The effect of *ABCB1* polymorphism on sirolimus in renal transplant recipients: a meta-analysis

Shuai Shao[1,2], Lei Hu[1], Zaigang Han[2], Kelu Hou[1], Huihui Fang[1], Guijie Zhang[1], Yufei Feng[1], Lin Huang[1]

[1]Department of Pharmacy, Peking University People's Hospital, Beijing 100044, China; [2]Department of Pharmacy, Affiliated Hospital of Beihua University, Jilin 132011, China

Contributions: (I) Conception and design: L Huang; (II) Administrative support: L Huang, Y Feng; (III) Provision of study materials: L Hu, K Hou; (IV) Collection and assembly of data: S Shao, ZG Han, H Fang, G Zhang; (V) Data analysis and interpretation: S Shao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Yufei Feng, MS; Lin Huang, PhD. Department of Pharmacy, Peking University People's Hospital, No. 11 Xizhimen South Street, Xicheng District, Beijing 100044, China. Email: fenyufei@126.com; huanglin@pkuph.edu.cn.

Background: Sirolimus (SRL) is an immunosuppressive drug and substrate of the P-glycoprotein (P-GP) encoded by *ABCB1*. The relationship between *ABCB1* polymorphism and the pharmacokinetics of SRL in different studies were conflicting in renal transplant recipients. Thus, this meta-analysis aims to investigate the influence of *ABCB1* C3435T, C1236T, and G2677T/A polymorphisms on the dose-adjusted trough level (C/D) of SRL in renal transplant recipients.

Methods: PubMed, Embase, and the Cochrane Library were searched for relevant studies. The quality of each eligible study was assessed according to Newcastle-Ottawa Scale. The STATA 15.0 was adopted to perform the meta-analysis. The fixed-effects model was used for pooled results with low heterogeneity ($I^2 \leq 50\%$); otherwise, the random-effects model was used.

Results: A total of 6 studies were included in the meta-analysis. Results of pooled analysis showed no significant association of SRL C/D ratio with *ABCB1* C3435T polymorphism. The subgroup analysis based on different ethnic groups and different time-points after SRL initiation in renal transplant recipients were also conducted. No significant association was observed in these subgroups. Significant associations were showed between *ABCB1* C1236T polymorphism and the C/D ratio of SRL in the homozygous model (TT *vs.* CC; WMD: –45.54; 95% CI: –75.15, –15.94; P=0.003), and also in subgroup of Caucasian (TT *vs.* CC; WMD: –46.57; 95% CI: –91.90, –1.25; P=0.044 and TT *vs.* CC + CT; WMD: –52.10; 95% CI: –95.38, –8.82; P=0.018). Significant differences were found in association between the *ABCB1* G2677T/A polymorphism and the C/D ratio of SRL, including the homozygous model (TT *vs.* GG; WMD: –76.47; 95% CI: –126.37, –26.58; P= 0.003), the heterozygous model (GT *vs.* GG, WMD: 178.62; 95% CI: 125.03, 232.22; P= 0.000), the dominant model (GT + TT *vs.* GG; WMD: 82.23; 95% CI: 36.28, 128.17; P=0.000), the recessive model (TT *vs.* GG + GT; WMD: –179.38; 95% CI: –283.33, –75.42; P=0.001), and the over-dominant model (GT *vs.* GG + TT; WMD: 199.44; 95% CI: 84.84, 314.05; P=0.001).

Conclusions: No significant association exists between *ABCB1* C3435T polymorphism and the C/D ratio of SRL in renal transplant recipients. To achieve target therapeutic concentrations, *ABCB1* C1236T homozygous mutant TT genotype will require a higher dose of sirolimus than wild type GG, especially in Caucasian renal transplant recipients. *ABCB1* G2677T/A TT genotype will also need a higher dose of sirolimus genotype. Genotyping of *ABCB1* might help to improve the individualization of SRL for renal transplant recipients. Further studies are expected to provide high-quality evidence.

Keywords: Sirolimus; *ABCB1*; pharmacokinetics; meta-analysis

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Introduction

Renal transplantation is one of the most effective treatments for end-stage renal disease (1). The emergence of immunosuppression drugs has dramatically improved the long-term survival of allografts and patients (2). Sirolimus (SRL), also known as rapamycin, which is a potent immunosuppressive drug used for prophylaxis of allograft rejection after renal transplantation (3). SRL shows substantial interindividual differences in pharmacokinetics (4). To achieve the desired efficacy and avoid the adverse reaction, monitoring the blood concentration of SRL is necessary (5). SRL is the substrate of P-glycoprotein (P-GP), an efflux transporter encoded by the *ABCB1* gene (6). P-GP transports SRL from the intracellular to the extracellular domain and influencing SRL pharmacokinetics (7). The expression and production of *ABCB1* are related to single nucleotide polymorphisms (SNPs) (8). The genetic polymorphisms of *ABCB1* have been considered as significant determinants of SRL pharmacokinetic (9).

Increasing studies have been conducted to investigate the influence of genetic polymorphisms of *ABCB1* on SRL trough blood concentrations and pharmacokinetic parameters in renal transplantation (4,10,11). While until now, the results of the *ABCB1* genotype on SRL pharmacokinetics are contradictory (12). Miao *et al.* (10) evaluated the relationship between the *ABCB1* 3435C>T genotype and C/D (trough concentrations/dose ratios) of SRL, but no significant association was observed. However, Sam *et al.* (13) reported that *ABCB1* 3435C>T genotype was significantly associated with log C/D of SRL. More than 50 genotypes have been studied in *ABCB1*, but most widely studied are the 3435C>T in exon 26, 1236C>T in exon 12, and three alleles 2677G>T/A in exon 21 (14). Although there are various studies on the correlation between *ABCB1* polymorphisms and dose-adjusted concentration of SRL, there is no systematical evidence about the effect of *ABCB1* polymorphisms on the dosage adjusted concentration of SRL. Therefore, to explore the relationship between *ABCB1* C3435T, C1236T, G2677T/A genotypes, and the SRL dose requirement in kidney transplant recipients, we performed the meta-analysis in related studies.

Methods

The report followed the guidelines set out in the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) Statement (15).

Search strategy

The studies were searched in the databases of PubMed, Embase, and the Cochrane Library up to November 2019. To investigate the association between ABCB1 polymorphism and pharmacokinetics of sirolimus in renal transplant recipients, we combined search terms as (kidney transplantation or renal transplantation) and (sirolimus or rapamycin or rapamune or AY-22989 or I-2190A) and (multidrug resistance-1 or ABCB1 or MDR1 or p-glycoprotein or P-GP or C3435T or C1236T or G2677T or G2677A or G2677T/A or rs1045642 or rs1128503 or rs2032582) and (polymorphism* or variant or mutation or genotype).

Study selection

Two reviewers evaluated studies for the titles, abstracts, and the full texts of the candidate articles (n=138) independently and in duplicate. Studies were enrolled according to the following inclusion criteria: (I) studies that assessed the association between *ABCB1* C3435T, C1236T or G2677T/A polymorphisms and sirolimus metabolism; (II) provided original data including sirolimus dosage adjusted concentration [C/D ratio = concentration (ng/mL)/dose (mg/kg)] after renal transplantation; (III) studies included detailed genotyping data of *ABCB1*. Exclusion criteria were (I) incomplete genotype data; (II) insufficient C/D data; (III) articles only with an abstract.

