*1* | 131 | 107.7±53.2 | | 111.2±62.2 | | 100.5±59.7 | |
| *CYP3A4*1/*1G* | 90 | 79.8±40.4 | $7.07\times10^{-5}$ | 76.6±45.0 | $9.58\times10^{-7}$ | 69.9±36.6 | $1.64\times10^{-6}$ |
| *CYP3A4*1G/*1G* | 19 | 85.6±32.1 | | 79.5±37.3 | | 65.3±35.8 | |
| *CYP3A4* rs4646437 TT | 10 | 68.7±29.2 | | 66.8±25.6 | | 53.8±12.8 | |
| *CYP3A4* rs4646437 TC | 80 | 81.9±40.0 | $4.32\times10^{-4}$ | 80.8±47.6 | $7.41\times10^{-4}$ | 71.5±39.1 | $3.48\times10^{-5}$ |
| *CYP3A4* rs4646437 CC | 150 | 104.5±52.3 | | 105.6±60.9 | | 96.3±57.8 | |
| *POR* rs1057868 CC | 101 | 96.2±45.1 | | 95.4±53.6 | | 84.7±52.9 | |
| *POR* rs1057868 CT | 107 | 93.9±52.7 | 0.714 | 99.0±61.4 | 0.422 | 90.1±54.7 | 0.444 |
| *POR* rs1057868 TT | 32 | 98.3±50.2 | | 85.8±52.7 | | 78.3±44.9 | |
| *POR* rs2868177 AA | 84 | 94.0±55.6 | | 94.6±62.8 | | 83.9±54.9 | |
| *POR* rs2868177 AG | 104 | 93.8±43.8 | 0.471 | 93.2±54.1 | 0.411 | 83.4±40.7 | 0.471 |
| *POR* rs2868177 GG | 52 | 101.1±48.7 | | 102.6±53.6 | | 95.7±68.2 | |
| *COMT* rs4646312 TT | 115 | 95.0±48.5 | | 97.5±55.0 | | 87.5±55.3 | |
| *COMT* rs4646312 TC | 98 | 97.3±53.0 | 0.994 | 93.562.9 | 0.469 | 84.1±52.5 | 0.620 |
| *COMT* rs4646312 CC | 27 | 90.9±36.6 | | 96.1±43.1 | | 88.5±42.3 | |
| *COMT* rs2239393 AA | 116 | 95.7±48.6 | | 97.6±54.9 | | 87.8±55.1 | |
| *COMT* rs2239393 AG | 96 | 96.7±53.2 | 0.966 | 93.4±63.4 | 0.413 | 83.9±53.0 | 0.577 |
| *COMT* rs2239393 GG | 28 | 90.5±36.0 | | 96.0±42.3 | | 88.0±41.6 | |
| *COMT* rs737865 TT | 126 | 94.7±47.3 | | 95.4±56.2 | | 86.0±57.0 | |
| *COMT* rs737865 TC | 92 | 95.4±53.1 | 0.749 | 93.1±60.8 | 0.153 | 85.4±48.1 | 0.632 |
| *COMT* rs737865 CC | 22 | 100.0±43.1 | | 108.1±45.2 | | 91.5±47.3 | |
| *COMT* rs6267 GG | 213 | 94.7±49.1 | | 96.1±59.1 | | 85.7±49.4 | |
| *COMT* rs6267 GT | 26 | 102.2±50.7 | 0.775 | 93.8±37.3 | 0.646 | 91.2±76.5 | 0.972 |
| *COMT* rs6267 TT | 1 | 84.5 | | 60.7 | | 74.4 | |
| *COMT* rs4680 GG | 133 | 98.9±52.6 | | 96.4±58.7 | | 87.2±56.5 | |
| *COMT* rs4680 GA | 86 | 90.2±42.7 | 0.594 | 94.8±54.0 | 0.936 | 84.2±47.1 | 0.962 |
| *COMT* rs4680 AA | 21 | 95.9±50.8 | | 95.3±61.0 | | 88.4±51.7 | |
| *COMT* rs165599 GG | 54 | 105.0±50.8 | | 103.2±58.0 | | 87.7±43.0 | |
| *COMT* rs165599 GA | 138 | 90.3±45.4 | 0.117 | 91.4±58.7 | 0.144 | 82.6±56.7 | 0.089 |
| *COMT* rs165599 AA | 48 | 99.7±55.9 | | 99.6±50.8 | | 95.3±50.4 | |
| *IL-10* rs1800871 CC | 15 | 88.1±40.1 | | 85.4±54.7 | | 75.7±40.2 | |
| *IL-10* rs1800871 CT | 111 | 92.1±47.8 | 0.330 | 91.1±58.5 | 0.104 | 82.6±57.2 | 0.129 |
| *IL-10* rs1800871 TT | 114 | 99.7±51.4 | | 101.5±55.8 | | 91.2±49.4 | |
| *IL-10* rs1800896 AA | 217 | 96.3±48.9 | 0.222 | 96.7±54.7 | 0.114 | 86.6±50.6 | 0.290 |
| *IL-10* rs1800896 AG | 23 | 88.1±51.5 | | 86.7±76.6 | | 83.2±70.6 | |
| *IL-10* rs1800872 CC | 15 | 88.0±40.1 | | 82.6±55.5 | | 74.0±40.9 | |
| *IL-10* rs1800872 CA | 112 | 92.1±47.8 | 0.352 | 91.6±58.0 | 0.086 | 83.4±56.8 | 0.174 |
| *IL-10* rs1800872 AA | 113 | 99.8±51.4 | | 101.6±56.1 | | 90.8±49.7 | |

