

RESEARCH ARTICLE

Effects of the *CYP3A4*1B* Genetic Polymorphism on the Pharmacokinetics of Tacrolimus in Adult Renal Transplant Recipients: A Meta-Analysis

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Abstract

Background and Objective

The association between the *CYP3A4*1B* single nucleotide polymorphism (SNP) and tacrolimus pharmacokinetics in different studies is controversial. Therefore, a meta-analysis was employed to evaluate the correlation between the *CYP3A4*1B* genetic polymorphism and tacrolimus pharmacokinetics at different post-transplantation times in adult renal transplant recipients.

Methods

Studies evaluating the *CYP3A4*1B* genetic polymorphism and tacrolimus pharmacokinetics were retrieved through a systematical search of Embase, PubMed, the Cochrane Library, ClinicalTrials.gov and three Chinese literature databases (up to Sept. 2014). The pharmacokinetic parameters (weight-adjusted tacrolimus daily dose and tacrolimus trough concentration/weight-adjusted tacrolimus daily dose ratio) were extracted, and the meta-analysis was performed using Stata 12.1.

Results

Seven studies (involving 1182 adult renal transplant recipients) were included in this meta-analysis. For the weight-adjusted tacrolimus daily dose, in all included renal transplant recipients (European & Indian populations), *CYP3A4*1/*1* recipients required a significantly lower weight-adjusted tacrolimus daily dose than did *CYP3A4*1B* carriers at 7 days (WMD -0.048; 95% CI -0.083 ~ -0.014), 6 months (WMD -0.058; 95% CI -0.081 ~ -0.036) and 12 months (WMD -0.061; 95% CI -0.096 ~ -0.027) post-transplantation. In light of the heterogeneity, the analysis was repeated after removing the only study in an Indian population, and *CYP3A4*1/*1* European recipients (mostly Caucasian) required a lower weight-adjusted tacrolimus daily dose within the first year post-transplantation. The tacrolimus trough concentration/weight-adjusted tacrolimus daily dose ratio (C_0 /Dose ratio) was significantly higher in *CYP3A4*1/*1* recipients than in *CYP3A4*1B* carriers at 6 months (WMD

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52.588; 95% CI 22.387 ~ 82.789) and 12 months (WMD 62.219; 95% CI 14.218 ~ 110.221) post-transplantation. When the only study in an Indian population was removed to examine European recipients (mostly Caucasian), the significant difference persisted at 1 month, 6 months and 12 months post-transplantation.

Conclusion

Based on our meta-analysis, the *CYP3A4*1B* genetic polymorphism affects tacrolimus dose requirements and tacrolimus trough concentration/weight-adjusted tacrolimus daily dose ratio within the first year post-transplantation in adult renal transplant recipients, especially in European recipients (mostly Caucasian).

Introduction

Renal transplantation is an effective treatment for the patients with end-stage renal disease. Tacrolimus, a macrolide antibiotic compound, is the most frequently used maintenance immunosuppressant after renal transplantation [1]. However, tacrolimus is characterized by its narrow therapeutic index and significant inter-individual variability in pharmacokinetics. Tacrolimus blood concentration below target trough levels can lead to rejection, and higher trough blood concentrations can lead to toxicity and infection [2,3]. Achieving a steady target blood concentration is critical to avoid rejection and adverse drug effects [4]. However, several factors influence the pharmacokinetics of tacrolimus, including hepatic dysfunction, post-transplantation time, hematocrit, serum albumin, age, race and drug interactions, especially gene polymorphism [5]. Single nucleotide polymorphisms (SNPs) in *cytochrome P450 3A* (*CYP3A*) play an important role in tacrolimus metabolism [6]. *CYP3A* enzymes in human liver microsomes play a major role in the oxidation of tacrolimus [7], and the tacrolimus metabolism within the small intestine contributes significantly to its bioavailability [8,9]. Many studies in renal transplant recipients focus on *CYP3A5*3* genetic polymorphism (rs776746, 6986A>G). There is a widespread view that *CYP3A5* nonexpressers (*CYP3A5*3/*3* carriers) required lower mean tacrolimus doses [10] and exhibit higher trough concentration/dose ratios [11,12]. The *CYP3A4*1B* genetic polymorphism (rs2740574, -392A>G), linked to enhanced *CYP3A4* activity, is likely related to the rapid metabolism of tacrolimus [6], but the effect of the *CYP3A4*1B* genetic polymorphism on tacrolimus pharmacokinetics (dose and concentration) in renal transplant recipients is controversial [13], and there has been no meta-analysis to assess the issue to date.

To evaluate the correlation between the *CYP3A4*1B* genetic polymorphism and tacrolimus pharmacokinetics (weight-adjusted tacrolimus daily dose and tacrolimus trough concentration/weight-adjusted tacrolimus daily dose ratio), a meta-analysis was employed to systematically review the published evidence of the relationship between the *CYP3A4*1B* genetic polymorphism and tacrolimus pharmacokinetics in adult renal transplant recipients.

