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LABORATORY STUDY

A deeper understanding of the association between *CTLA4* +49A/G and acute rejection in renal transplantation: an updated meta-analysis

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Abstract

To reevaluate the association between the costimulatory molecule cytotoxic T lymphocyte-associated antigen4 (*CTLA4*) single nucleotide polymorphism (SNP) +49A/G and acute rejection (AR) in renal transplantation, nine studies published before June 2013 were analyzed. Meta-analysis and cumulative meta-analysis (metacum) were performed for each genotype in a random/fixed effect model. The combined odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the strength of the association. In the sensitivity analysis, a single study involved in the meta-analysis was deleted each time to investigate the influence of the individual data sets on the pooled ORs. Meta-analysis regression was used for some influence factors, such as year of publication, total number in each group (AR group and control group), ethnicity, the ratio of GG to GA + AA, the ratio of G to A in *CTLA4* +49A/G. Overall, a significant correlation was noted between the *CTLA4* SNP (+49A/G) and the risk of AR (for GG vs. AG + AA: OR = 1.35, 95% CI = 1.05–1.73, $p = 0.02$; for G vs. A: OR = 1.21, 95% CI = 1.03–1.42, $p = 0.02$), especially in the Asian subgroup (for GG vs. AG + AA: OR = 1.79, 95% CI = 1.15–2.78, $p = 0.009$; for G vs. A: OR = 1.47, 95% CI = 1.04–2.07, $p = 0.03$). Of the influence factors, the ratio of GG to GA+AA ($p = 0.046$) and the ratio of G to A ($p = 0.017$) were significant factors. In conclusion, our results suggest that *CTLA4* +49A/G contribute to the risk of AR following renal transplantation.

Keywords

Acute rejection, cytotoxic T lymphocyte-associated antigen4, meta-analysis, single nucleotide polymorphism

History

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Introduction

Cytotoxic T lymphocyte-associated antigen 4 (*CTLA4*) is a key element in the immune system that induces immune tolerance and is one of the critical negative regulators of the T cell-mediated immune response.[1] It is also expressed constitutively on the surface of regulatory T cells (Tregs) and is detectable on approximately 50% of Tregs; it is only found on <1% of naive helper T cells.[2] *CTLA4* ligation on Tregs results in a significant decrease in the presentation capacity of the antigen-presenting cells and effector T cell downregulation in mice.[3] *CTLA4* plays an important role in the downregulation of the immune response. The rs231775 (+49A/G) single nucleotide polymorphism (SNP) is located within the signal peptide of the molecule and influences the expression of the full-length isoform on the T cell membrane. The expression pattern of the *CTLA4* protein is also changed by polymorphisms of the rs4553808 (−1661A/G) and rs5742909 (−318C/T) loci, which are located in the *CTLA4* gene promoter.[4] Similarly, the rs733618 (−1772T) allele was found to decrease transcription of the *CTLA4* gene by influencing the binding of transcription factors.[5] The rs3087243 (+6230G/A) SNP is situated within the 3′ untranslated region of the *CTLA4* gene and was found to be associated with susceptibility to autoimmune diseases.[6] The +49A/G (rs231775) and the +6230G/A (rs3087243) SNPs of the *CTLA4* gene play influential roles in graft rejection and the long-term clinical outcome of organ transplantation.[7–12] Among these polymorphisms, the +49A/G (rs231775) polymorphism is the most widely investigated in renal transplantation. Over the last few years, numerous studies have been conducted concerning the relationship between the *CTLA4* +49A/G polymorphism, found in exon 1, and acute rejection (AR) following renal transplantation indifferent races and ethnicities. However, these studies have yielded inconsistent results.[13,14] To produce more precise results, we evaluated these associations using a meta-analysis.

Materials and methods

Identification of eligible studies

The relevant literature was extracted from databases including MEDLINE and EMBASE; the last updated search was

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performed before July 2013. The following search terms were used: ''*CTLA4*'', ''polymorphism'', ''kidney'', and ''AR''. Only published articles were included in this study. The references of the selected papers were also manually checked for other relevant articles that might have been missed in the initial search. No limitation was set on the language of the literature. The following inclusion criteria were used: (1) the study discussed the association between the SNP *CTLA4* +49A/G and the risk of AR; (2) the study described useful genotype frequencies; and (3) when a study reported results indifferent subpopulations, these subpopulations were considered as separate studies. The following exclusion criteria were used: (1) the study was conducted on animals; (2) there was no control group; (3) the study was not associated with the polymorphism; and (4) for studies with overlapping or repeated data, the most recent or complete studies with the largest numbers of cases and controls were included.

Data extraction

Two investigators (Yifeng Guo and Fang Guo) independently reviewed and extracted the information from all eligible publications according to the inclusion and exclusion criteria listed above. In the case of a conflict, an agreement was reached after a discussion between the two reviewers. The following characteristics were extracted from each study: name of the first author, year of publication, country of origin, racial descent of participants, polymorphisms, number of cases and controls, *p* value for HWE, and genotyping methods.

Statistical analysis

The allele frequencies of the *CTLA4* gene polymorphisms were determined using the allele counting method. Hardy–Weinberg equilibrium (HWE) was assessed in each study using the goodness-of-fittest (chi-square test or Fisher exact