Data extraction

Two independent researchers extracted the following information from each study: lead author, publication year, country of origin, ethnicity, mean or range of age, sample size, sex, therapy time (the time of renal transplant recipients treated with SRL), weight-adjusted dosage of sirolimus [the daily dose of SRL (mg) divided by the weight (mg/kg/day)], target therapeutic window (rang of dosage adjusted trough steady-state blood levels of SRL), method of genotype measured, method of concentration measured. Furthermore, the C/D ratios were shown by the form of mean ± standard deviation (SD). If the studies only provided minimum and maximum; instead, the mean ± standard
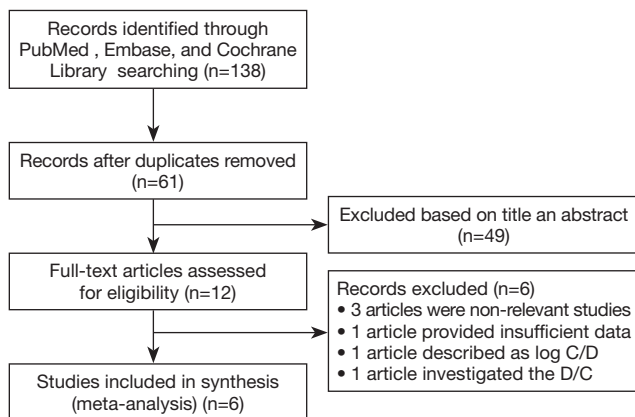
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Records identified through
PubMed , Embase, and Cochrane
Library  searching (n=138)
                │
                ▼
Records after duplicates removed
(n=61)
                │                    ──►  Excluded based on title an abstract
                ▼                              (n=49)
Full-text articles assessed
for eligibility (n=12)                ──►  Records excluded (n=6)
                │                          • 3 articles were non-relevant studies
                ▼                          • 1 article provided insufficient data
Studies included in synthesis             • 1 article described as log C/D
(meta-analysis) (n=6)                      • 1 article investigated the D/C
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Figure 1 Flow chart of the study selection process.

deviation was estimated by the method, which was reported by Jiang *et al.* (16).

Quality assessment

The quality assessment of included eligible studies was conducted by the Newcastle-Ottawa Scale (NOS) (17). It consisted of three parts: a selection of participants (four items), comparability of cases, and control groups (two items), adequacy of Outcome (three items). Thus, the quality assessment score ranged from zero to nine-point. The score seven points or more were expressed high quality and insignificant risk of bias, and if less than seven points represent low or moderate quality, considered as high or moderate risk of bias.

Statistical analysis

Statistical analyses were conducted with Stata (release 15.0; Stata Corporation, College Station, TX, USA) software. The weighted mean difference (WMD) and 95% confidence intervals (CIs) on forest plots of sirolimus C/D ratio among different C1236T, C3435T and G2677T/A genotypes were evaluated. We examined the value of WMD for the allelic model, homozygous model, heterozygous model, dominant model, recessive model, over-dominant model. I-squared (I^2) statistics estimated the heterogeneity. The fixed effects model was initially applied. When heterogeneity existed as $I^2 > 50\%$, and the random effects model was used. To evaluate the influence of ethnicity and therapy time differences in heterogeneity, subgroup analysis based on ethnicity and therapy time was performed.

Moreover, deviation from the Hardy-Weinberg equilibrium (HWE) of each eligible study was assessed, and if $P < 0.05$ was considered as disequilibrium. Studies not in HWE were subjected to sensitivity analysis. We performed a sensitivity analysis for the influence of each study on the stability of the results. Publication bias was examined by the symmetry of the funnel plot and evaluated by Egger's test ($P < 0.05$ was considered as significant publication bias).

Results

Studies selection and characteristics

The flow diagram for the study selection process is shown in *Figure 1*. After a preliminary online search, a total of 138 potentially relevant articles, with 58 from PubMed, 36 from Embase, and 44 from the Cochrane Library, were named for further evaluations. There were 61 studies removed after duplicates. Then 49 studies were screened for inclusion by the titles and abstracts articles not associated with the *ABCB1* polymorphisms and the C/D ratio of SRL. Six studies were excluded: 3 articles were non-relevant studies; 1 article supplied insufficient data; 1 article described as log C/D; 1 article investigated the D/C ratio. Thus, there were 6 eligible studies (10,11,18-21) described the association of *ABCB1* polymorphism with the C/D ratio of SRL. These studies were conducted in different countries including China (10,18,19), Spain (20), Belgium (21), France (11). The detailed characteristics, *ABCB1* genotype distributions, and dose-adjusted concentration of sirolimus of these included studies were shown in *Tables 1* and *2*.

Study quality assessment

The quality of the included eligible studies was evaluated according NOS. The scores of these studies were between 6 and 9, which represented high quality and minimal risk of bias. The results of the quality assessment were showed in *Table 2*. The distribution of the genotypes of all included studies was in HWE except for C1236T (P-HWE =0.042) of Lee *et al.* (18).

Association between ABCB1 C3435T polymorphism and C/D ratio of sirolimus

A total of six studies analyzed the association between *ABCB1* C3435T polymorphism and the C/D ratio of SRL. As shown in *Table 3*, three studies were conducted

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Table 1 The characteristics of included eligible studies

Study	Year	Country	Ethnicity	N	M/F	Age [years]	Therapy time (month)	Weight-adjusted dosage of sirolimus (mg/kg/day)	Target therapeutic window (ng/mL)	Genotype	Method of genotype measured	Method of concentration measured
Rodríguez-Jiménez (20)	2017	Spain	Caucasian	36	28/8	58±9	≥1	CC: 0.077±0.053; CT: 0.042±0.012; TT: 0.052	CC: 11.219±7.884; CT: 10.957±4.586; TT: 11.660±5.352	C3435T	PCR-RFLP	Microparticle enzyme immunoassay technique
Li (19)	2015	China	Asian	43	30/13	35 [34–46]	>1	0.04–0.06	5–10	C1236T G2677T C3435T	PCR	Automated enzyme immunoassay analyzer
Lee (18)	2014	China	Asian; Han nationality	85	65/20	42.9±10.4	≥3	–	5–10	C1236T G2677T/A C3435T	PCR	HPLC
Miao (10)	2008	China	Asian; Han nationality	50	39/11	42±15	≥6	CC: 0.025±0.006; CT: 0.024±0.004; TT: 0.025±0.003	CC: 7.86±3.09; CT: 9.05±2.79; TT: 8.27±2.35	C3435T	PCR-RFLP	HPLC
Mourad (21)	2005	Belgium	Caucasian; Africans; South Asian	–	–	–	6.2–285.3	0.11±0.06	5–15	C1236T G2677T C3435T	PCR	LC-MS/MS
Anglicheau (11)	2005	France	Caucasian; Black; Caribbean	51	30/21	43.7 [19.9–61.0]	3	0.025–0.476	10–20	C1236T G2677T/A C3435T	PCR	HPLC

M/F, male/female.

Table 2 The genotype distributions and dose-adjusted concentration of sirolimus of included eligible studies

Study	Postoperative time (month)	C3435T					C1236T					G2677T					G2677 mutant					NOS Score
		Cases (n)	CC	CT	TT	HWE	Cases (n)	CC	CT	TT	HWE	Cases (n)	GG	GT	TT	HWE	Cases (n)	GG	G/mutant	mutant/mutant	HWE	
Rodríguez-Jiménez	3	3/13/1	183.70±166.67	301.18±238.95	159.31	0.060	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Li	>1	18/20/5	442.45±65.93	338.1±80.25	383.85±82.98	0.096	8/20/15	246.6±59	446.85±91.03	163.75±-57.43	0.770	7/16/11	246.6±59	446.85±91.03	163.75±57.43	0.790	–	–	–	–	–	7
Lee	≥3	29/43/13	262.79±118.37	260.63±103.67	272.16±88.77	0.652	6/47/32	257.03±62.79	269.24±84.49	271.23±106.95	0.042	–	–	–	–	–	18/34/33	261.45±58.12	258.49±81.57	267.37±112.60	0.108	8
Miao	≥6	12/27/8	334.59±133.69	377.88±127.97	344.92±121.26	0.281	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Mourad	6.2–285.3	26/44/15	447.21±194.58	375.13±156.55	569.79±261.77	0.626	30/41/14	447.21±194.58	582.90±255.22	408.83±181.30	0.998	32/39/14	457.56±189.40	575.70±258.82	408.83±181.30	0.717	–	–	–	–	–	7
Anglicheau	3	21/21/9	186±128	139±77	176±75	0.360	116/23/12	198±146	150±70	150±73	0.967	–	–	–	–	–	22/20/9	184±125	144±80	166±77	0.250	9

Values are given as concentration/dose (ng/ml per mg/kg) by mean ± standard deviation. Mutant type includes TT, TA, or AA. NOS, Newcastle-Ottawa Scale; HWE, Hardy-Weinberg equilibrium; CC, wild type.