Methods

Search strategy and study selection

Embase, PubMed, the Cochrane Library, ClinicalTrials.gov and three Chinese databases (CNKI, Sinomed and WanFang Data) were searched from their date of inception to September

2014, without language and publication status restrictions, for published studies that evaluated the effects of the *CYP3A4*1B* genetic polymorphism on tacrolimus pharmacokinetics. The search terms (“tacrolimus” or “FK506”) and “CYP3A4”) as well as related Chinese keywords in the Chinese databases were used. In addition, the reference lists of the included articles and relevant reviews were searched manually. In cases of missing data, the original authors were contacted for more detailed information by e-mail.

The inclusion criteria for the included studies were as follows: (a) studies focus on the effects of the *CYP3A4*1B* genetic polymorphism on adult renal transplant recipients treated with tacrolimus; (b) tacrolimus pharmacokinetics parameters was described separately according to different *CYP3A4*1B* genotypes; and (c) tacrolimus pharmacokinetic parameters were measured at explicit post-transplantation times. According to the above criteria, studies were assessed independently by two reviewers (S.W.L. and T.H.L.) for inclusion in the meta-analysis.

Data extraction and quality assessment

Relevant data from all eligible studies were extracted independently by the two reviewers (S.W.L. and T.H.L.), and discrepancies in the data extraction were resolved through consensus. The following information was collected: first author, publication information, design of the study, demographic data, immunosuppressive protocol, method of concentration measured, genotype frequency, post-transplantation time, weight-adjusted tacrolimus daily dose (Dose), tacrolimus trough concentration (C_0), C_0 /Dose ratio. For continuous data, information was collected as mean (SD), if the studies provided the median (range), the method reported by Hozo *et al.* [14] was employed to estimate the mean (SD).

The quality of the included studies was assessed by two reviewers (S.W.L. and T.H.L.) through a checklist derived from the Strengthening the Reporting of Genetic Association (STREGA) recommendations for reports on genetic association studies [15], and modified according to the quality checklist described elsewhere [16,17].

Statistical analysis

The Dose and C_0 /Dose ratio values were compared between *CYP3A4*1/*1* recipients and *CYP3A4*1B* carriers, and a random-effect model was used for all meta-analyses. The data of the *CYP3A4*1B* carriers were calculated from the *CYP3A4*1/*1B* and the *CYP3A4*1B/*1B* groups using the method provided by Table 7.7.a of the Cochrane handbook 5.1.0 [18]. The continuous data were pooled by weighted mean difference (WMD) or standard mean difference (SMD) and 95% confidence interval (CI), and Z-tests were performed to determine the statistical significance of the results. Statistical significance was defined as $P < 0.05$.

The heterogeneity across the included studies was assessed using the I^2 statistic, with significance defined as $I^2 > 50\%$. In case of substantial heterogeneity ($I^2 > 50\%$), meta-regression was performed to explore the sources of heterogeneity [post-transplantation time (7 days, 1 month, 3 months, 6 months, 12 months), ethnicity (Caucasian, Indian, mixed race), location (Europe, India), method of concentration measured (MEIA, CMIA, EMIT), initial dose (0.1–0.16 mg/kg/day, 0.2–0.3 mg/kg/day), and Hardy-Weinberg equilibrium (equilibrium or disequilibrium)]. Further subgroup analysis was performed according to the results of the meta-regression. A sensitivity analysis was performed to assess the validity of the outcomes by excluding each observation successively. A publication bias analysis was not performed because less than 10 studies were included. All statistical analysis was performed using Stata 12.1.

Results

Characteristics of the articles included in the meta-analysis

A total of 683 publications were identified by the literature search. After screening the titles and abstracts, the full texts of the remaining 93 studies were further assessed, and 7 studies [19–25] were included in the final meta-analysis. The details of identification of the eligible studies and the reasons for the exclusion of studies are presented in Fig 1. All 7 studies were published in English. A total of 1182 adult renal transplant recipients were included in the studies, and the characteristics of the 7 included studies are presented in Table 1. The results of quality assessment are presented in Table 2. Five original authors [20,21,23–25] were contacted for missing or specific demographic data, and three authors [20,21,25] replied.

Effects of the CYP3A4*1B genetic polymorphism on the weight-adjusted tacrolimus dose (Dose)

All 7 studies [19–25] evaluated the association between the CYP3A4*1B genetic polymorphism and weight-adjusted tacrolimus daily dose (Dose) at different post-transplantation time. The result of the meta-analysis revealed that CYP3A4*1/*1 recipients required a lower Dose than CYP3A4*1B carriers (WMD -0.047; 95% CI -0.062 ~ -0.031; $P < 0.001$). However, substantial heterogeneity existed ($I^2 = 75.3\%$), and a meta-regression was performed to explore the sources of heterogeneity with respect to the following factors: post-transplantation time, ethnicity, location, method of concentration measured, initial dose and Hardy-Weinberg equilibrium. The results of the meta-regression are presented in Table 3. The tacrolimus daily dose varied by post-transplantation time, ethnicity and location, although only “method of concentration measured” ($r = 0.027$, $P = 0.008$) and “initial dose” ($r = -0.077$, $P < 0.001$) contributed to heterogeneity. We performed subgroup analyses for all of the above covariates with the exception of the “Hardy-Weinberg equilibrium” because of collinearity, and the results are presented in Table 4. As Table 4 indicated, although the heterogeneity persisted, most subgroups demonstrated that CYP3A4*1/*1 recipients required a lower Dose than CYP3A4*1B carriers, but there was no statistical significance detected in subgroup analyses by post-transplantation time (1 month & 3 months), ethnicity (North Indian), location (India) or initial dose (0.1–0.16 mg/kg/day) involving the India population.