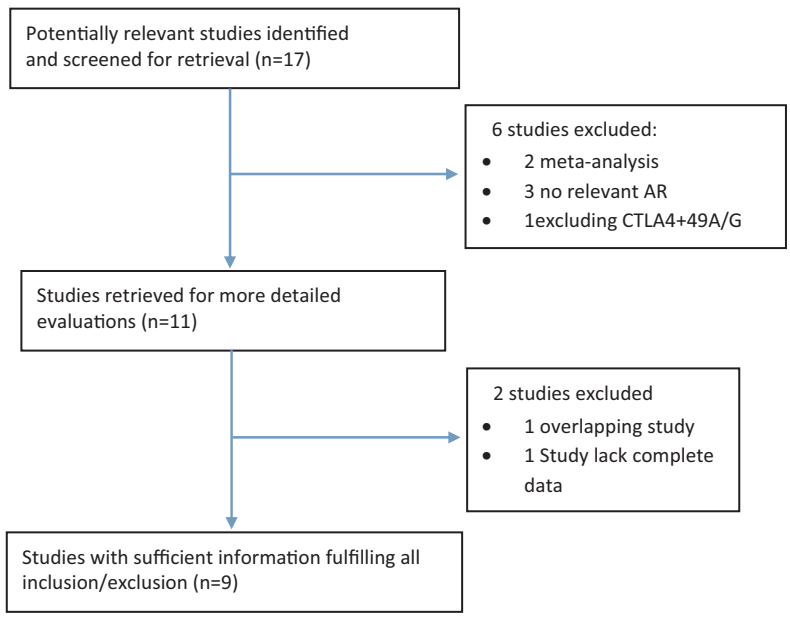


Figure 1. Flow diagram of search strategy and study selection.

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

First author	Year	Ethnicity	Polymorphisms	Cases (AR) 481	Controls (Non-AR) 1335	*p* Value for HWE	Genotyping method
Dmitrenko	2005	European	rs5742909 (−318 C/T), rs231775 (+49 G/A)	50	50	Yes	PCR-RFLP
Wisniewski	2006	European	rs5742909 (−318 C/T), rs231775 (+49G/A)	38	53	Yes	PCR-RFLP
Gorgi	2006	African	rs5742909 (−318 C/T), rs231775 (+49G/A)	31	39	Yes	PCR-RFLP/PCR-SSP
Gendzekhadze	2006	European	rs733618 (−1722T/C), rs4553808 (−1661A/G), rs5742909 (−318 C/T), rs231775 (+49G/A)	30	33	Yes	PCR-RFLP
Haimila	2009	European	rs4553808 (−1661A/G), rs5742909 (−318C/T), rs231775 (+49G/A), rs3087243 (+6230 G/A)	109	546	Yes	
Ruhi	2010	European	rs4553808 (−1661A/G), rs5742909 (−318C/T), rs231775 (+49G/A), rs3087243 (+6230 G/A)	49	47	Yes	PCR-RFLP
Kim	2010	Asian	rs5742909 (−318 C/T), rs231775 (+49G/A)	59	266	Yes	PCR
Gao	2012	Asian	rs733618 (−1722T/C), rs4553808 (−1661A/G), rs5742909 (−318C/T), rs231775 (+49G/A), rs3087243 (+6230 G/A)	45	122	Yes	DNA sequencing
Domanski	2012	European	rs231775 (+49G/A)	70	179	Yes	RT-PCR

Notes: PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction with restriction fragment length polymorphism; PCR-SSP, polymerase chain reaction with sequence-specific primers; RT-PCR, real-time polymerase chain reaction; LT, liver transplantation; RT, renal transplantation; p_{Het}; *p* value for heterogeneity; G, guanine; A, adenine; C, cytosine; T, thymine.

test) in case-control groups. The pooled odds ratios (ORs) with 95% confidence intervals (95% CI) were used to assess the strength of the association. Analysis of the association between the *CTLA4* polymorphism and AR was performed using a dominant model, a recessive model, a co-dominant model, and an allele model. Statistical heterogeneity among the studies was assessed using a chi-square test; a corresponding p value below 0.05 was considered to represent significant heterogeneity. Meta-regression analysis and metan-based influence analysis were performed to analyze the heterogeneity more deeply. If there was a significant difference in terms of heterogeneity, the ORs were pooled according to the random effect model (the Der Simonian and Laird model). Otherwise, a fixed effect model (the Mantel–Haenszel model) was used. Subgroup analysis was performed based on race. To assess the publication bias, the Egger's regression test and the Begg–Mazumdar test based on Kendall'stau were carried out. Cumulative meta-analysis was performed by year of publication. All the statistical analyses were performed using Review Manerge 5.0 (Cochrane Collaboration, Oxford, UK) and Stata 12.0 (StataCorp LP, College Station, TX).

Results

Study characteristics and eligible studies

In total, 43 papers were identified after an initial search. After screening the articles (Figure 1), nine of these articles were included. All nine studies (Table 1) contained data for the rs231775 (+49G/A) polymorphism. When categorized by ethnicity, the subjects of six studies were European,[15–20] two were Asian,[21,22] and the remaining subjects were African.[7] The characteristics of these studies are shown in Table 1.

The rs231775 A/G (+49A/G) polymorphism

The eligible studies for the analysis of the +49A/G polymorphism included 481 cases with AR and 1324 non-AR controls. Overall, nine case-control studies were included in the meta-analysis of the association between the +49A/G polymorphism and the risk of AR. Among these case-control studies, six were from Europe, two were from Asia, and one was from Africa. While a significant association was observed for the GG versus AG + AA genotype (OR = 1.35, 95% CI = 1.05–1.73, $p = 0.02$) and the G versus A allele (OR = 1.21, 95% CI = 1.03–1.42, $p = 0.02$), the comparisons of the other genotypes did not reveal any statistical association (Table 2, Figures 1–6). In the subgroup meta-analysis, a significant association was observed in the analysis of the GG versus AG + AA genotype (OR = 1.79, 95% CI = 1.15–2.78, $p = 0.009$) and the G versus A allele (OR = 1.47, 95% CI = 1.04–2.07, $p = 0.03$) genotype in the Asian group (Table 2, Figures 3 and 6).

Sensitivity analysis

A single study involved in the meta-analysis was deleted each time to investigate the influence of the individual data sets on the pooled ORs. As expected, the results of the +49A/G analysis were sensitive because of the marginal p value. After excluding one study, we identified a statistically significant change in the OR (95% CI). For the GG versus AG + AA genotype, after removing the Gorgi et al.[7] study, the p value changed from 0.02 to 0.007; after removing the Kim et al.[21]

Table 2. Results from different comparative genetic models in *CTLA4* +49A/G (rs231775).