Table 3 Results of association between *ABCB1* C3435T polymorphism and C/D ratio of sirolimus

Genetic models	Studies included	Effects model	WMD (95% CI)	P	I^2 (%)
Allelic model (T *vs.* C)					
Overall	6	F	−10.93 [−28.45, 6.58]	0.221	48.4
Asian	4	R	−4.30 [−42.07, 33.47]	0.823	68.5
Caucasian	3	F	1.70 [−28.92, 32.31]	0.913	16.0
≥3 months	5	F	3.25 [−17.08, 23.58]	0.754	0
≥6 months	2	F	22.01 [−16.92, 60.94]	0.268	0
Heterozygous model (CT *vs.* CC)					
Overall	6	R	−33.27 [−85.42, 18.89]	0.211	66.1
Asian	4	R	−37.42 [−105.03, 30.19]	0.278	76.5
Caucasian	3	F	−47.27 [−97.69, 3.15]	0.066	12.8
≥3 months	5	F	−16.03 [−49.88, 17.82]	0.353	30.0
≥6 months	2	R	−14.76 [−127.82, 98.31]	0.798	69.1
Homozygous model (TT *vs.* CC)					
Overall	6	F	−4.15 [−41.77, 33.47]	0.829	15.8
Asian	4	F	−2.08 [−45.88, 41.72]	0.926	36.4
Caucasian	3	R	38.87 [−86.49, 164.22]	0.543	57.7
≥3 months	5	F	11.90 [−30.91, 54.70]	0.586	0
≥6 months	2	F	50.27 [−40.48, 141.01]	0.278	25.8
Dominant model (CT + TT *vs.* CC)					
Overall	6	R	−23.64 [−71.21, 23.92]	0.330	62.3
Asian	4	R	−25.49 [−87.84, 36.86]	0.423	74.2
Caucasian	3	F	−25.06 [−74.25, 24.14]	0.318	0
≥3 months	5	F	−5.72 [−38.35, 26.91]	0.731	0
≥6 months	2	F	7.36 [−54.61, 69.34]	0.816	0
Recessive model (TT *vs.* CC + CT)					
Overall	6	F	13.87 [−18.29, 46.03]	0.398	26.8
Asian	4	F	14.03 [−24.47, 52.53]	0.475	45.1
Caucasian	3	R	77.42 [−71.62, 226.46]	0.309	75.3
≥3 months	5	F	17.65 [−17.80, 53.10]	0.329	42.5
≥6 months	2	R	66.87 [−116.35, 250.09]	0.474	79.4
Over-dominant model (CT *vs.* CC + TT)					
Overall	6	R	−35.41 [−85.44, 14.62]	0.165	71.2
Asian	4	R	−43.13 [−108.58, 22.33]	0.197	79.9
Caucasian	3	R	−49.66 [−136.55, 37.24]	0.263	61.3
≥3 months	5	R	−20.45 [−73.82, 32.92]	0.453	63.0
≥6 months	2	R	−37.89 [−190.84, 115.06]	0.627	86.9

WMD, weighted mean difference; F, fixed model; R, random model; 95% CI, 95% confidence interval.

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in China (10,18,19), and the others were respectively in Spain (20), Belgium (21), and France (11). According to the statistical analysis in total populations via different genetic models, no significant association was observed between *ABCB1* C3435T polymorphism and C/D ratio of SRL. The subgroup analyses were performed according to the ethnicity of recipients (grouped as Asian or Caucasian) and the interval after transplantation (grouped as over 3 months or over 6 months). No significant association was found in subgroups of ethnicity and the interval after transplantation. Overall, there was no significant effect of *ABCB1* C3435T polymorphism on the dose-adjusted trough level of SRL.

Association between ABCB1 C1236T polymorphism and C/D ratio of sirolimus

Four included studies evaluated the association between *ABCB1* C1236T polymorphism and C/D ratio of SRL. As shown in *Table 4*, two studies were conducted in China (18,19), 1 in Belgium (21), and 1 in France (11). According to the statistical analysis, significant association were observed in the homozygous model of all patients (TT *vs.* CC; WMD: –45.54; 95% CI: –75.15, –15.94; P=0.003), subgroup of Caucasian in the homozygous model (TT *vs.* CC; WMD: –46.57; 95% CI: –91.90, –1.25; P=0.044), subgroup of Asian in the dominant model (CT + TT *vs.* CC; WMD: 55.11; 95% CI: 21.34, 88.87; P=0.001), and subgroup of Caucasian in the recessive model (TT *vs.* CC + CT; WMD: –52.10; 95% CI: –95.38, –8.82; P=0.018). The forest plots were shown in *Figures S1-S4*. The subjects with TT genotype in Caucasian subgroup *ABCB1* C1236T had a lower C/D ratio and needed higher sirolimus dose than those with CC genotype.

Association between ABCB1 G2677T/A polymorphism and C/D ratio of sirolimus

The *ABCB1* 2677G>T/A mutation could lead to two changes of an amino acid (from alanine to serine or threonine) (22). The genotypes for the *ABCB1* 2677G>T/A SNP were classified as follows: wild type (G/G), heterozygous (G/T or G/A) and homozygous for the variant (T/T, T/A or A/A). Due to the diversity of this genotype, data can not be merged simply.

Two studies (19,21) assessed the influence of *ABCB1* G2677T polymorphism on the dose-adjusted trough level of SRL, and the summarized results were shown in *Table 5*. According to the statistical analysis, significant differences were found in association between the *ABCB1* G2677T polymorphism and the C/D ratio of SRL in the heterozygous model (GT *vs.* GG, WMD: 178.62; 95% CI: 125.03, 232.22; P=0.000), the homozygous model (TT *vs.* GG; WMD: –76.47; 95% CI: –126.37, –26.58; P=0.003), the dominant model (GT + TT *vs.* GG; WMD: 82.23; 95% CI: 36.28, 128.17; P=0.000), the recessive model (TT *vs.* GG + GT; WMD: –179.38; 95% CI: –283.33, –75.42; P=0.001), and the over-dominant model (GT *vs.* GG + TT; WMD: 199.44; 95% CI: 84.84, 314.05; P=0.001). The forest plots were shown in *Figures S5-S9*.

Two studies (11,18) assessed the influence of *ABCB1* G2677mutant polymorphism on the C/D ratio of SRL. Mutant type included TT, TA or AA in both of these studies. The summarized results were shown in *Table 6*. The results of heterogeneity within all genetic models were 0. Moreover, no significant difference was found in association with the *ABCB1* G2677mutant polymorphism with the C/D ratio of SRL.

Sensitivity analysis

As shown in *Table 2*, only one study (18) included in the meta-analysis was a departure from HWE (P<0.05). A sensitivity analysis was performed by sequential omission of each eligible study to assess the influence of the individual data on the pooled WMDs. The results revealed that the departure from HWE of study has no major impact. Sensitivity analysis to evaluate the ethnicity and therapy time showed that no individual study influenced the pooled estimate significantly. The results are shown in *Figures S10-S13*. None of the studies had an individually considerable influence on the impact of *ABCB1* C3435T, C1236T, G2677T/A. Sensitivity analyses suggested that this meta-analysis was steady.

Estimation of publication bias

The potential publication bias of eligible studies was assessed by the funnel plot, Egger's test, and Begg's test. As shown in *Figures S14-S17*, the funnel plots did not provide evidence of obvious asymmetry. The Egger's test and Begg's test for publication bias were not statistically significant in all the genetic models of *ABCB1* C3435T, C1236T (*Table S1*), because of the number of G2677T/A studies was small, the Egger's test cannot be displayed.