Considering the above results, we removed the study involving the Indian population [23] and performed the subgroup analysis according to post-transplantation time. Unlike before, the 1 month (WMD -0.023; 95% CI -0.045 ~ -0.000; $P = 0.047$; $I^2 = 0.0\%$) and 3 months (WMD -0.065; 95% CI -0.119 ~ -0.010; $P = 0.021$; $I^2 = 70.4\%$) subgroups exhibited significant difference between CYP3A4*1/*1 and CYP3A4*1B carriers (Table 5). Thus, in all included populations, CYP3A4*1/*1 recipients required lower Dose than CYP3A4*1B carriers at 7 days, 6 months and 12 months post-transplantation; in European recipients (mostly Caucasian), CYP3A4*1/*1 recipients required lower Dose than CYP3A4*1B carriers within the first year post-transplantation.

Effects of the CYP3A4*1B genetic polymorphism on the tacrolimus trough blood concentration/Dose ratio (C₀/Dose ratio)

Six studies [19–23,25] evaluated the association between the CYP3A4*1B genetic polymorphism and the C₀/Dose ratio at different post-transplantation times. The result of the meta-analysis revealed that CYP3A4*1/*1 recipients exhibited higher C₀/Dose ratios than CYP3A4*1B carriers (WMD 37.127; 95% CI 18.202 ~ 56.051; $P < 0.001$). Similar to the above analysis, substantial heterogeneity existed ($I^2 = 83.4\%$), and a meta-regression was performed