				Statistical method				Test of heterogeneity			Publication bias	
Genetic t model	Overall or subgroup	Numbers of studies	Numbers of participants	OR (95% CI)	Z value	p Value	Model	Chi^2	p_{Het}	I^2 (%)	Begg's	Egger's
GG + AG versus AA	All	9	1805	1.19 (0.92, 1.55)	1.31	0.19	Fixed	9.80	0.28	18	0.602	0.670
	European	6	1243	1.28 (0.96, 1.71)	1.68	0.09	Fixed	4.41	0.49	0	–	–
	Asian	2	492	1.20 (0.58, 2.48)	0.49	0.56	Fixed	0.34	0.56	0	–	–
	African	1	70	0.19 (0.04, 0.97)	2.00	0.05	Fixed	–	–	–	–	–
GG versus AG + AA	All	9	1805	1.35 (1.05, 1.73)	2.36	0.02	Fixed	5.64	0.69	0	0.009	0.021
	European	6	1243	1.26 (0.92, 1.74)	1.43	0.15	Fixed	0.87	0.97	0	–	–
	Asian	2	492	1.79 (1.15, 2.78)	2.60	0.009	Fixed	0.59	0.44	0	–	–
	African	1	70	0.64 (0.25, 1.64)	0.93	0.35	Fixed	–	–	–	–	–
GG versus AA	All	9	982	1.31(0.94, 1.82)	1.58	0.11	Fixed	7.59	0.47	0	0.048	0.081
	European	6	657	1.41 (0.96, 2.08)	1.74	0.08	Fixed	1.49	0.91	0	–	–
	Asian	2	280	1.58 (0.75, 3.35)	1.20	0.23	Fixed	0.61	0.44	0	–	–
	African	1	45	0.18 (0.03, 1.00)	1.58	0.11	Fixed	–	–	–	–	–
AG versus AA	All	9	1261	1.08 (0.82, 1.43)	0.55	0.59	Fixed	8.43	0.39	5	0.754	0.473
	European	6	962	1.19 (0.88, 1.62)	1.13	0.26	Fixed	3.81	0.58	0	–	–
	Asian	2	265	0.85 (0.39, 1.86)	0.40	0.69	Fixed	0.16	0.69	0	–	–
	African	1	34	0.19 (0.03, 1.11)	0.55	0.59	Fixed	–	–	–	–	–
G versus A	All	9	3610	1.21(1.03, 1.42)	2.30	0.02	Fixed	9.84	0.28	19	0.118	0.204
	European	6	2486	1.20 (1.00, 1.45)	1.93	0.05	Fixed	2.61	0.76	0	–	–
	Asian	2	984	1.47 (1.04, 2.07)	2.18	0.03	Fixed	0.61	0.44	0	–	–
	African	1	140	0.51 (0.25, 1.05)	1.82	0.07	Fixed	–	–	–	–	–

Notes: OR, odds ratio; CI, confidence interval; Z, test for overall effect; I^2, index of heterogeneity; p_{Het}, p value for heterogeneity; G, guanine; A, adenine; C, cytosine; T, thymine; Fixed, fixed effect model; Random, random effect model.