Table 4 Results of the association between *ABCB1* C1236T polymorphism and dose-adjusted concentration of sirolimus

Genetic models	Studies included	Effects model	WMD (95% CI)	P	I^2 (%)
Allelic model (T *vs.* C)					
Overall	4	R	−31.26 [−72.53, 10.02]	0.138	82.5
Asian	3	R	−25.02 [−88.97, 38.93]	0.443	87.8
Caucasian	2	R	−26.66 [−75.58, 22.26]	0.285	50.4
≥3 months	3	R	−14.48 [−52.09, 23.13]	0.450	69.4
Heterozygous model (CT *vs.* CC)					
Overall	4	R	72.25 [−50.25, 194.74]	0.248	94.4
Asian	3	R	115.00 [−15.13, 245.12]	0.083	90.8
Caucasian	2	R	37.16 [−142.37, 216.69]	0.685	90.4
≥3 months	3	R	20.64 [−64.28, 105.56]	0.634	82.8
Homozygous model (TT *vs.* CC)					
Overall	4	F	−45.54 [−75.15, −15.94]	0.003	47.1
Asian	3	F	−37.36 [−106.40, 31.67]	0.289	64.6
Caucasian	2	F	−46.57 [−91.90, −1.25]	0.044	0
≥3 months	3	F	−25.60 [−62.27, 11.08]	0.171	16.9
Dominant model (CT + TT *vs.* CC)					
Overall	4	R	28.66 [−41.23, 98.55]	0.422	85.6
Asian	3	F	55.11 [21.34, 88.87]	0.001	47.4
Caucasian	2	R	14.61 [−121.27, 150.49]	0.833	86.5
≥3 months	3	R	8.15 [−62.63, 78.93]	0.821	78.2
Recessive model (TT *vs.* CC + CT)					
Overall	4	R	−34.26 [−86.68, 18.16]	0.200	58
Asian	3	R	−45.78 [−161.53, 69.96]	0.438	75.1
Caucasian	2	F	−52.10 [−95.38, −8.82]	0.018	37.4
≥3 months	3	R	−34.26 [−86.68, 18.16]	0.200	58
Over-dominant model (CT *vs.* CC + TT)					
Overall	4	R	88.11 [−56.48, 232.69]	0.232	97.2
Asian	3	R	133.58 [−47.99, 315.15]	0.149	97.0
Caucasian	2	R	46.86 [−140.42, 234.13]	0.624	92.4
≥3 months	3	R	20.95 [−56.30, 98.20]	0.595	85.3

WMD, weighted mean difference. F, fixed model; R, random model; 95% CI, 95% confidence interval.

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Table 5 Results of association between *ABCB1* G2677T polymorphism and C/D ratio of sirolimus

Genetic models	Studies included	Effects model	WMD (95% CI)	P	I² (%)
Allelic model (T *vs.* G)	2	R	−39.51 [−111.15, 32.14]	0.280	71.0
Heterozygous model (GT *vs.* GG)	2	F	178.62 [125.03, 232.22]	0.000	42.8
Homozygous model (TT *vs.* GG)	2	F	−76.47 [−126.37, −26.58]	0.003	0
Dominant model (GT + TT *vs.* GG)	2	F	82.23 [36.28, 128.17]	0.000	0
Recessive model (TT *vs.* GG + GT)	2	R	−179.38 [−283.33, −75.42]	0.001	68.6
Over-dominant model (GT *vs.* GG + TT)	2	R	199.44 [84.84, 314.05]	0.001	77.1

WMD, weighted mean difference. F, fixed model; R, random model; 95% CI, 95% confidence interval.

Table 6 Results of association between *ABCB1* G2677mutant polymorphism and C/D ratio of sirolimus

Genetic models	Studies included	Effects model	WMD (95% CI)	P	I² (%)
Allelic model (mutant *vs.* G)	2	F	−2.70 [−24.09, 18.69]	0.805	0
Heterozygous model (G/mutant *vs.* GG)	2	F	−13.0 [−45.77, 19.76]	0.437	0
Homozygous model (mutant/mutant *vs.* GG)	2	F	−1.13 [−40.49, 38.24]	0.955	0
Dominant model (G/mutant + mutant/mutant *vs.* GG)	2	F	−7.70 [−38.31, 22.92]	0.622	0
Recessive model (mutant/ mutant *vs.* GG + G/mutant)	2	F	5.50 [−29.61, 40.60]	0.759	0
Over-dominant model (G/mutant *vs.* GG + mutant/mutant)	2	F	−16.32 [−47.39, 14.75]	0.303	0

Mutant type included TT, TA, or AA. WMD, weighted mean difference; F, fixed model; R, random model. 95% CI, 95% confidence interval.

Discussion

Sirolimus (SRL) is a necessary immunosuppressive drug after renal transplantation. Nevertheless, SRL exhibit significant interindividual variability in pharmacokinetics (23). It is necessary for therapeutic drug monitoring to avoid under or over-immunosuppression. It has been suggested that *ABCB1* polymorphisms contribute to the variability of SRL pharmacokinetics and therapeutic outcome (24). Although the influence of *ABCB1* polymorphisms on SRL metabolism has been studied focusing on C3435T, C1236T, and G2677T/A, the relationship between ABCB1 polymorphism and SRL metabolism in patients is still unclear. Therefore, our study was to explore the relationship between *ABCB1* polymorphisms and the pharmacokinetics of SRL in renal transplantation by a meta-analysis of existing data. Our work is helpful to evaluate that whether *ABCB1* genetic testing is expected to play a role in guiding the individualized treatment of SRL.

The AUC is challenging to apply in clinical practice, so other indicators such as trough concentration (C_0) replace the AUC (25). That is why AUC is rarely reported in these included studies. To make a comparison between the different doses, the dosage adjusted trough concentration C/D ratio was adopted in our study.

ABCB1 C3435T, a silent SNP localized in exon 26, has been found to be associated with altered P-GP function. It was reported that the homozygosity for the T allele resulted in a 2-fold reduction in intestinal P-GP expression (26). However, our overall analysis of pooled results demonstrated no statistically significant association between the C/D ratio of SRL and *ABCB1* C3435T polymorphism in different genetic models. In addition, relatively obvious heterogeneities existed in our study. With the aim of detecting the source of heterogeneity, we conducted stratified analysis according to the ethnicity and the interval after transplantation. The results were consistent with the overall analysis. Therefore, so far, there was no enough evidence showing the clinical relevance of the *ABCB1* C3435T polymorphism and the dosage adjusted trough concentration of SRL in Caucasians or Asians.

Significant association were observed between *ABCB1* C1236T polymorphism and C/D ratio of sirolimus in all patients via the homozygous model (TT *vs.* CC). The following subgroup analysis indicated the ethnicity of the renal transplant recipients might be one of the most critical covariates that could influence the dose adjusted concentration of SRL. The result showed that homozygous mutated genotype TT had a significant impact on the C/D ratio of sirolimus in Caucasians but nor in Asians. It was also found that the dose adjusted concentrations of SRL in Caucasian patients with *ABCB1* C1236T CC carriers are significantly higher than TT carriers. Therefore, Caucasian renal transplant recipients *ABCB1* C1236T TT carriers might need higher doses of SRL than CC carriers recipients.

The triallelic SNP G2677T/A results in a change of the amino acid alanine into serine or threonine (27) and may alter drug transport (28), whereas the synonymous SNP C3435T and C1236T are a silent mutation that do not lead to an amino acid change. The pooled analysis of studies focusing on G2677T polymorphism(alleles G and T) suggested that the polymorphism has significant influence on the C/D ratio of SRL. Patients carrying G2677T homozygous genotype TT would require higher doses of SRL to reach target levels compared with the wild genotype GG. However, The results of the pooled analysis about G2677mutant polymorphism (alleles G, A and T) showed no significant differences between *ABCB1* G2677mutant and the C/D ratio of SRL within all the genetic models. The small sample size may limit the analysis.

While each of the polymorphisms in the *ABCB1* haplotype may be independent, they may produce a much more salient phenotype when they appear together. One study was performed associated between *ABCB1* C1236T/G2677T/C3435T haplotypes analyses and the C/D ratio of SRL. Among the haplotypes, TTT, TGC, and CGC were the most frequently observed (29). Lee *et al.* (18) showed that patients carrying the CGC/CGC diplotype had a significantly lower C/D ratio of SRL compared with those carrying the CGC/TTT and TTT/TTT diplotype (P<0.05).

This meta-analysis pooled available data from eligible studies and significantly increased the statistical reliability. Also, there are some advantages to this meta-analysis. Firstly, this research is the first one to estimate the association between *ABCB1* polymorphism and the dosage adjusted concentration of SRL in renal transplant recipients. Secondly, the subgroup for the stratified analysis of potential sources of heterogeneity was performed based on ethnicity and the interval after transplantation. Thirdly, this study systematically analyzed the six genetic models to explore the association between the dosage adjusted concentration of SRL and *ABCB1* polymorphism.

Although the meta-analysis conducted considerable retrieval and analysis, there are still several limitations existed. First of all, high heterogeneity existed in more than half of outcomes, and lots of factors could lead to heterogeneity, such as differences among various therapy regimens, disease staging, age, sex and method of genotype and concentration detecting. However, the complete data were hardly accessed to perform subgroup analysis. Some of these factors might further influenced the results. Second, several eligible studies are excluded due to the absence of available original data, which may have an impact on this meta-analysis. Third, the sample sizes of the included studies were relatively small. Further studies are expected to provide high-quality evidence.