doi:10.1371/journal.pone.0086206.t003

SNaPshot assay using the Applied Biosystems Multiplex Kit (Life Technologies Corporation, Shanghai, China) [22].All SNPs of 240 patients tested in this study were successfully genotyped and passed quality control. Haplotypes were inferred by a Bayesian statistical method with the PHASE 2.1 software (Stephens and Donnelly 2003). Reconstructed haplotypes were inserted into the Haploview v. 4.2 program to find $r^2$.

## Statistical Analysis

The dose-adjusted tacrolimus trough concentration ($C_0/D$) is the ratio of the measured tacrolimus trough concentration $C_0$ divided by the corresponding daily tacrolimus dose $D$ expressed as mg/kg body weight. All values are expressed as mean ± SD. Sample size and statistical power were evaluated using one-way analysis of variance model (unequal n's) based on nQuery advisor

**Table 4.** Comparison of the tacrolimus $C_0/D$ in the different genotypic groups on the time achieving target blood tacrolimus concentrations.

| Genotype | $n=183$ | Stable conditions ($C_0/D$) | $p$ |
|---|---|---|---|
| *CYP3A5*1/*1* | 17 | 67.3±29.9 | |
| *CYP3A5*1/*3* | 81 | 69.5±27.9 | $3.01\times10^{-13}$ |
| *CYP3A5*3/*3* | 85 | 115.7±52.0 | |
| *CYP3A4*1/*1* | 98 | 105.0±51.4 | |
| *CYP3A4*1/*1G* | 73 | 74.3±35.5 | $1.12\times10^{-6}$ |
| *CYP3A4*1G/*1G* | 12 | 74.6±34.4 | |
| *CYP3A4* rs4646437 TT | 6 | 60.6±9.4 | |
| *CYP3A4* rs4646437 TC | 63 | 78.8±38.8 | $1.11\times10^{-3}$ |
| *CYP3A4* rs4646437 CC | 114 | 99.0±50.4 | |
| *POR* rs1057868 CC | 67 | 92.4±46.6 | |
| *POR* rs1057868 CT | 90 | 90.9±49.5 | 0.728 |
| *POR* rs1057868 TT | 26 | 86.1±40.5 | |
| *POR* rs2868177 AA | 65 | 92.0±50.8 | |
| *POR* rs2868177 AG | 85 | 86.5±39.4 | 0.563 |
| *POR* rs2868177 GG | 33 | 99.4±56.9 | |
| *COMT* rs4646312 TT | 92 | 91.1±48.6 | |
| *COMT* rs4646312 TC | 73 | 87.3±46.0 | 0.152 |
| *COMT* rs4646312 CC | 18 | 103.2±43.2 | |
| *COMT* rs2239393 AA | 93 | 91.4±48.4 | |
| *COMT* rs2239393 AG | 71 | 87.2±46.5 | 0.2 |
| *COMT* rs2239393 GG | 19 | 101.1±43.0 | |
| *COMT* rs737865 TT | 99 | 89.6±49.8 | |
| *COMT* rs737865 TC | 70 | 90.2±44.1 | 0.328 |
| *COMT* rs737865 CC | 14 | 101.4±43.0 | |
| *COMT* rs6267 GG | 163 | 92.3±48.6 | |
| *COMT* rs6267 GT | 19 | 78.1±31.0 | 0.561 |
| *COMT* rs6267 TT | 1 | 74.4 | |
| *COMT* rs4680 GG | 97 | 89.0±45.5 | |
| *COMT* rs4680 GA | 69 | 94.3±49.9 | 0.749 |
| *COMT* rs4680 AA | 17 | 86.6±45.9 | |
| *COMT* rs165599 GG | 38 | 94.3±42.1 | |
| *COMT* rs165599 GA | 106 | 89.351.3 | 0.363 |