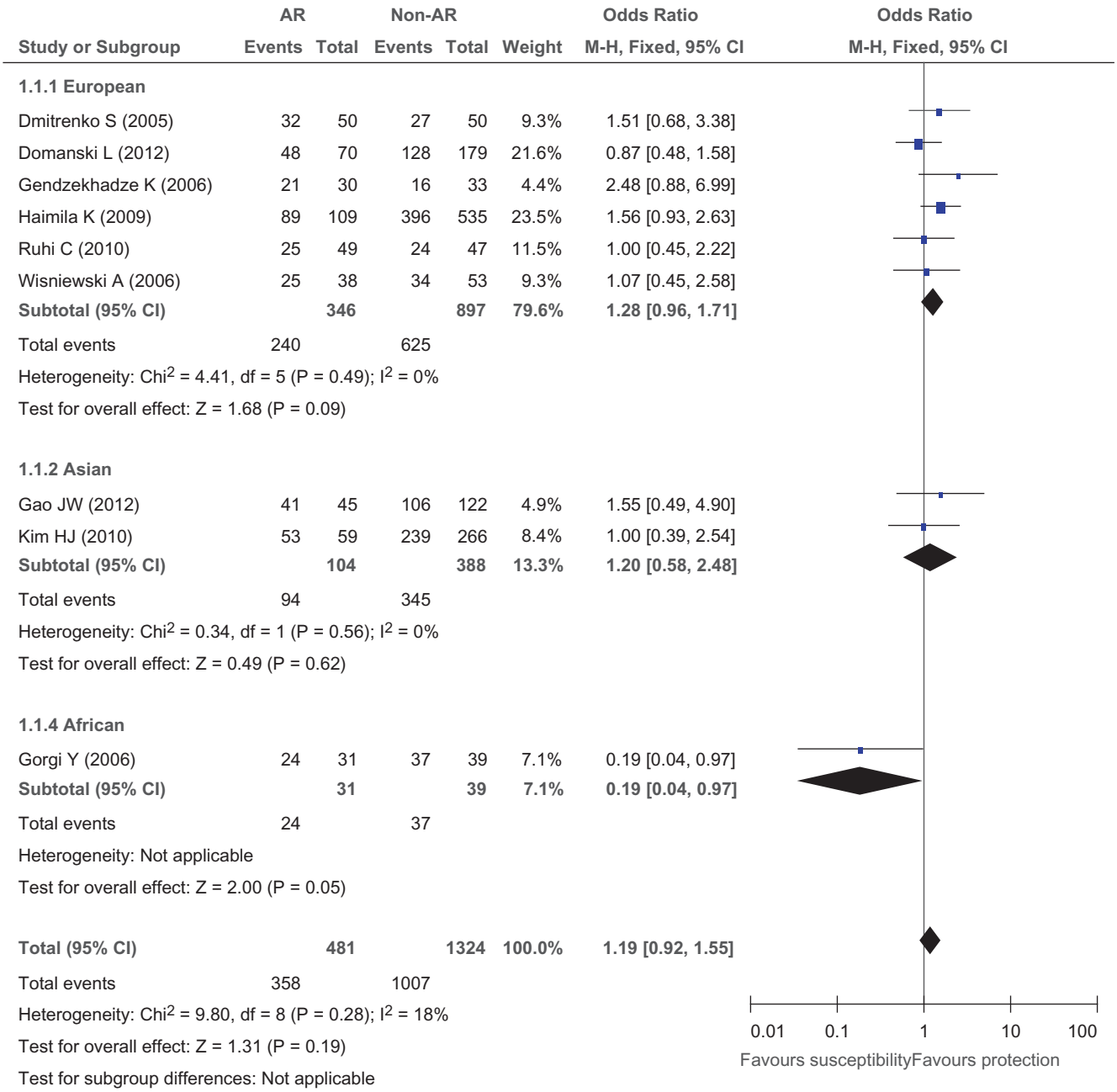


Figure 2. Meta-analysis for the association between AR risk in renal transplantation and *CTLA4* +49A/G (GG + AG vs. AA).

study, $p = 0.02$ changed to $p = 0.06$. Removal of the Gao et al.[22] study, $p = 0.02$ changed to $p = 0.10$. For the GG versus AA genotype, the removal of the Gorgi et al.[7] study, the p value changed from 0.11 to 0.03. For the G versus A allele, after removing the Gorgi et al.[7] study, the p value changed from 0.02 to 0.006; removal of the Domanski et al.[20] study, $p = 0.02$ changed to $p = 0.009$, and the removal of the Gao et al.[22] study, the p value changed from 0.02 to 0.08. Other corresponding pooled ORs were not significantly altered (data not shown).

Heterogeneity analysis

To assess the stability of the results of the meta-analysis, statistical heterogeneity among the studies was assessed using a chi-square test; a corresponding p value below 0.05 was considered to represent significant heterogeneity. As shown in Table 2, all the results did not show significant heterogeneity (GG + GA vs. AA: $I^2 = 18\%$, $p = 0.28$; GG vs. GA + AA: $I^2 = 0$, $p = 0.69$; GG vs. AA: $I^2 = 0$, $p = 0.47$; GG vs. GA: $I^2 = 5\%$, $p = 0.39$; G vs. A: $I^2 = 19\%$, $p = 0.28$).

Meta-analysis regression

To model the GG versus GA + AA and the G versus A effects, meta reg analysis was used for some influence factors, such as year of publication, total number in each group (such as the AR group and control group), ethnicity, the ratio of GG to GA + AA, or the ratio of G to A for the *CTLA4* +49A/G SNP. Of these factors, the ratio of GG to GA + AA ($p = 0.046$) and the ratio of G to A ($p = 0.017$) were significant.