Conclusions

In summary, this meta-analysis showed that no significant association exists between *ABCB1* C3435T polymorphisms and the C/D ratio of SRL in renal transplant recipients. However, compared with *ABCB1* C1236T CC carriers, those with TT genotype will require a higher dose of sirolimus to achieve target therapeutic concentrations in Caucasian renal transplant recipients. *ABCB1* G2677T/A TT genotype will require a higher dose of sirolimus than wild type GG genotype. Performing *ABCB1* C1236T and G2677T genotyping before transplantation may guide to improve the individual immunosuppressive therapy. Further studies with large sample size are expected to confirm the relationship of *ABCB1* polymorphisms and the pharmacokinetics of SRL in renal transplant recipients.

Acknowledgments

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related

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to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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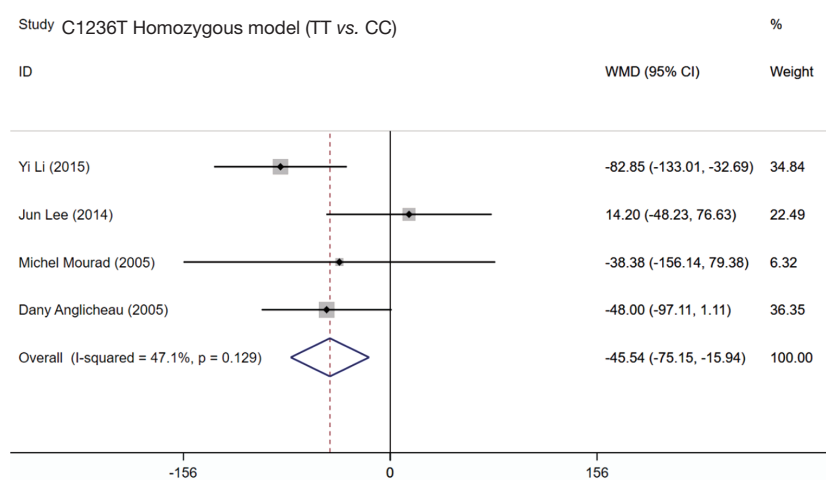
Figure S1 Forest plot of sirolimus dose-adjusted concentration between subjects carrying *ABCB1* C1236T TT genotype and CC genotype by the fixed-effects model in a homozygous model.
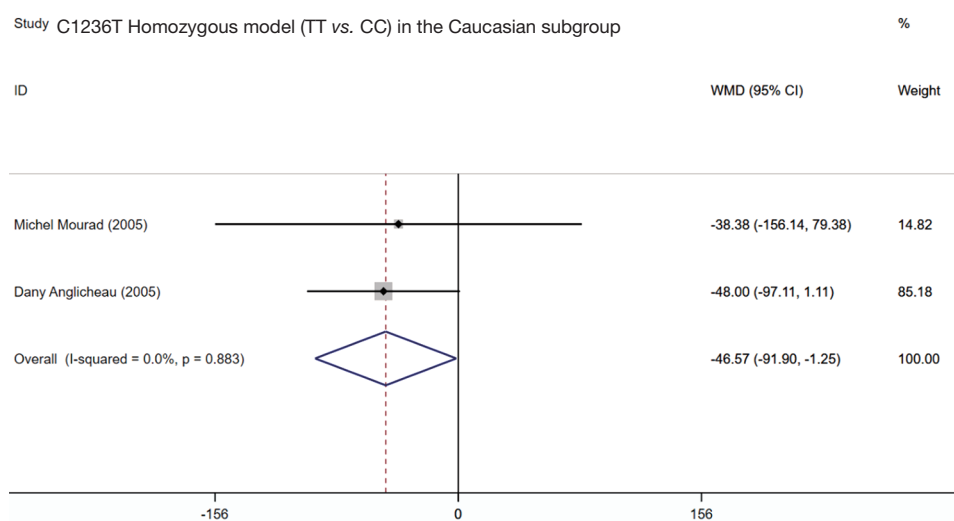
Figure S2 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying TT genotype and carrying CC genotype at *ABCB1* C1236T by the fixed-effects model in the Caucasian subgroup.
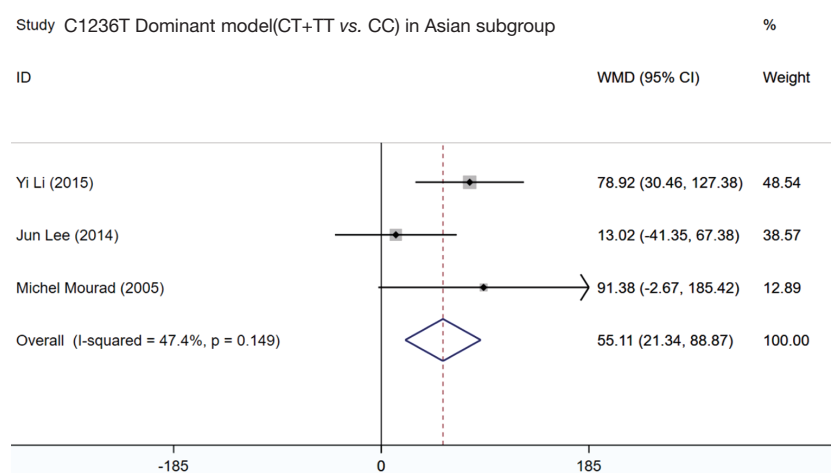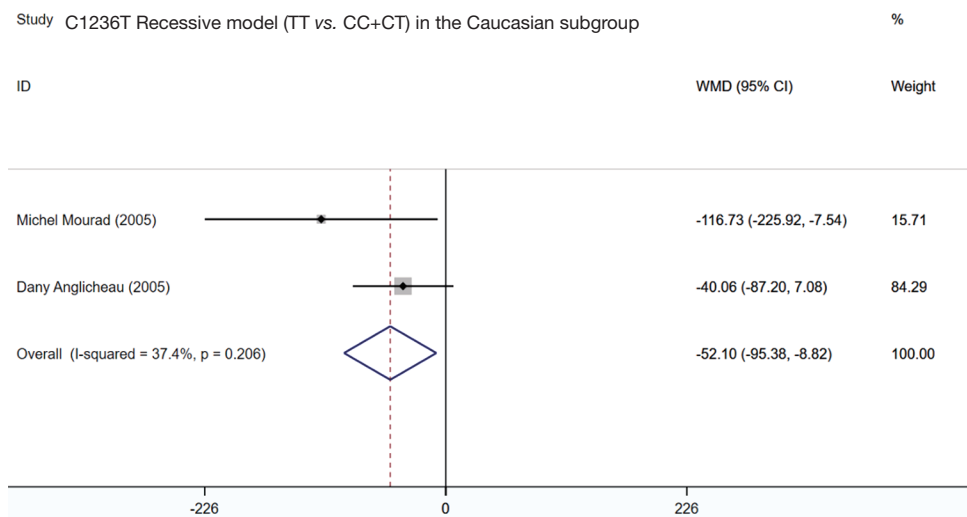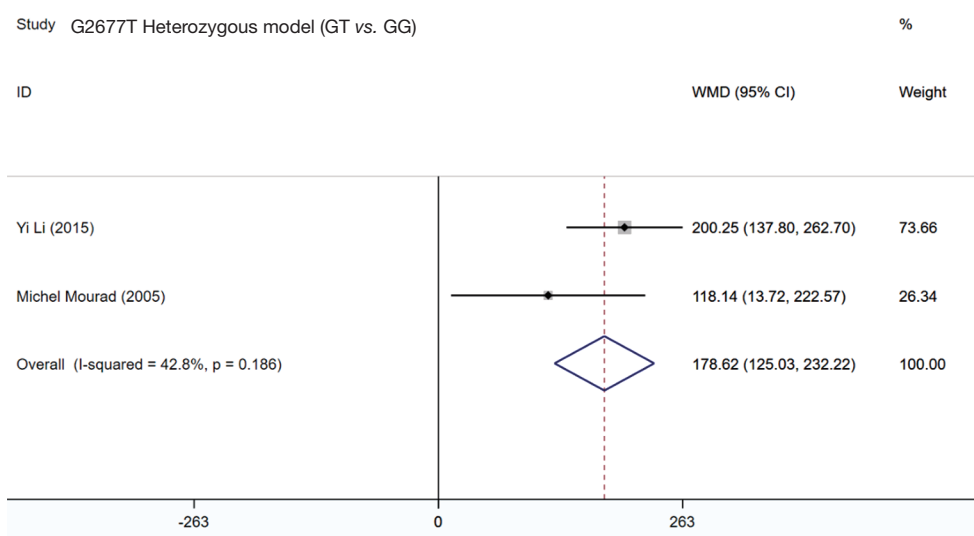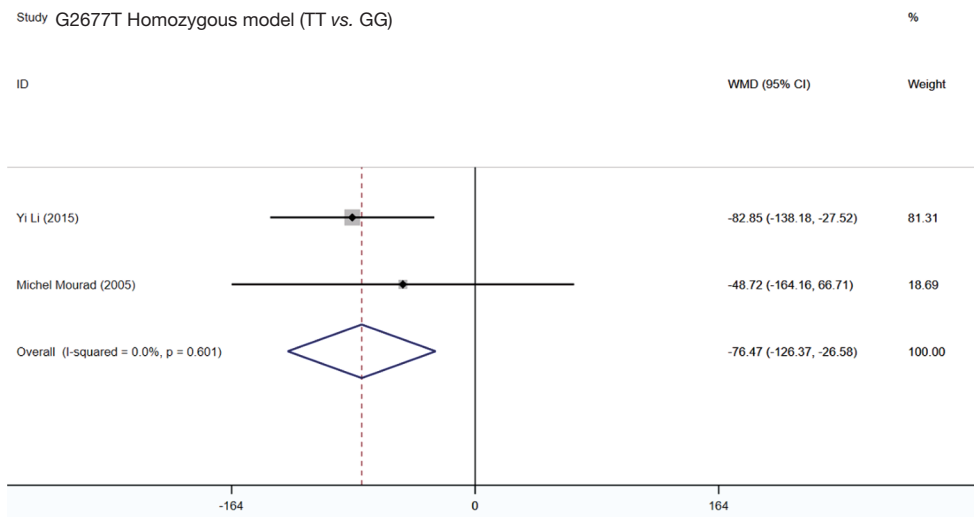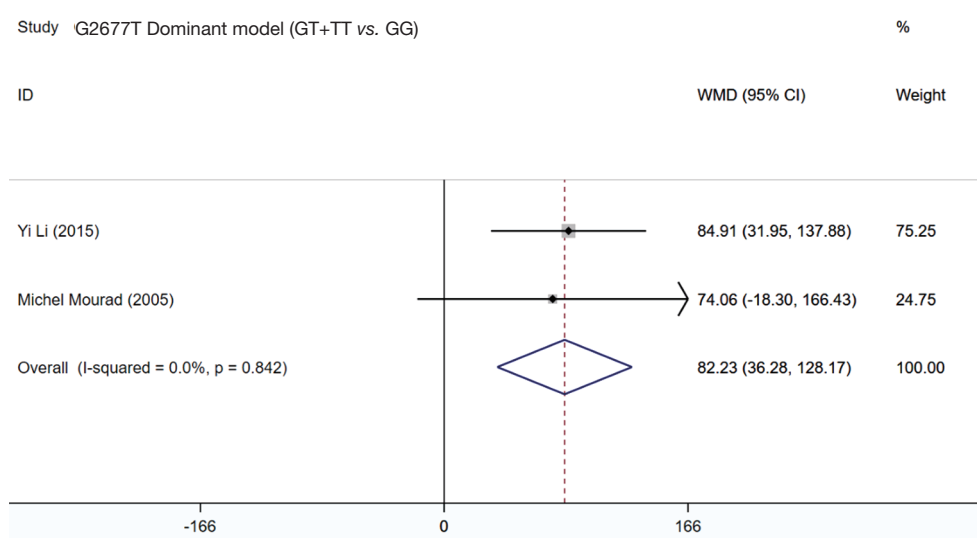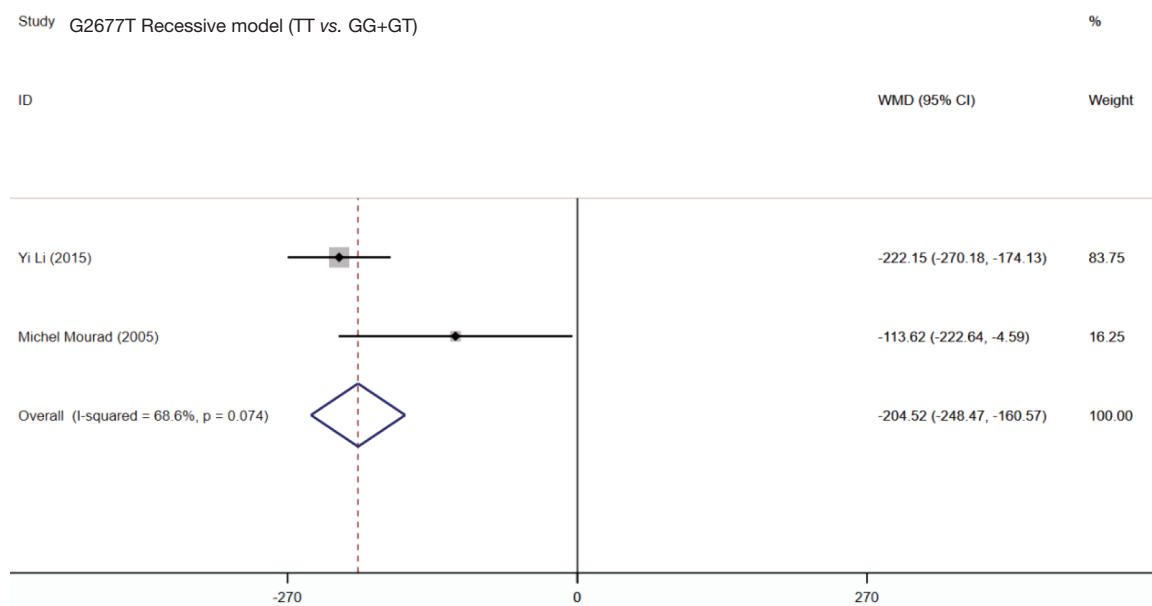
Figure S3 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying CT + TT genotype and carrying CC genotype at *ABCB1* C1236T by the fixed-effects model in Asian subgroup.