| *COMT* rs165599 AA | 39 | 91.3±40.1 | |
| *IL-10* rs1800871 CC | 8 | 73.5±36.8 | |
| *IL-10* rs1800871 CT | 84 | 84.1±46.9 | 0.017 |
| *IL-10* rs1800871 TT | 91 | 98.4±47.1 | |
| *IL-10* rs1800896 AA | 168 | 90.4±44.5 | 0.710 |
| *IL-10* rs1800896 AG | 15 | 95.4±72.0 | |
| *IL-10* rs1800872 CC | 8 | 76.5±36.8 | |
| *IL-10* rs1800872 CA | 85 | 84.5±46.6 | 0.046 |
| *IL-10* rs1800872 AA | 90 | 98.0±47.6 | |

doi:10.1371/journal.pone.0086206.t004

version 7.0 (Statistical Solutions, Cork, Ireland). To account for multiple testing, the Bonferroni correction was applied. $P$ values for SNPs less than 0.05/N (N = number of SNPs to be analyzed) were considered as significant. All SNPs identified were tested for deviations from Hardy–Weinberg disequilibrium with the use of a $\chi^2$ test. The following analyses were used to evaluate the impact of each SNP on $C_0/D$ and the length of time required to reach the target $C_0$ range. $C_0/D$ among the three genotypes of these SNPs was compared using the Kruskal–Wallis test. $C_0/D$ between the two genotypes of these SNPs was compared using the Mann–Whitney test. SNPs that were associated significantly with $C_0/D$ were examined for association with the length of time required to reach the target $C_0$ range. The proportion of patients who achieved the target $C_0$ range among the different genotypic groups at different time points was analyzed with the $\chi^2$ test. All statistical analyses were performed using the SPSS software package (version 13.0, SPSS Inc., Chicago, IL).

## Results

### Patient characteristics and genotype frequencies

A total of 240 renal transplant recipients were included in this retrospective study. Of these, 183 finally achieved the target $C_0$ range through drug monitoring and dosage adjustment. The other 57 patients who hardly achieved the target $C_0$ range would undergo further therapy. Of the 17 SNPs, 14 (except *CYP3A5* rs28365085 T>C, *CYP3A4**22 and *CYP3A4* rs33972239 delT) were identified in the renal transplant recipients. Finally, the 14 SNPs were analyzed in this study. The allele frequencies of the 14 SNPs in 240 patients were in accordance with Hardy–Weinberg equilibrium, and the same results were found in 183 patients with the stable condition. The demographics, clinical characteristics and genotype frequencies of the patients on days 1–3, 6–8 and 12–14 after transplantation, as well as during the period of the predefined tacrolimus therapeutic range are given in Tables 1 and 2.