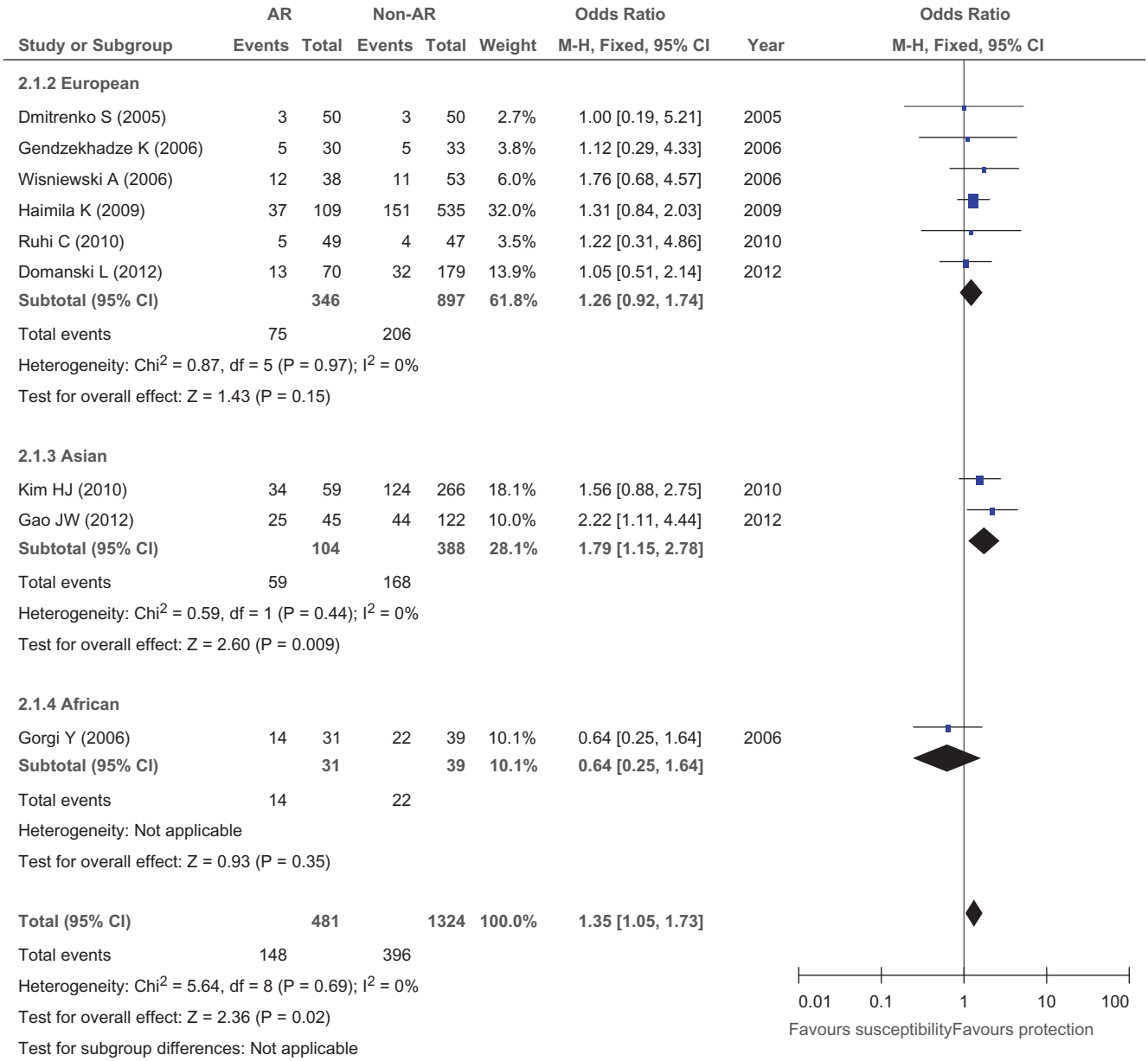


Figure 3. Meta-analysis for the association between AR risk in renal transplantation and *CTLA4* +49A/G (GG vs. AG + AA).

Cumulative meta-analysis

Cumulative meta-analysis was used based on the year of publication (from 2005 to 2012) to assess the *CTLA4* +49GG versus GA + AA and the G versus A genotypes (Figures 7 and 8). OR point estimates and CI stabilized, and there was a good change in the trend situation.

Publication bias

Publication bias was assessed using Begg's test and Egger's test. Funnel plot asymmetry was assessed using Egger's linear regression test. If the line passed through the origin, this indicated that publication bias did not exist. Excluding the +49GG + AG versus AA (Begg's test, $p = 0.009$; Egger's test, $p = 0.021$) and +49GG versus AA (Begg's test, $p = 0.048$; Egger's test, $p = 0.081$) comparisons, Begg's test and Egger's test suggested no publication bias (Table 2).

Discussion

The *CTLA4* gene has been widely studied, and many studies have evaluated the effect of polymorphisms in the *CTLA4* gene on AR in transplant recipients. Some studies have yielded conflicting results. Duan and Zhu performed a meta-analysis to evaluate these associations in AR following renal and liver transplantation, respectively.[13,14] In Duan's study,[13] the association between the *CTLA4* +49G allele and AR was weakly significant (OR = 0.805, 95% CI = 0.677–0.957, $p = 0.014$), but in the meta-analysis from Zhu,[14] no significant association was discovered between the +49G/A SNP and AR in kidney transplantation. To produce more precise results, we enlarged the number of studies used in the meta-analysis to include a total of nine articles published by 2013; among these papers, six were from Europe, two were from Asia, and one was from Africa. For the +49A/G SNP, no significant results have been discovered from the present statistical data