Figure S4 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying TT genotype and carrying CC + CT genotype at *ABCB1* C1236T by the fixed-effects model in the Caucasian subgroup.
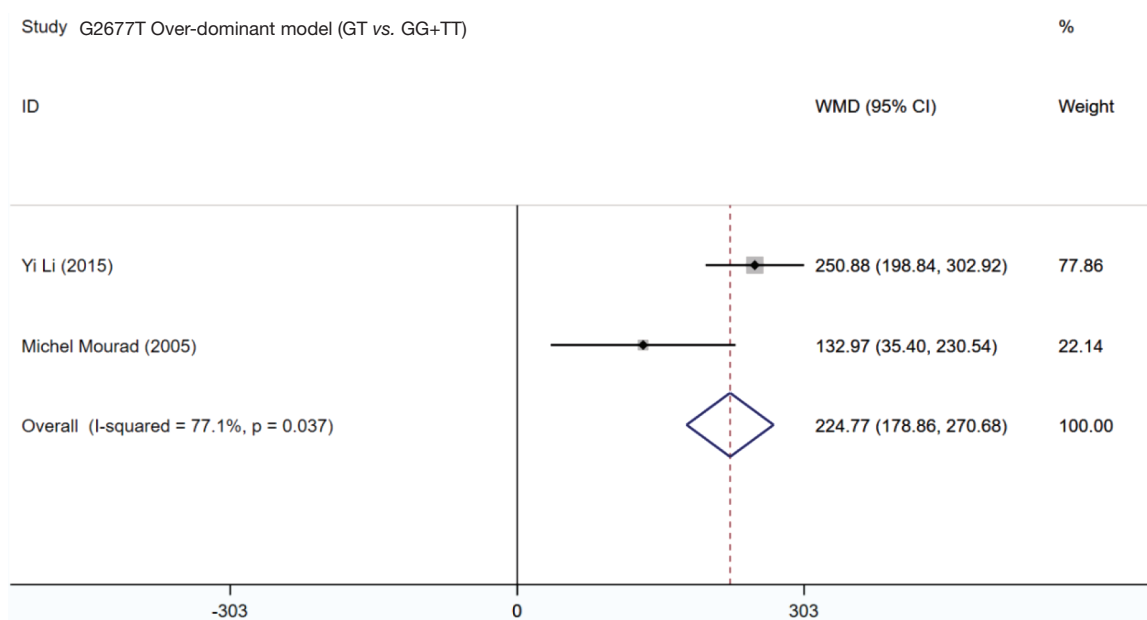
Figure S5 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying GT genotype and carrying GG genotype at *ABCB1* G2677T by fixed-effects model.