### Single genetic polymorphism analysis for association with tacrolimus $C_0/D$

We examined the association of the 14 genotypic variants with tacrolimus $C_0/D$ at different time points after transplantation. The level of significance has been adjusted according to the Bonferroni correction ($p_{bonf}$ <0.0036). Of the 14 variants, *CYP3A5*3*, *CYP3A4*1G* and *CYP3A4* rs4646437 T>C presented a significant association with tacrolimus $C_0/D$ at different time points after transplantation (Tables 3 and 4; Figure 1). Tacrolimus $C_0/D$ of the patients with *CYP3A5 *3/*3* was highest among the different genotypic groups of *CYP3A5*3* (Figure 1A). $C_0/D$ of the patients with *CYP3A4 *1/*1*was highest among the different genotypic groups of *CYP3A4*1G* (Figure 1B). $C_0/D$ of the patients with *CYP3A4 rs4646437-CC* was highest among the different genotypic groups of *CYP3A4* rs4646437 T>C (Figure 1C). Moreover, the *IL-10* rs1800871 C>T and *IL-10* rs1800872 C>A presented a marginal association ($p$<0.05) with $C_0/D$ at the time point when the patients achieved the maintenance dose (Table 4). However, impact of *IL-10* rs1800871 C>T and *IL-10* rs1800872 C>A on $C_0/D$ was not statistically significant after applying Bonferroni correction. None of the other 9 variants demonstrated a significant association with $C_0/D$ at any time point. In addition, the minimum sample sizes needed for 80% power for analysis of *CYP3A5*3*, *CYP3A4*1G* and *CYP3A4* rs4646437 T>C were estimated, and the sample size (240 patients) is enough to assure the statistical power and conclusion (Table S1).

### Difference in the length of time required to reach the target $C_0$ range

According to the above data, *CYP3A5*3*, *CYP3A4*1G*, *CYP3A4* rs4646437 T>C, *IL-10* rs1800871 C>T and *IL-10* rs1800872 C>A might be associated with $C_0/D$. We also evaluated the relationships between the five variants and the length of time

**Table 5.** The impact of the genetic variants on the time to achieve the target blood tacrolimus concentrations.

| | Week 1 | | Week 2 | | Week 3 | | Week 4 | |
|---|---|---|---|---|---|---|---|---|
| | Stable conditions | | Stable conditions | | Stable conditions | | Stable conditions | |
| | Yes/No (*n*) | *p* | Yes/No (*n*) | *p* | Yes/No (*n*) | *p* | Yes/No (*n*) | p |
| *CYP3A5*3/*3* | 9/107 | 0.058 | 39/77 | 0.298 | 72/44 | 0.802 | 79/37 | 0.369 |
| *CYP3A5*1/*3* or **1/*1* | 3/121 | | 34/90 | | 75/49 | | 91/33 | |
| *CYP3A4*1/*1* | 10/121 | 0.041 | 43/88 | 0.296 | 79/52 | 0.855 | 89/42 | 0.280 |
| *CYP3A4*1/*1G* or *1G/*1G* | 2/107 | | 29/80 | | 67/42 | | 81/28 | |
| *CYP3A4* rs4646437 CC | 9/141 | 0.360 | 49/101 | 0.329 | 95/55 | 0.307 | 106/44 | 0.942 |
| *CYP3A4* rs4646437 TC or TT | 3/87 | | 24/66 | | 51/39 | | 64/26 | |
| *IL-10* rs1800871 TT | 5/109 | 0.679 | 38/76 | 0.351 | 77/37 | 0.004 | 87/27 | 0.076 |
| *IL-10* rs1800871 CT or CC | 7/119 | | 35/91 | | 62/64 | | 83/43 | |
| *IL-10* rs1800872 AA | 5/108 | 0.700 | 37/76 | 0.461 | 75/38 | 0.098 | 86/27 | 0.091 |
| *IL-10* rs1800872 CA or CC | 7/120 | | 36/91 | | 71/56 | | 84/43 | |