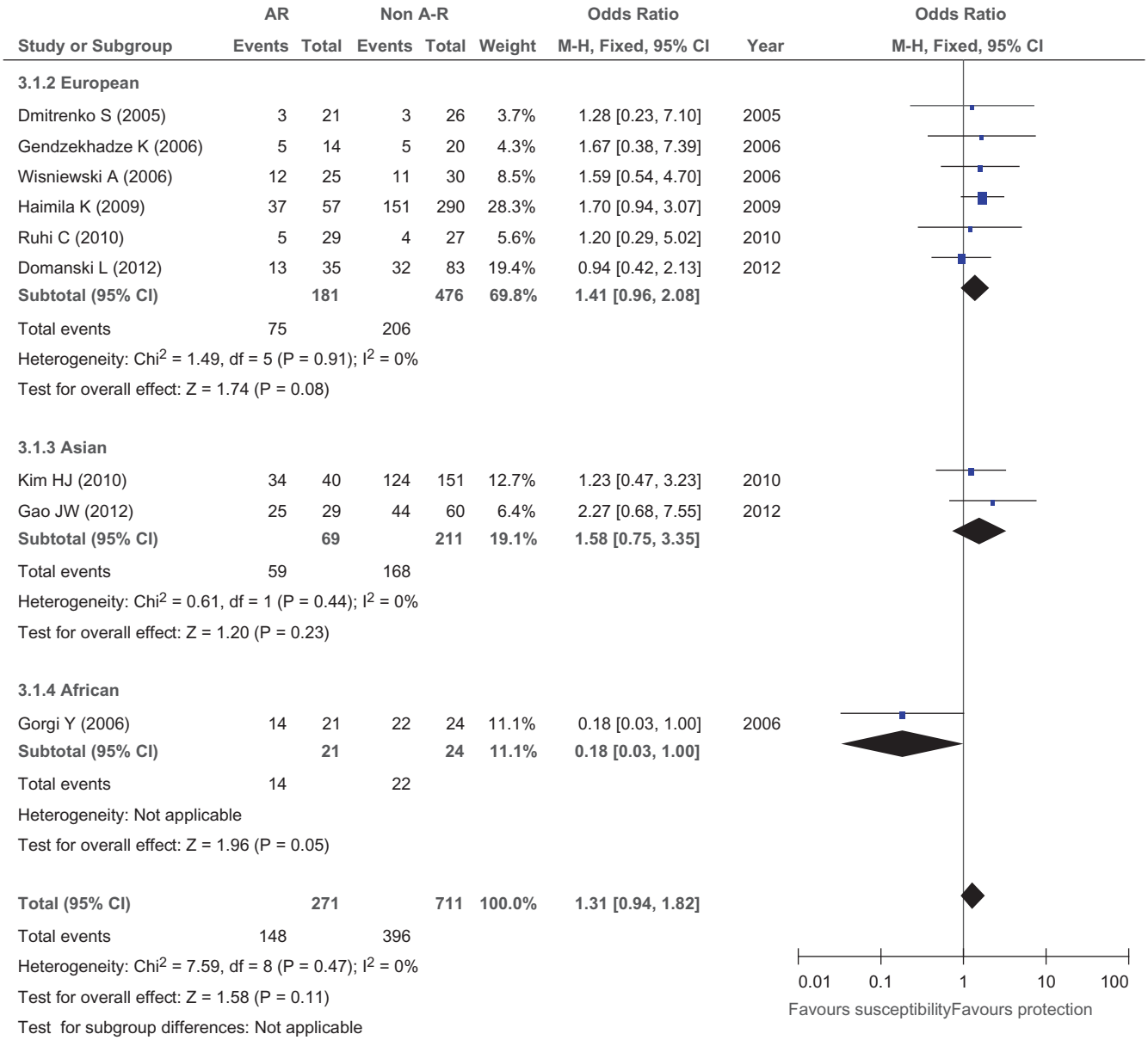


Figure 4. Meta-analysis for the association between AR risk in renal transplantation and *CTLA4* +49A/G (GG vs. AA).

from a single study. Only in Korean patients[21] was the *CTLA4* +49A/G SNP (rs231775) statistically associated with late acute rejection (LAR) in the dominant model (OR = 0.48, 95% CI = 0.25–0.93, corrected $p = 0.026$); the allele frequency of the same SNP (rs231775) was also statistically associated with a risk of LAR (OR = 2.02, 95% CI = 1.15–3.52, corrected $p = 0.013$), where the presence of the G allele increased the risk of LAR in kidney transplantation. However, meta-analysis of the renal transplantation data shows that recipients carrying the GG genotype and the G allele had an increased risk of AR (GG vs. GA + AA and G vs. A; $p = 0.02$).

The metareg analysis was used for some influencing factors, such as year of publication, total number in each group (AR group and control group), ethnicity, the ratio of GG to GA + AA, and the ratio of G to A alleles. Of these factors, we discovered that there were statistically significant differences between studies for the ratio of GG to GA + AA ($p = 0.046$) and the ratio of G to A ($p = 0.017$). In a meta-analysis, examining the association of the *CTLA4* gene with Graves' disease in the Chinese Han population in the large samples, the ratio of the G and A alleles was 70.5/29.5 = 2.39 in Chinese populations (healthy controls).[23] In meta-analyses, examining the association between the *CTLA4* exon1 +49A/G polymorphism and systemic lupus erythematosus[24] in the Korean population, the ratio of G to A was 1.950 (Pyo et al.[25]), 2.053 (Hudson et al.[5]), and 2.822 (Lee et al.[26]); in the Japanese population, the G/A ratio was 1.339 (Ahmed et al.[27]), 1.586 (Matsushita et al.[28]), and 2.014 (Takeuchi et al.[29]). The Spanish population had a G/A ratio of 0.352 (Aguilar et al.[30]), whereas the Portuguese population had one of 0.378 (Barreto et al.[31]), and the English population was 0.458 (Heward et al.[32]). In another paper, the G/A ratio was 0.876 in the Italian population (healthy controls) (Brozzetti et al.[33]). In other words, the MAF (minor allele frequency) of the G and A alleles at the +49 locus of the *CTLA4* gene may