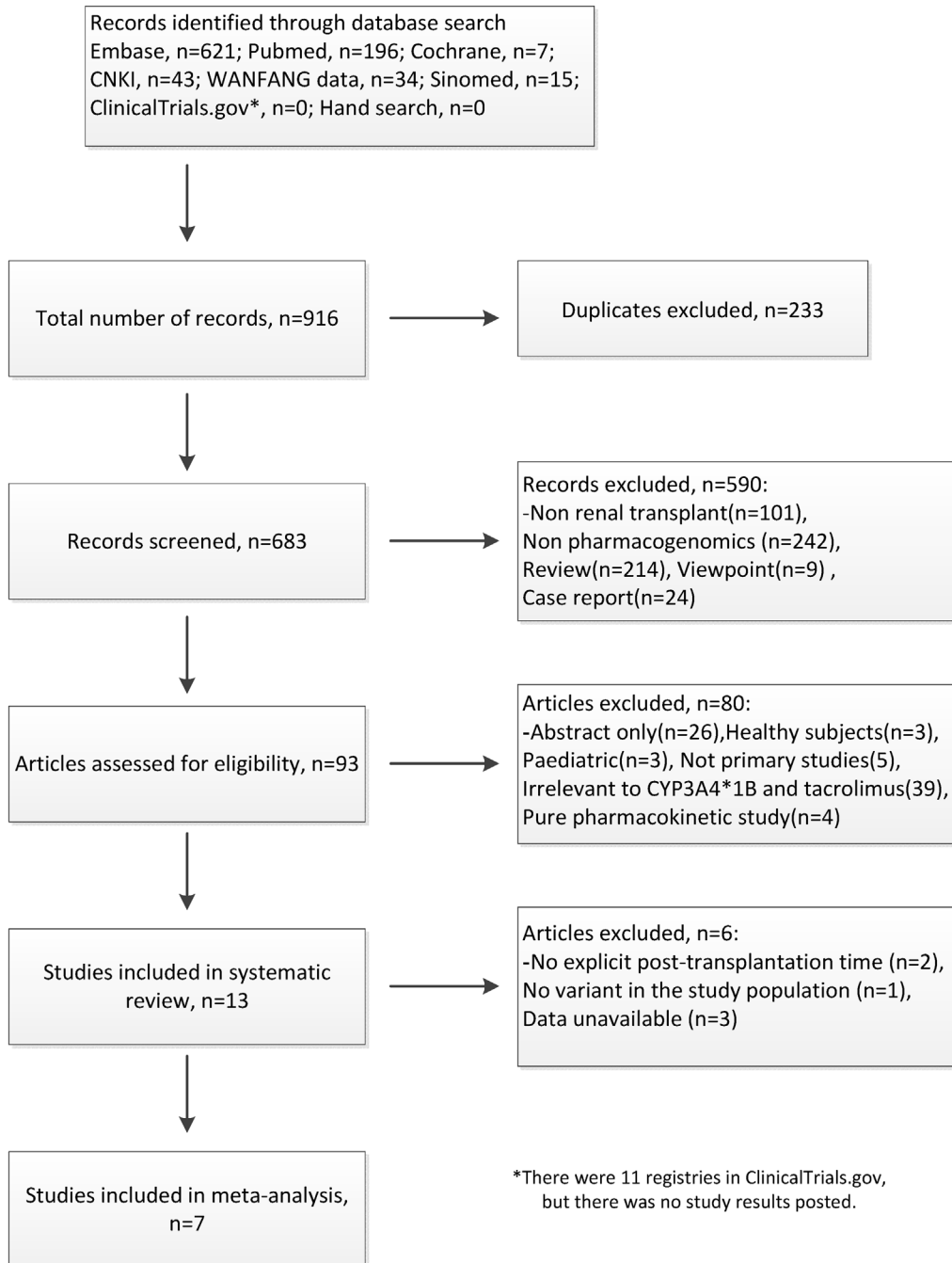


Fig 1. Flow diagram of the systematic review.

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to explore the sources of heterogeneity. As shown in [Table 3](#), post-transplantation time ($r = 13.320, P < 0.001$), ethnicity ($r = -39.806, P < 0.001$), method of concentration measured ($r = 57.668, P < 0.001$) and initial dose ($r = -85.672, P < 0.001$) contributed to heterogeneity. Subgroup analyses of post-transplantation time, ethnicity, method of concentration measured and initial dose were performed. *CYP3A4*1/1* recipients exhibited higher C_0/Dose ratios than

Table 1. Characteristics of the studies included in the meta-analysis.

Study	Cases/ Male(n)	Location/ Ethnicity	Age (years)	Immunosuppressive protocol ^a	Initial dose of tacrolimus (mg/kg/day)	Desired trough concentration(ng/ ml)	Hardy- Weinberg equilibrium	Allele frequencies of CYP3A4 (%)	
								*1	*1B
Kurzawski 2014 ^[19]	241/134	Poland/ Caucasian	45.8 +/-12.4	Tac+MMF+steroids	0.1	1 st month:10–15; Subsequent:8–10	Yes	96.9	3.1
Tavira 2013 ^[20]	206/126	Spain/ Caucasian	48.6 +/-13.5	Tac+MMF+prednisone	0.2	0–3 months:10–15; Subsequent:5–15	Yes	97.1	2.9
Gervasini 2012 ^[21]	103/62	Spain/ Caucasian	48.7 +/-16.9	Tac+MMF+steroids	0.2	0–3 months:10–15; Subsequent:5–10 ^b	Yes	97.6	2.4
Tavira 2011 ^[22]	400/242	Spain/ Caucasian	48.02 +/-13.29	Tac+MMF+prednisone	0.2	0–3 months:10–15; Subsequent:5–10	Yes	96.9	3.1
Singh 2009 ^[23]	73/NA	India/North Indian	NA	Tac+MMF/Aza +prednisolone	0.16	1 st month: 10– 12;3 rd month:8–10	No	97.3	2.7
Kuypers 2007 ^[24]	95/57	Belgium/ Caucasian	51.3 +/-14.1	Tac+MMF +methylprednisolone	0.2	8–15	Yes	96.3	3.7
Hesselink 2003 ^[25]	64/34	Netherlands/ Asian,Black & White	NA	NA	0.2–0.3	NA	No	89.8	10.2

NA: not available.

^aTac: tacrolimus; MMF: mycophenolate mofetil; Aza: Azathioprine.

^bBetween October 2001 and February 2003, 7–15 ng/ml between June 2000 and September 2001.

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CYP3A4*1B carriers in the subgroups of post-transplantation time (6 months & 12 months), ethnicity (Caucasian), method of concentration measured (EMIT) and initial dose (0.2–0.3 mg/kg/day) (Table 4), but the heterogeneity persisted in some subgroups.

When the study concerning the India population was removed, the results of the 1 month post-transplantation exhibited significant differences (WMD 58.129; 95% CI 40.584 ~ 75.675; $P < 0.001$; $I^2 = 0.0\%$) (Table 5). Thus, in all included population, CYP3A4*1/*1 recipients exhibited higher C₀/Dose ratios than CYP3A4*1B carriers at 6 and 12 months; in European recipients (mostly Caucasian), CYP3A4*1/*1 recipients exhibited higher C₀/Dose ratios than CYP3A4*1B carriers at 1 month, 6 months and 12 months post-transplantation.

Effects of the CYP3A4*1B genetic polymorphisms on Dose and the C₀/Dose ratio stratified by the CYP3A5 genotype

We performed subgroup analyses at different post-transplantation times stratified by the CYP3A5 genotype. In CYP3A5*3/*3 recipients, CYP3A4*1/*1 recipients required lower Dose than CYP3A4*1B carriers at 7 days, 6 months and 12 months post-transplantation; in CYP3A5*1 carriers, CYP3A4*1/*1 recipients required lower Dose than CYP3A4*1B carriers at 6 months and 12 months post-transplantation. Except for CYP3A5*3/*3 recipients at the time point of 6 months post-transplantation, there was no significant difference in the C₀/Dose ratio between the CYP3A4*1/*1 recipients and CYP3A4*1B carriers between different CYP3A5 genotypes (Table 5). The results suggested that the effect of the CYP3A4*1B genetic polymorphism on tacrolimus pharmacokinetics was independent at 7 days, 6 months and 12 months post-transplantation in CYP3A5*3/*3 recipients; and at 6 months and 12 months post-transplantation in CYP3A5*1 carriers.