doi:10.1371/journal.pone.0086206.t005

required to reach the target $C_0$ range. The proportion of patients who achieved the target $C_0$ range was compared for the different genotypic groups at weeks 1, 2, 3 and 4 after transplantation (Table 5). The level of significance has been adjusted according to the Bonferroni correction ($p_{bonf} < 0.01$). The proportion of patients in *CYP3A4*1/*1* group who achieved the target $C_0$ range at week 1 was higher ($p = 0.041$) compared to the *CYP3A4*1/*1G* and *CYP3A4*1G/*1G* groups. However, the significance was lost after Bonferroni correction. The proportion of patients in the *IL-10 rs1800871-TT* group who achieved the target $C_0$ range at week 3 was higher ($p = 0.004$) compared to the *IL-10 rs1800871-CT* and *IL-10 rs1800871-CC* groups. There was no significant difference among the other variant groups at any time point.

### Linkage between *CYP3A4* SNPs and *CYP3A5*3* in tacrolimus metabolism

The *CYP3A4* and *CYP3A5* genes are located in 7q21.1. We analyzed the linkage disequilibrium (LD) between the *CYP3A4* and *CYP3A5* variants. There was a moderate degree of LD between *CYP3A4*1/*1G* (rs2242480 C>T) and *CYP3A5*1/*3* (rs776746 A>G) ($r^2 = 0.502$) and a low degree of LD between *CYP3A4* rs4646437 T>C and *CYP3A5*1/*3* (rs776746 A>G) ($r^2 = 0.244$). We investigated the effect of the *CYP3A4*1/*1G* and *CYP3A4* rs4646437 T>C polymorphisms on the dose-adjusted tacrolimus concentration ($C_0/D$) among CYP3A5 expressers and nonexpressers (Tables 6 and 7). There was no significant difference in $C_0/D$ between patients with the *CYP3A4*1G* allele and the **1/*1* genotype. The same results were found between patients with the *CYP3A4 rs4646437 T* allele and the *CYP3A4 rs4646437 CC* genotype.

## Discussion

This retrospective study examined the contribution of gene polymorphisms to the dose-adjusted tacrolimus concentration ($C_0/D$) and the length of time required to reach the target trough blood concentration range ($C_0$) in Chinese renal transplant recipients. In accord with the results of earlier studies [7–11], we found that *CYP3A5*3* presented a significant association ($p < 0.0036$) with tacrolimus $C_0/D$ at different time points after transplantation (Figure 1A). This result further validated that the *CYP3A5*3* allele was strongly associated with tacrolimus pharmacokinetics. In addition, the *CYP3A4 *1G* allele and *CYP3A4* rs4646437 T>C were associated ($p < 0.0036$) with $C_0/D$ at different time points after transplantation (Figure 1B and 1C). This is the first report of association between *CYP3A4* rs4646437 T>C and tacrolimus pharmacokinetics. Because the *CYP3A4* and *CYP3A5* genes are both located in 7q21.1, the LD between *CYP3A4* SNPs and *CYP3A5* 6986A>G might influence the impact of *CYP3A4* SNPs on the tacrolimus $C_0/D$. Crettol et al. reported that the *CYP3A4* rs4646437C>T influenced cyclosporine pharmacokinetics, the *rs4646437-T* carriers requiring higher cyclosporine dose. They

**Table 6.** Tacrolimus $C_0/D$ in *CYP3A4*1/*1G* genotypes classified by different CYP3A5 expressers.

| | CYP3A5 expresser | | *p* | CYP3A5 nonexpresser | | *p* |
|---|---|---|---|---|---|---|
| | *CYP3A4*1/*1* | *CYP3A4*1/*1G+ *1G/*1G* | | *CYP3A4*1/*1* | *CYP3A4*1/*1G+ *1G/*1G* | |
| N | 25 | 99 | | 106 | 10 | |
| (days1 to 3) $C_0/D$ | 75.4±37.4 | 77.6±36.6 | 0.681 | 115.3±53.7 | 112.4±50.0 | 0.875 |
| (days6 to 8) $C_0/D$ | 73.1±46.0 | 71.9±37.4 | 0.988 | 121.0±62.3 | 127.9±66.6 | 0.791 |
| (days 12 to 14) $C_0/D$ | 58.3±23.8 | 66.0±35.4 | 0.480 | 110.5±61.2 | 100.2±32.1 | 0.890 |