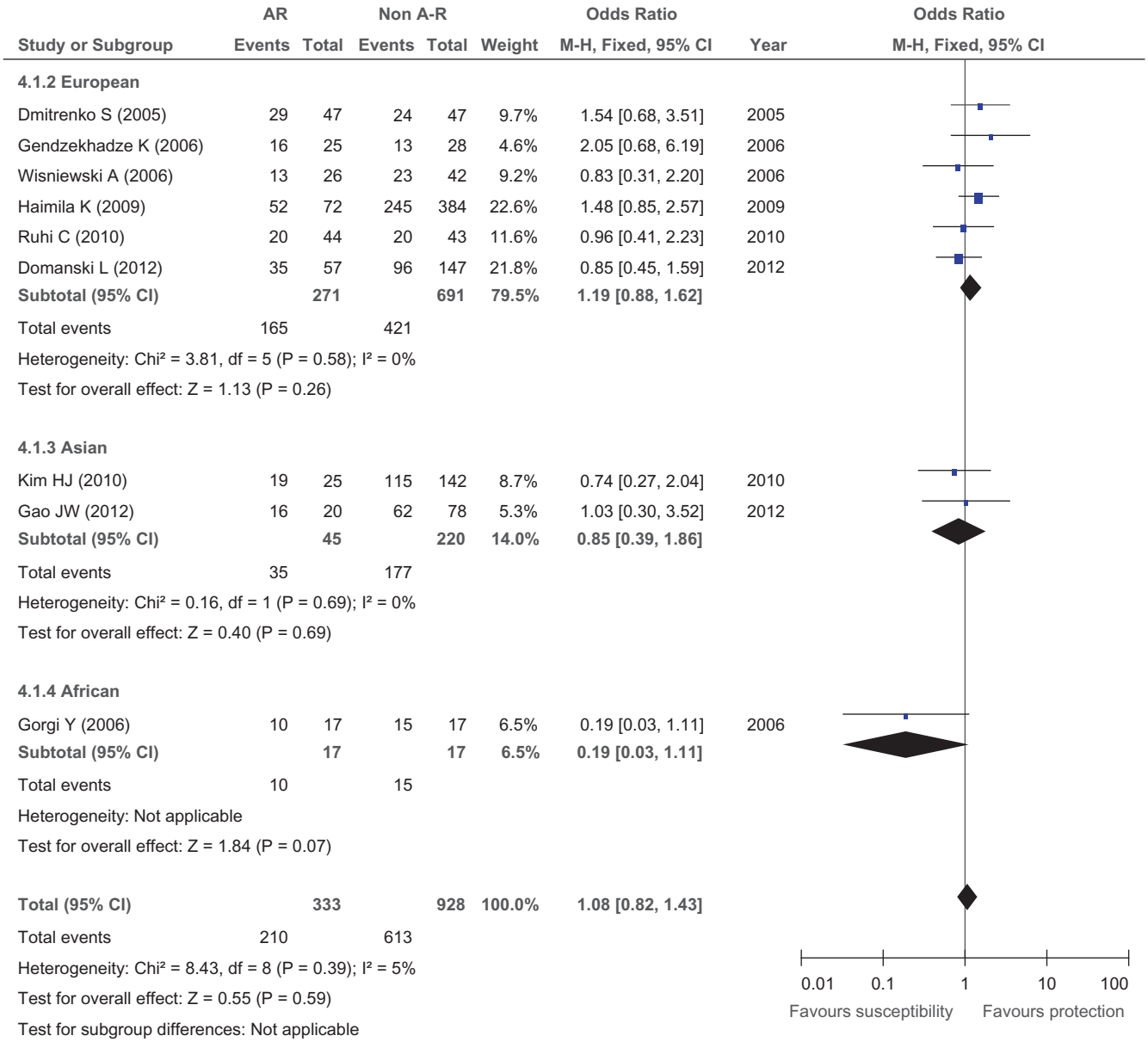


Figure 5. Meta-analysis for the association between AR risk in renal transplantation and the *CTLA4* +49A/G (AG vs. AA).

be different between Asian and European populations; the minor allele may be A in Asia, but G in Europe.

The subgroup analysis (GG vs. GA + AA and G vs. A) in the Asian subgroup, there were significant results (GG vs. GA + AA, $p = 0.009$; G vs. A, $p = 0.03$), but there were no statistically significant results in the European subgroup (GG vs. GA + AA, $p = 0.15$; G vs. A, $p = 0.05$). In the sensitivity analysis, when the Asian studies were removed one by one the statistics became meaningless. Therefore, the ethnicity and the ratio of the G and A alleles may be internal factors that influence the development of AR in renal transplant recipients.

The cell-surface expression of *CTLA4* was significantly increased in individuals carrying the AA genotype compared to the expression in carriers of the AG and GG genotypes.[34] T cells with the +49GG genotype had higher activation and proliferation rates compared to those with the +49AA genotype.[35] *CTLA4* +49G > A caused a ^{17}Ala > ^{17}Thr substitution in the leading peptide of *CTLA4*.[36] The ^{17}Thr substitution increased the binding of *CTLA4* to B7.1, causing stronger inhibition of T cell activation than *CTLA4* ^{17}Ala.[35] Recently, the G allele of the +49A/G polymorphism was reported to have a strong association with autoimmune diseases.[37–39] In our paper, the G allele was associated with AR in kidney transplantation; there may be a similar immune mechanism involved in autoimmune diseases.

Heterogeneity and publication bias can influence the results of meta-analyses. There was no significant heterogeneity in the overall comparisons for all five polymorphisms. Therefore, heterogeneity did not appear to influence the results, suggesting that our results were reliable. In our meta-analysis, only studies indexed by the selected databases were included. Negative studies were less likely to be published in journals or to be available in computerized databases, resulting in potential overestimation of effect sizes.[40] In this meta-analysis, Begg's test and Egger's test showed significant