Table 2. Quality assessment of the studies included in the meta-analysis.

First author	Year	Clear statement of background, objectives and hypothesis	Describe the studies and information	Clear eligibility criteria	Clear definition of variables	Credible method of concentration measured	Credible genetic testing method	Replicability of statistical methods	Assessment of H-W equilibrium	Sufficient descriptive demographic data	Report the withdrew person and reasons	Statement of outcome data	Funding
Kurzwanski, M	2014	+	+	+	+	+	+	+	+	+	±	+	+
Tavira, B	2013	+	±	+	+	+	+	+	+	±	+	+	+
Gervasini, G	2012	+	+	+	+	+	+	+	+	+	+	+	+
Tavira, B	2011	+	+	+	+	+	+	+	+	+	+	+	+
Singh, R	2009	+	+	+	+	+	+	+	-	±	+	+	+
Kuyppers, R, J	2007	+	+	+	+	+	+	+	+	+	±	+	+
Hesselink, D A	2003	+	±	+	+	+	+	+	-	±	+	+	+

“+”: detailed description; “±”: incomplete description; “-”: no description.

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Table 3. Results of the meta-regression in all included renal transplant recipients. ^a

Subjects	Covariates	r	P	Tau ²	I ² -res	Adjusted R ²
Dose	post-transplantation time	-0.005	0.177	0.000134	19.47%	86.02%
Dose	ethnicity	-0.011	0.280	0.000134	19.47%	86.02%
Dose	location	0.036	0.083	0.000134	19.47%	86.02%
Dose	method ^b	0.027	0.008	0.000134	19.47%	86.02%
Dose	initial dose	-0.077	< 0.001	0.000134	19.47%	86.02%
C ₀ /Dose	post-transplantation time	13.320	< 0.001	0	0.00%	100.00%
C ₀ /Dose	ethnicity	-39.806	< 0.001	0	0.00%	100.00%
C ₀ /Dose	location	-48.820	0.099	0	0.00%	100.00%
C ₀ /Dose	method*	57.668	< 0.001	0	0.00%	100.00%
C ₀ /Dose	initial dose	-85.672	< 0.001	0	0.00%	100.00%

r: coefficient of correlation; Tau²: REML estimate of between-study variance; I²-res: residual variation due to heterogeneity; Adjusted R²: proportion of between-study variance explained.

^a covariate “Hardy-Weinberg equilibrium” dropped because of collinearity.

^b method: method of concentration measured;

*method: method of concentration measured.

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Discussion

The findings of our meta-analysis suggest that the *CYP3A4*1B* genetic polymorphism influences the weight-adjusted tacrolimus daily dose and the C₀/Dose ratio in adult renal transplant recipients.

In all included renal transplant recipients, relative to *CYP3A4*1B* carriers, *CYP3A4*1/*1* recipients required a lower weight-adjusted tacrolimus daily dose (Dose) at 7 days, 6 months and 12 months post-transplantation, and exhibited a higher C₀/Dose ratio at 6 and 12 months post-transplantation. This finding suggests that *CYP3A4*1/*1* recipients required a lower initial dose within 7 days post-transplantation and a lower maintenance dose to achieve the target blood concentration relative to *CYP3A4*1B* carriers at 6 and 12 months post-transplantation.

In the meta-regression and subgroup analyses, we determined that the ethnicity and location influenced the pooled estimate results significantly. Therefore, the meta-analysis was stratified by post-transplantation time and was performed in the European recipients. The results revealed that *CYP3A4*1/*1* recipients exhibited a lower weight-adjusted tacrolimus daily dose (Dose) within the entire first year and a higher C₀/Dose ratio at 1 month, 6 months and 12 months post-transplantation compared with *CYP3A4*1B* carriers. Thus, the *CYP3A4*1B* genetic polymorphism plays a more important role in European renal transplant recipients. The results of the sensitivity analyses were consistent with the meta-regression results; excluding the Indian population changed the pooled estimate at 1 month and 3 months with respect to Dose and at 1 month with respect to the C₀/Dose ratio post-transplantation, but there was no effect on the overall estimate. Although there was no significant difference in the C₀/Dose ratio between *CYP3A4*1/*1* recipients and *CYP3A4*1B* carriers stratified by *CYP3A5* genotype, the dose requirement differed significantly at 6 and 12 months post-transplantation, which indicated that different doses were required in *CYP3A4*1/*1* recipients and *CYP3A4*1B* carriers stratified by the *CYP3A5* genotype; these data further indicate that *CYP3A4*1B* and *CYP3A5*3* may have independent effects on tacrolimus pharmacokinetics.