Figure S6 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying TT genotype and carrying GG genotype at *ABCB1* G2677T by fixed-effects model.

Figure S7 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying GT+TT genotype and carrying GG genotype at *ABCB1* G2677T by fixed-effects model.

Figure S8 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying TT genotype and carrying GG + GT genotype at *ABCB1* G2677T by fixed-effects model.

Figure S9 Forest plot of meta-analysis of dose-adjusted concentration of sirolimus administration between subjects carrying GT genotype and carrying GG + TT genotype at *ABCB1* G2677T by fixed-effects model.
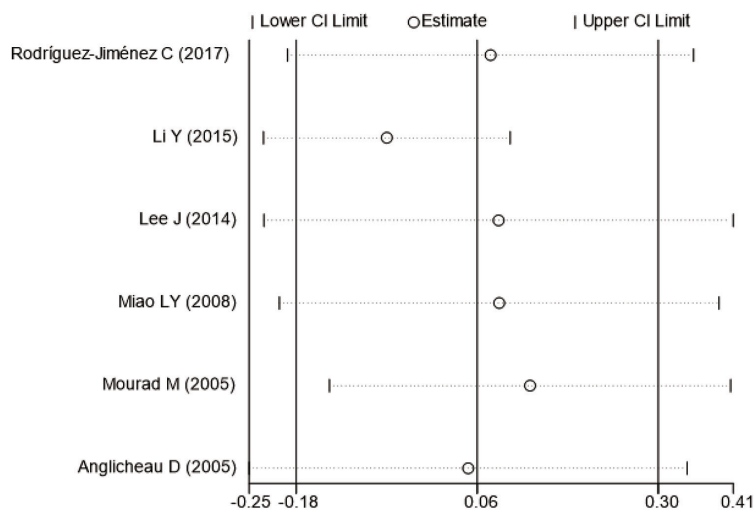
Figure S10 Sensitivity analysis for *ABCB1* C3435T polymorphism with the dose-adjusted concentration of sirolimus.
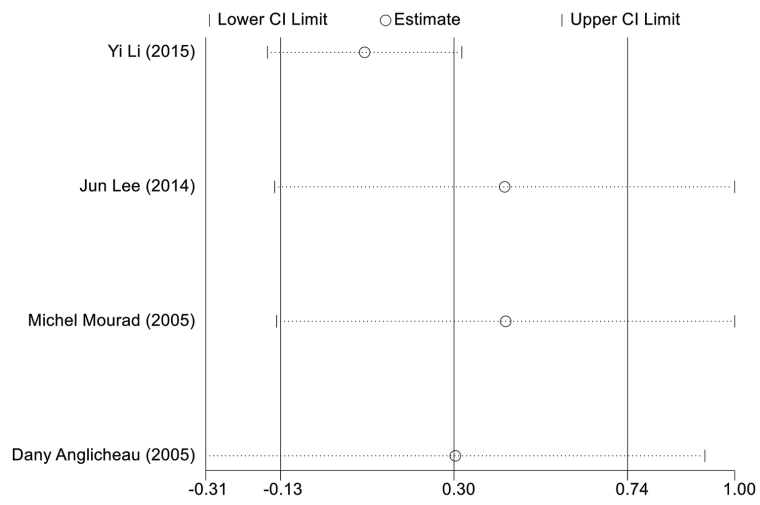
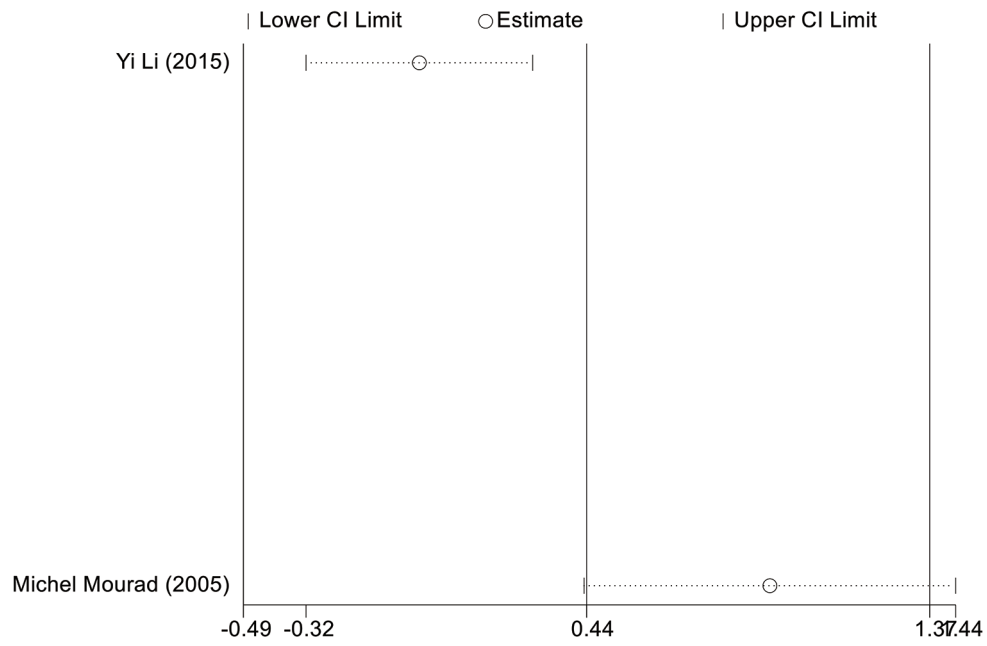**Figure S11** Sensitivity analysis for *ABCB1* C1236T polymorphism with the dose-adjusted concentration of sirolimus.

Figure S12 Sensitivity analysis for *ABCB1* G2677T polymorphism with the dose-adjusted concentration of sirolimus.
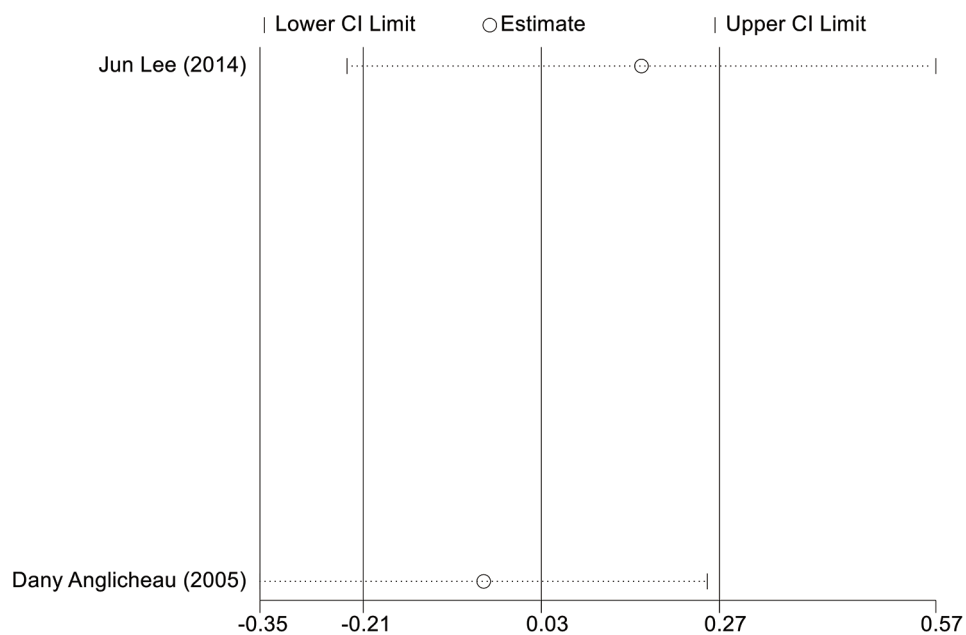
Figure S13 Sensitivity analysis for *ABCB1* G2677mutant polymorphism with the dose-adjusted concentration of sirolimus.
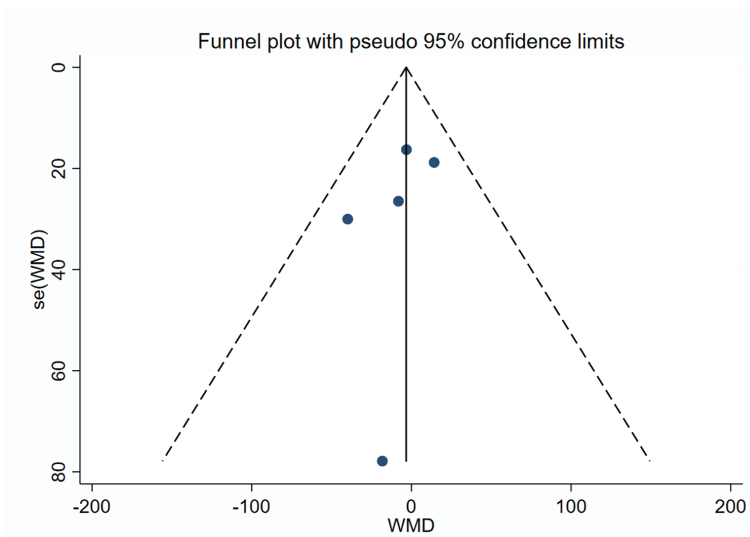