doi:10.1371/journal.pone.0086206.t006

**Table 7.** Tacrolimus $C_0/D$ in *CYP3A4* rs4646437 genotypes classified by different CYP3A5 expressers.

| | CYP3A5 expresser | | p | CYP3A5 nonexpresser | | p |
|---|---|---|---|---|---|---|
| | *CYP3A4 rs4646437 CC* | *CYP3A4 rs4646437 TC + TT* | | *CYP3A4 rs4646437 CC* | *CYP3A4 rs4646437 TC + TT* | |
| N | 46 | 78 | | 104 | 12 | |
| (days1 to 3) $C_0/D$ | 79.0±37.1 | 76.1±36.5 | 0.658 | 115.8±54.2 | 108.4±44.9 | 0.744 |
| (days6 to 8) $C_0/D$ | 70.9±38.7 | 73.0±39.5 | 0.668 | 121.7±62.7 | 120.4±62.1 | 0.878 |
| (days 12 to 14) $C_0/D$ | 64.1±28.8 | 64.6±36.1 | 0.649 | 110.6±61.7 | 101.0±32.0 | 0.935 |

doi:10.1371/journal.pone.0086206.t007

found also that the *rs4646437-T* allele was in strong LD ($r^2 = 0.82$) with the *CYP3A5*1* allele in Caucasian renal transplant recipients [23]. In this study, there was a moderate degree of LD between *CYP3A4*1/*1G* (rs2242480 C>T) and *CYP3A5*1/*3* (rs776746 A>G) ($r^2 = 0.502$) and a low degree of LD between *CYP3A4* rs4646437 T>C and *CYP3A5*1/*3* (rs776746 A>G) ($r^2 = 0.244$). Miura et al. reported that the *CYP3A4*1/*1G* might affect interindividual variability in tacrolimus pharmacokinetics among CYP3A5 expressers [24]. Zuo et al. reported that *CYP3A4*1G* can influence the oral clearance (CL/F) of tacrolimus in CYP3A5 expressers or nonexpressers among Chinese renal transplant recipients [25]. We divided the patients into CYP3A5 expressers and nonexpressers, and examined the impact of *CYP3A4* variants on $C_0/D$ in different CYP3A5 expresser groups. There was no significant difference of $C_0/D$ between patients with the *CYP3A4*1G* allele and the **1/*1* genotype among the different CYP3A5 expresser groups (Table 6). The same result was found between patients with the *CYP3A4 rs4646437-T* allele and the *CYP3A4 rs4646437-CC* genotype (Table 7). This results indicated that the LD with *CYP3A5*1/*3* might be one reason for the association between the *CYP3A4* SNPs and $C_0/D$ although the LD was not strong in our study population. So, the impact of the two SNPs on tacrolimus metabolism needs further investigation. Zhang et al. reported that liver transplantation recipients with donors who had the *IL-10 rs1800896-AA* genotype had higher $C_0/D$ values compared to donors with the *IL-10 rs1800896-AG* genotype [20]. They found also that the $C_0/D$ values of liver transplantation recipients with donors who had a low IL-10 production genotype (*rs1800871-TT*, *rs1800872-AA*) were higher compared to a high IL-10 production genotype (*rs1800871-CC* or *CT*, *rs1800872-CC* or *AC*) and they suggested that the expression level of the *IL-10* gene could influence $C_0/D$. In this study, *IL-10* gene variants (*IL-10* rs1800871 C>T, *IL-10* rs1800872 C>A) presented a marginal association ($p<0.05$) with $C_0/D$ of renal recipients during the period of the predefined tacrolimus therapeutic range. However, the difference was not significant after correction by Bonferroni method. Since the Bonferroni method is very conservative, the effect of *IL-10* rs1800871 C>T and *IL-10* rs1800872 C>A on tacrolimus needs further investigation. In addition, six susceptible *COMT* variants and two susceptible *POR* variants were analyzed; however, none of these variants had a significant association with $C_0/D$. Moreover, the variants of *CYP3A5 rs28365085 C*, *CYP3A4*22* and *CYP3A4 rs33972239 delT* were not found in this study, although there are reports that they can affect tacrolimus pharmacokinetics [26–28]. This phenomenon revealed that the genetic background of tacrolimus metabolism varies among ethnic groups.