In clinical settings, the initial tacrolimus dose is given according to the weight of different renal recipients, and the maintenance dose is adjusted according to the blood concentration

Table 4. Subgroup analysis of the CYP3A4*1B genetic polymorphism on weight adjusted tacrolimus daily dose (Dose) and C₀/Dose by various factors in all included renal transplant recipients.

Subgroup(CYP3A4*1/*1 vs. CYP3A4*1B carriers)	Studies included (observations)	WMD(95% CI)	P	I ² (%)	SMD(95% CI)	P	I ² (%)
Dose							
By time of PT^c							
7 days	19–22,24(5)	-0.048(-0.083,-0.014)	0.006	78.2	-0.781(-1.281,-0.281)	0.002	69.5
1 month	19,21,23(3)	-0.012(-0.036,0.012)	0.322	34.8	-0.204(-0.626,0.217)	0.342	0.0
3 months	19,23–25(4)	-0.043(-0.092,0.007)	0.091	82.8	-0.928(-1.812,-0.044)	0.040	81.2
6 months	19–22,24(5)	-0.058(-0.081,-0.036)	<0.001	43.4	-1.033(-1.465,-0.601)	<0.001	58.6
12 months	19,21,22,24,25(5)	-0.061(-0.096,-0.027)	0.001	79.3	-1.241(-1.973,-0.508)	0.001	84.2
By ethnicity							
Caucasian	19–22,24(18)	-0.051(-0.067,-0.035)	<0.001	69.5	-0.932(-1.214,-0.650)	<0.001	73.4
North Indian	23(2)	0.010(-0.010,0.030)	0.330	0.0	0.252(-0.566,1.070)	0.546	0.0
Mixed [#]	25(2)	-0.066(-0.094,-0.038)	<0.001	0.0	-1.356(-1.863,-0.850)	<0.001	0.0
By location							
Europe	19–22,24,25(20)	-0.052(-0.067,-0.038)	<0.001	67.3	-0.971(-1.236,-0.707)	<0.001	72.1
India	23(2)	0.010(-0.010,0.030)	0.330	0.0	-0.252(-0.566,1.070)	0.546	0.0
By method[*]							
EMIT	21,25(6)	-0.046(-0.067,-0.025)	<0.001	51.7	-1.103(-1.530,-0.675)	<0.001	36.2
MEIA	19,23,24(11)	-0.031(-0.052,-0.009)	0.005	71.1	-0.837(-1.332,-0.342)	0.001	81.9
CMIA	20,22(5)	-0.074(-0.100,-0.049)	<0.001	65.2	-0.873(-1.155,-0.592)	<0.001	44.4
By initial dose							
0.2–0.3mg/kg/day	20–22,24,25(15)	-0.065(-0.080,-0.049)	<0.001	59.5	-1.203(-1.501,-0.906)	<0.001	66.7
0.1–0.16mg/kg/day	19,23(7)	-0.008(-0.020,0.004)	0.204	0.0	-0.322(-0.553,-0.090)	0.006	0.0
C₀/Dose							
By time of PT^c							
7 days	19–22(4)	19.971(-7.707,47.650)	0.157	81.1	0.189(-0.156,0.533)	0.283	26.6
1 month	19,21,23(3)	34.966(-6.988,76.920)	0.102	79.4	0.247(-0.738,1.232)	0.623	75.4
3 months	19,23,25(3)	7.676(-20.134,35.485)	0.589	0.0	0.151(-0.239,0.541)	0.448	0.0
6 months	19–22(4)	52.588(22.387,82.789)	0.001	59.1	0.344(-0.026,0.715)	0.069	41.6
12 months	19,21,22,25(4)	62.219(14.218,110.221)	0.011	86.5	0.350(0.037,0.663)	0.028	14.0
By ethnicity							
Caucasian	19–22(14)	47.245(26.341,68.150)	<0.001	85.1	0.335(0.160,0.510)	<0.001	23.7
North Indian	23(2)	-42.457(-91.044,6.131)	0.087	0.0	-0.821(-1.645,0.002)	0.051	0.0
Mixed ^a	25(2)	15.651(-9.785,41.087)	0.228	0.0	0.179(-0.299,0.657)	0.462	0.0
By method^b							
EMIT	21,25(6)	62.270(28.801,95.739)	<0.001	86.1	0.692(0.276,1.107)	0.001	35.4
MEIA	19,23(7)	23.487(-2.353,49.327)	0.075	50.1	0.230(-0.060,0.519)	0.120	28.7
CMIA	20,22(5)	24.973(-0.538,50.484)	0.055	80.0	0.120(-0.084,0.323)	0.248	0.0
By initial dose							
0.2–0.3mg/kg/day	20–22,25(11)	45.395(20.373,70.417)	<0.001	88.9	0.326(0.094,0.559)	0.006	38.0
0.1–0.16mg/kg/day	19,23(7)	23.487(-2.353,49.327)	0.075	50.1	0.230(-0.060,0.519)	0.120	28.7

^a Mixed: Asian, Black & White.

^b method: method of concentration measured;

^c PT: post-transplantation.