Figure S14 Funnel plots of the association between *ABCB1* C3435T polymorphism and dose-adjusted concentration of sirolimus.
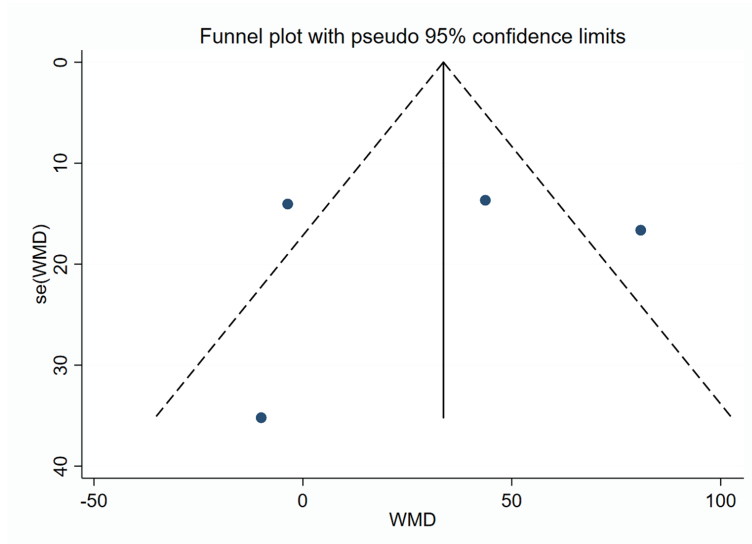
Figure S15 Funnel plots of the association between *ABCB1* C1236T polymorphism and dose-adjusted concentration of sirolimus.
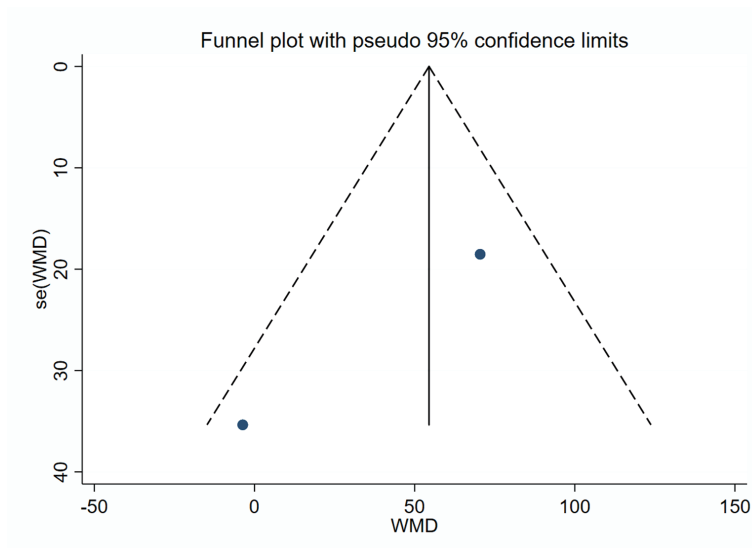

Figure S16 Funnel plots of the association between *ABCB1* G2677T polymorphism and dose-adjusted concentration of sirolimus.
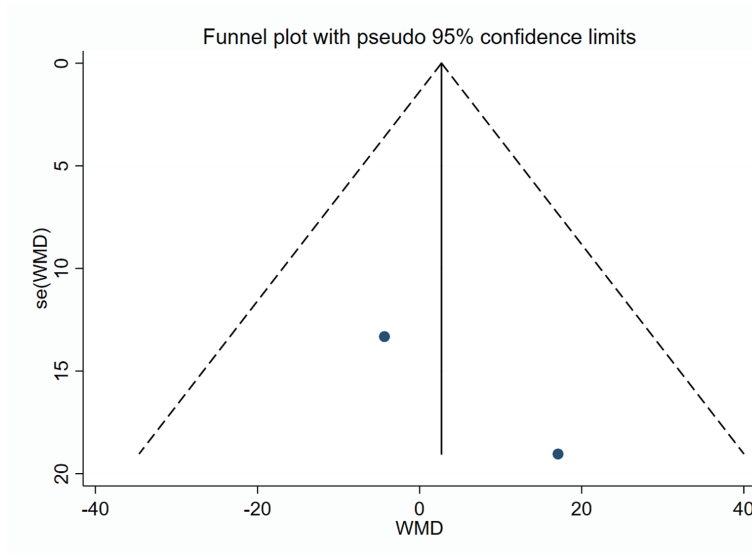
Figure S17 Funnel plots of the association between *ABCB1* G2677mutant polymorphism and dose-adjusted concentration of sirolimus.

Table S1 The P value of Egger's test and Begg's test for publication bias

Genetic models	Begg's test-P	Egger's test-P
C3435T		
Allelic model (T *vs.* C)		
Overall	0.707	0.415
Asian	0.734	0.433
Caucasian	1.000	0.641
≥3 months	0.462	0.411
≥6 months	1.000	-
Heterozygous model (CT *vs.* CC)		
Overall	0.452	0.246
Asian	0.308	0.521
Caucasian	1.000	0.397
≥3 months	0.806	0.528
≥6 months	1.000	-
Homozygous model (TT *vs.* CC)		
Overall	0.806	0.297
Asian	0.734	0.415
Caucasian	1.000	-
≥3 months	0.308	0.217
≥6 months	1.000	-
Dominant model (CT + TT *vs.* CC)		
Overall	0.452	0.175
Asian	1.000	0.378
Caucasian	0.296	0.102
≥3 months	0.806	0.325
≥6 months	1.000	-
Recessive model (TT *vs.* CC + CT)		
Overall	1.000	0.300
Asian	1.000	0.385
Caucasian	1.000	-
≥3 months	0.734	0.355
≥6 months	1.000	-
Over-dominant model (CT *vs.* CC + TT)		
Overall	0.707	0.518
Asian	0.734	0.952
Caucasian	1.000	0.707
≥3 months	1.000	0.751
≥6 months	1.000	-
C1236T		
Allelic model (T *vs.* C)		
Overall	1.000	0.868
Asian	1.000	0.957
Caucasian	1.000	-
≥3 months	1.000	0.767
Heterozygous model (CT *vs.* CC)		
Overall	0.308	0.380
Asian	1.000	0.898
Caucasian	1.000	-
≥3 months	0.296	0.072
Homozygous model (TT *vs.* CC)		
Overall	0.734	0.673
Asian	1.000	0.786
Caucasian	1.000	-
≥3 months	1.000	0.891
Dominant model (CT + TT *vs.* CC)		
Overall	0.734	0.291
Asian	1.000	0.847
Caucasian	1.000	-
≥3 months	0.296	0.100
Recessive model (TT *vs.* CC + CT)		
Overall	1.000	0.992
Asian	1.000	0.992
Caucasian	1.000	-
≥3 months	0.296	0.100
Over-dominant model (CT *vs.* CC + TT)		
Overall	0.308	0.573
Asian	1.000	0.826
Caucasian	1.000	-
≥3 months	0.296	0.220