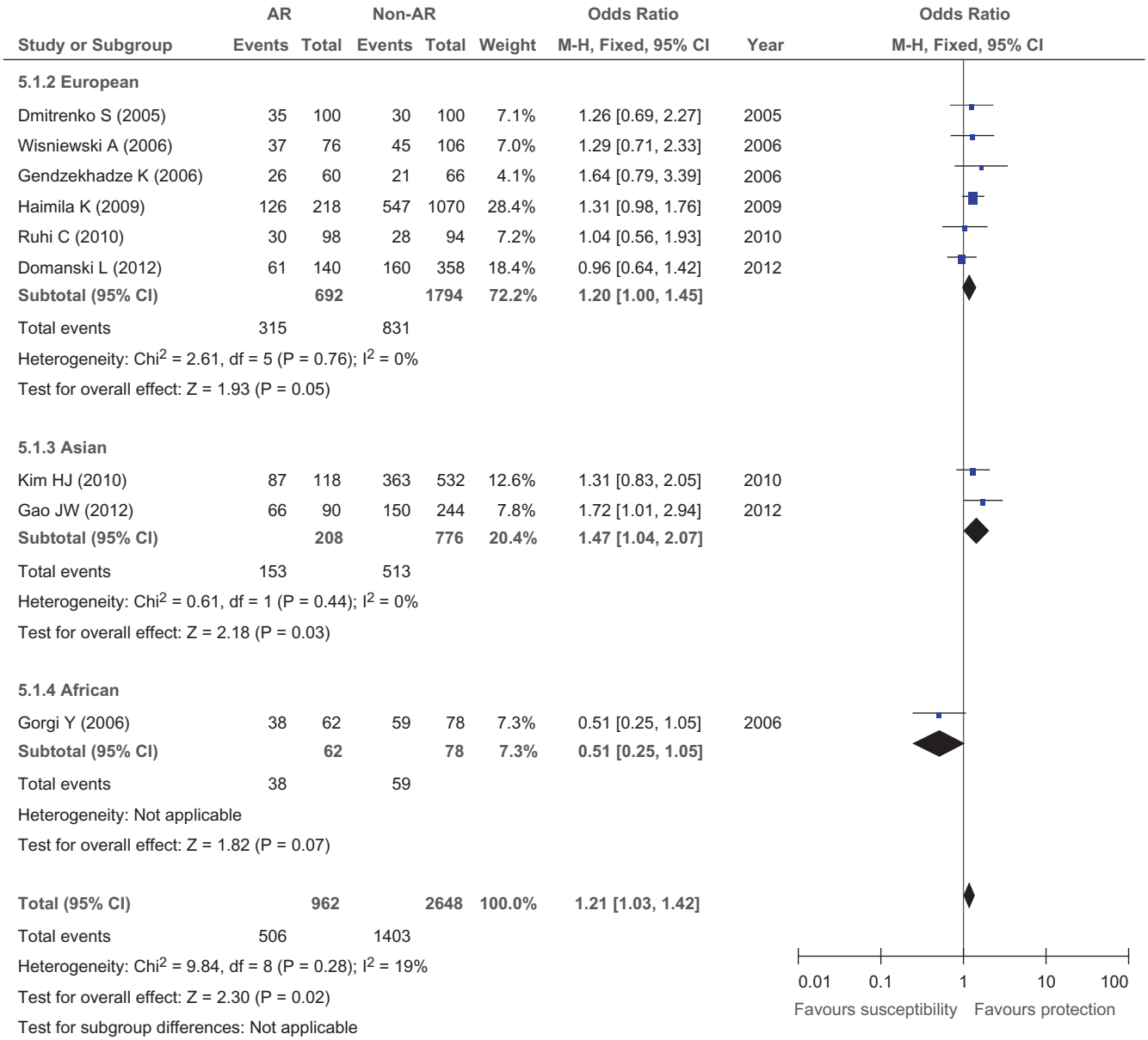
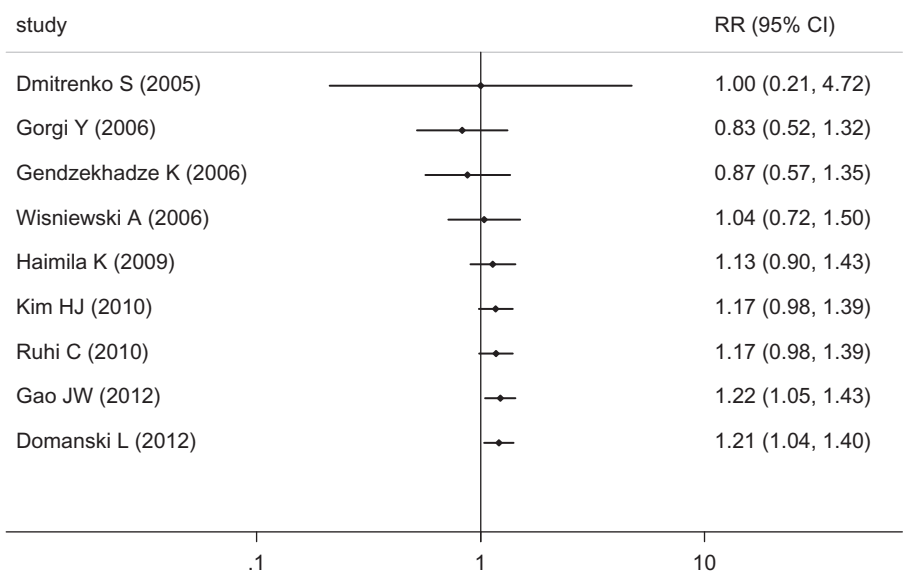


Figure 6. Meta-analysis for the association between AR risk in renal transplantation and *CTLA4* +49A/G (G vs. A).

Figure 7. A metacum analysis in *CTLA4* +49 GG versus GA+AA.



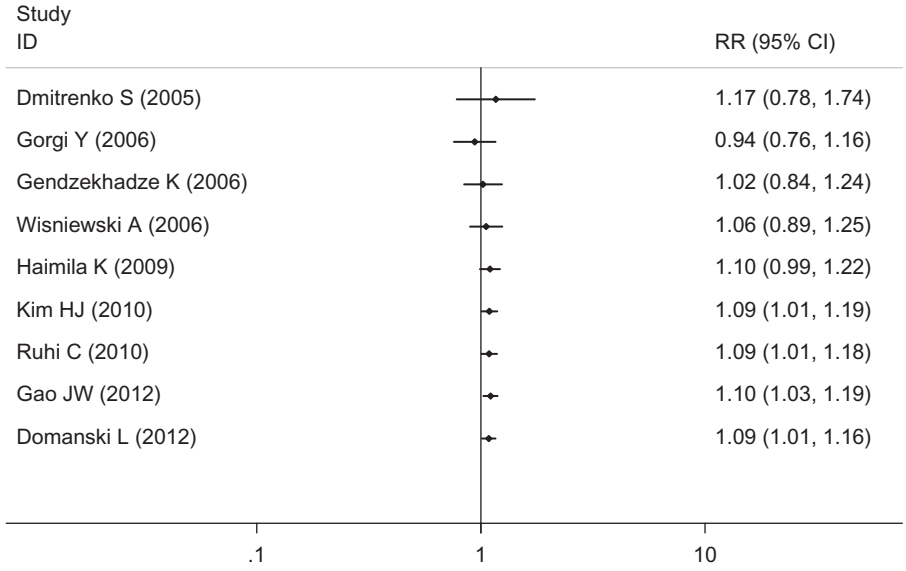


Figure 8. A metacum analysis in *CTLA4* +49 G versus A.

publication bias in the model for GG versus AG + AA and GG versus AA, so the current results should be interpreted cautiously. The limitations of this meta-analysis should be considered. First, the number of available studies that could be included was relatively small. Second, only two of the nine studies were conducted in Asian populations, and only one of the nine studies was conducted in an African population. Third, the overall outcomes were based on individual unadjusted ORs. Fourth, there was a lack of a general allele survey of the *CTLA4* +49A/G locus in the various populations, and the inconsistency of the minor allele of A/G between the European and Asian populations needs to be confirmed.

Overall, the current meta-analysis suggests that the +49A/G polymorphism in the *CTLA4* gene may be associated with the risk of rejection after renal transplantation, especially in the Asian population. Well-designed, unbiased prospective studies with larger sample sizes that address the gene–ethnicity interactions should be conducted to confirm these results.

Declaration of interest

The authors report no conflicts of interest.

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