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Table 5. Subgroup analysis of the CYP3A4*1B genetic polymorphism on weight adjusted tacrolimus daily dose (Dose) and C₀/Dose by time of post-transplantation in European (Indian population removed).

Subgroup(CYP3A4*1/*1 vs. CYP3A4*1B carriers)	Studies included (observations)	WMD(95% CI)	P	I ² (%)	SMD(95% CI)	P	I ² (%)
Dose							
By time of PT^c							
7 days	19–22,24(5)	-0.048(-0.083,-0.014)	0.006	78.2	-0.781(-1.281,-0.281)	0.002	69.5
1 month	19,21(2)	-0.023(-0.045,-0.000)	0.047	0.0	-0.274(-0.727,0.179)	0.235	0.0
3 months	19,24,25(3)	-0.065(-0.119,0.010)	0.021	70.4	-1.231(-2.188,-0.273)	0.012	83.0
6 months	19–22,24(5)	-0.058(-0.081,-0.036)	<0.001	43.4	-1.033(-1.465,-0.601)	<0.001	58.6
12 months	19,21,22,24,25(5)	-0.061(-0.096,-0.027)	0.001	79.3	-1.241(-1.973,-0.508)	0.001	84.2
By CYP3A5 & time of PT^c							
CYP3A5*3/*3							
7 days	20,22(2)	-0.031(-0.059,-0.003)	0.033	13.2	-0.444(-0.818,-0.071)	0.020	0.0
6 months	20,22(2)	-0.037(-0.050,-0.024)	<0.001	0.0	-0.604(-1.005,-0.204)	0.003	10.6
12 months	22(1) ^a	-0.040(-0.064,-0.016)	0.001	NA	-0.278(-0.908,0.352)	0.388	NA
CYP3A5*1 carriers							
7 days	20–22,24(4)	-0.059(-0.141,0.022)	0.154	88.7	-0.793(-1.800,0.215)	0.123	76.9
1 month	21(1) ^a	0.020(-0.024,0.064)	0.371	NA	0.566(-0.705,1.836)	0.383	NA
3 months	24(1) ^a	-0.030(-0.145,0.085)	0.608	NA	-0.272(-1.292,0.748)	0.601	NA
6 months	20–22,24(4)	-0.032(-0.059,-0.004)	0.025	1.7	-0.622(-1.054,-0.190)	0.005	0.0
12 months	21,22,24(3)	-0.050(-0.092,-0.008)	0.020	47.7	-0.991(-1.557,-0.424)	0.001	17.2
C₀/Dose							
By time of PT^c							
7 days	19-22(4)	19.971(-7.707,47.650)	0.157	81.1	0.189(-0.156,0.533)	0.283	26.6
1 month	19,21(2)	58.129(40.584,75.675)	<0.001	0.0	0.643(0.176,1.109)	0.007	3.0
3 months	19,25(2)	14.790(-15.089,44.668)	0.332	0.0	0.244(-0.170,0.658)	0.247	0.0
6 months	19-22(4)	52.588(22.387,82.789)	0.001	59.1	0.344(-0.026,0.715)	0.069	41.6
12 months	19,21,22,25(4)	62.219(14.218,110.221)	0.011	86.5	0.350(0.037,0.663)	0.028	14.0
By CYP3A5 & time of PT^c							
CYP3A5*3/*3							
7 days	19,20,22(3)	-5.800(-17.709,6.109)	0.340	0.0	-0.075(-0.490,0.340)	0.723	0.0
1 month	19(1) ^a	25.100(-8.015,58.215)	0.137	NA	0.225(-0.586,1.036)	0.587	NA
3 months	19(1) ^a	-37.400(-178.178,103.378)	0.603	NA	-0.376(-1.188,0.435)	0.364	NA
6 months	19,20,22(3)	49.715(6.896,92.533)	0.023	54.0	0.192(-0.224,0.607)	0.366	0.0
12 months	19,22(2)	21.241(-72.999,115.480)	0.659	72.0	0.028(-0.495,0.551)	0.918	8.5
CYP3A5*1 carriers^b							
7 days	19–22,24(5)	NA	NA	NA	0.365(-0.021,0.750)	0.064	0.0
1 month	19,21(2)	NA	NA	NA	0.222(-0.523,0.966)	0.559	0.0
3 months	19,24(2)	NA	NA	NA	0.323(-0.369,1.015)	0.360	0.0
6 months	19–22,24(5)	NA	NA	NA	0.142(-0.390,0.674)	0.601	36.3
12 months	19,21,22,24(4)	NA	NA	NA	0.619(-0.031,1.269)	0.062	45.7

NA: not available.

^a only one observation.

^b the units of C₀/Dose are different, so the data were pooled by SMD;

^c PT: post-transplantation.

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[26]. Acute rejection and nephrotoxicity are unavoidable, especially in the early post-transplantation stage because the clinicians must adjust the dose frequently to achieve the target blood concentration. According to our meta-analysis, the *CYP3A4*1B* genetic polymorphism should be considered when determining the initial tacrolimus dose and adjusting the maintenance dose, which may be helpful to achieve the target concentration in a shorter time and reduce the concentration fluctuations. Because of the therapeutic drug monitoring (TDM), even though the *CYP3A4*1B* carriers required a higher Dose than the *CYP3A4*1/1* recipients, the C_0 /Dose ratio were not stable within 3 months post-transplantation.