We examined the relationships between the five SNPs associated with the $C_0/D$ and the length of time required to reach the target $C_0$ range. Of the five SNPs, *IL-10* rs1800871 C>T influenced the proportion of patients who achieved the target $C_0$ range at weeks 3. MacPhee et al. reported that CYP3A5 nonexpressers achieved the target tacrolimus concentration easily, whereas there was a significant delay for CYP3A5 expressers [12]. In this study, there was no significant difference between the CYP3A5 expressers and CYP3A5 nonexpressers in the proportion of patients who achieved the target $C_0$ range (Table 5). However, it appeared the genotypic groups with the higher $C_0/D$, such as the *IL-10 rs1800871-TT* groups, were able to achieve the target $C_0$ more easily. According to our data, the proportion of patients in the *IL-10 rs1800871-TT* group who achieved the target $C_0$ range was higher ($p = 0.004$) compared to the *IL-10 rs1800871-CT* and *IL-10 rs1800871-CC* groups at week 3. A large proportion of patients achieved the target $C_0$ range during week 3 after transplantation. So, it appears *IL-10* rs1800871 C>T was very important for the ease with which patients were able to achieve the target $C_0$ range.

Owing to the strict inclusion and exclusion criteria, 97 patients with disease states that might affect tacrolimus pharmacokinetics were excluded. The exclusion of patients with some disease states is necessary because those diseases might affect tacrolimus metabolism and, thus, the results of the study. In addition, we selected days 1–3, 6–8 and 12–14 and the period of the predefined tacrolimus therapeutic range for analysis of the association between genetic polymorphisms and $C_0/D$. Several time points were selected for the analysis, which was necessary because analysis of one genetic polymorphism at a single time point could produce an unreliable result.

There are several limitations to our study. The number of patients in several genotypic groups was small when the patients were divided into different groups according to genotype, which could influence the study results because of insufficient statistical power. Moreover, we can't confirm that *CYP3A4*1G* allele and *CYP3A4* rs4646437 T>C have independent effect on tacrolimus $C_0/D$. The mechanism by which IL-10 affects the length of time required to reach the target $C_0$ range is also unclear and further investigations are needed.

In clinical practice, the immunosuppressive effect of tacrolimus is not equivalent to tacrolimus $C_0$. However, tacrolimus $C_0$ is an important parameter to evaluate the immune status of transplant recipients. The latest insight into the genetic mechanism underlying tacrolimus metabolism has proved useful for tacrolimus individualization of organ transplantation patients. Some recent studies have individualized the dosage of tacrolimus on the basis of the *CYP3A5* genotype and obtained effective results [29,30]. In this study, we found a significant association between tacrolimus $C_0/D$ and genotypes *CYP3A5*3*, *CYP3A4*1G* and *CYP3A4* rs4646437 T>C in Chinese renal transplant recipients. We observed increased proportions of patients with *IL-10 rs1800871-TT* genotypes who achieved the target $C_0$ range. Therefore, genotyp-

ing of these genetic polymorphisms could potentially benefit Chinese renal transplant recipients by reducing the risk and the length of time needed to reach the target $C_0$ range, and the results could be useful for the tacrolimus individualization of other organ transplant recipients.

## Supporting Information

**Table S1 Sample size and statistical power evaluation based on the different genetic variants.**
(DOC)

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## Acknowledgments

We thank all the patients who participated in this study. We thank all of our colleagues past and present who contributed to this project.

## Author Contributions

Conceived and designed the experiments: L. Li. Performed the experiments: C-JL W-ML H-XJ PZ. Analyzed the data: L. Li L. Lin Z-YZ Y-JZ X-HT LZ. Contributed reagents/materials/analysis tools: C-JL. Wrote the paper: L. Li C-JL.