Several limitations should be noted in our meta-analysis. First, only 7 observational studies (involving 1182 adult renal transplant recipients) were included, and there were only 77 *CYP3A4*1B* carriers. Considering the influence of large sample size from one single study, a sensitivity analysis had been performed to assess the validity of the pooled estimates. There was no single study which have a significant influence on the pooled estimate except for the study in the Indian population, which had been analyzed in Results. And our meta-analysis had tried to include all the studies that evaluated the effects of the *CYP3A4*1B* genetic polymorphism on tacrolimus pharmacokinetics, which could be retrieved from electronic database at present. The limitation of sample size influenced the pooled estimate possibly. Because of only 7 studies were included, the publication bias analysis was not performed. Second, the formulae for estimating the mean using the values of the median provided by Hozo *et al.* introduced some uncertainty [14], and the formulae for combining groups provided by the Cochrane handbook 5.1.0 Table 7.7.a provided a slight underestimate of the desired standard deviation [18]; bias resulting from the methods is unavoidable. Third, the results of our meta-analysis should be interpreted with caution because of the substantial heterogeneity across all current available studies, even though we performed meta-regression to explore the source of heterogeneity and subgroup analyses to minimize the heterogeneity. According to our meta-regression results, the method of concentration measured was the source of heterogeneity, because different methods of concentration measured have different properties in the cross-reaction with tacrolimus metabolites [27], a further subgroup analyses by method of concentration measured revealed that *CYP3A4*1B* carriers required higher Dose in all three subgroups, and had lower C_0 /Dose in EMIT subgroup (Table 4). Although the included studies had stated the demographic information and immunosuppressive protocol, different steroid tapering schedules may have influenced on the pooled estimate due to the interaction between steroids and tacrolimus [28]. Therefore, we listed the SMD of the pooled estimate to minimize the influence of the method of concentration measured and the steroids tapering schedules in various studies, which should be considered when the conclusions were interpreted by different transplantation centers based on different situations.

Furthermore, even though several studies [13] had demonstrated linkage disequilibrium between *CYP3A4*1B* and *CYP3A5*1*, in the combined *CYP3A4*1B/CYP3A5*1* genotype analysis, Gervasini, G *et al.* [21] and Chitnis, S D *et al.* [29] reported that *CYP3A4*1/1* recipients exhibited higher tacrolimus trough concentrations and C_0 /Dose ratios than *CYP3A4*1B* carriers in *CYP3A5* expressers (*CYP3A5*1/1* or *CYP3A5*1/3*); Tavira, B *et al.* [22] reported that, relative to *CYP3A4*1B* carriers stratified by *CYP3A5* (expressers or non-expressers respectively), *CYP3A4*1/1* recipients required lower tacrolimus doses, and exhibited higher tacrolimus trough concentrations and C_0 /dose ratios, which suggests a significant role of the *CYP3A4*1B*. Our meta-analysis revealed the *CYP3A4*1B* and *CYP3A5*3* may have independent effects on tacrolimus pharmacokinetics. Furthermore, tacrolimus is a substrate for both P-glycoprotein (P-gp, coded by *ABCB1*) and *CYP3A*, *ABCB1* genetic polymorphisms (such as *C3435T*) have an influence on the activity of P-gp and tacrolimus pharmacokinetics [30]. The intestinal *CYP3A4* and P-gp work together in a coordinated manner to serve as an absorption barrier

against tacrolimus [31], and the altered activity of P-gp has a significant influence on tacrolimus metabolism by CYP3A4 in both gut and liver [32,33]. However, lack of studies focused on the interaction between *ABCB1* and *CYP3A4* genetic polymorphisms on tacrolimus in renal transplant recipients, which limited analyzing the issues further. It is possible that a combination of *CYP3A4/5* [34] and *ABCB1* genotypes would be more helpful in making predictions than any single gene.

In conclusion, our meta-analysis suggests that the *CYP3A4*1B* genetic polymorphism may affect the tacrolimus dose requirements and the C_0 /Dose ratio within the first year post-transplantation in adult renal transplant recipients, especially in European recipients (mostly Caucasian). *CYP3A4* genotyping before transplantation would be helpful to provide an appropriate initial dose and adjust the maintenance dose in adult renal transplant recipients.

Supporting Information

S1 Checklist. PRISMA checklist.

(DOC)

S2 Checklist. Meta-analysis on genetic association studies form checklist.

(DOCX)

S1 File. Excluded articles list.

(DOCX)

S2 File. PRISMA flow diagram.

(DOC)

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Author Contributions

Conceived and designed the experiments: WLS HLT SDZ. Performed the experiments: WLS HLT. Analyzed the data: WLS HLT. Contributed reagents/materials/analysis tools: WLS HLT. Wrote the paper: WLS HLT.

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