REVIEW ARTICLE

Association of the genetic polymorphisms in inhibiting and activating molecules of immune system with rheumatoid arthritis: A systematic review and meta-analysis

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Several studies have demonstrated that the genetic polymorphisms in the genes encoding immune regulatory molecules, namely cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and CD28, play a fundamental role in susceptibility to rheumatoid arthritis (RA). Several disperse population studies have resulted in conflicting outcomes regarding the genetic polymorphisms in these genes and RA risk. This systematic review and meta-analysis study was performed to reach a conclusive understanding of the role of single-nucleotide polymorphisms (SNPs) of CTLA4-rs231775, CTLA4-rs5742909, and CD28-rs1980422 in susceptibility to RA. Databases (ISI Web of Science, MEDLINE/PubMed, and Scopus) were searched to find the case–control studies surveying the association of CTLA4 gene rs231775, CTLA4 gene rs5742909, and CD28 gene rs1980422 polymorphisms and RA susceptibility in different population until August 2020. Association comparison between the polymorphisms and RA proneness was assessed using pooled odds ratio (OR) and their corresponding 95% confidence interval. This study was conducted on 16 population studies, comprising 1078 RA patients and 1118 healthy controls for CTLA4-rs231775, 2193 RA patients and 2580 healthy controls for CTLA4-rs5742909, and 807 RA patients and 732 healthy controls for CD28-rs1980422. Analysis indicated that G-allele, GG and GA genotypes, and dominant model for rs231775, recessive model for rs5742909, and C-allele, CC and CT genotypes, and recessive model for rs1980422 were significantly associated with increased RA risk. This meta-analysis showed that genetic polymorphisms of both immune inhibitory and activating genes, including CTLA4-rs231775, CTLA4-rs5742909, and CD28-rs1980422 polymorphisms, may increase susceptibility to RA.

Key words: CD28, CTLA4, meta-analysis, polymorphism, rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disorder, primarily occurs in the synovial joints, which causes remarkable disability in body limbs, reduced life expectancy, and even mortality.[1] Although the exact etiology and pathogenesis of RA have not been disclosed yet, it has been suggested that interactions between genetic and environmental contribute factors play a major role in the susceptibility to RA.[2] Immune system has repetitively been blamed as the major culprit in the development of RA. In the disease, overactivation of CD4 + T-cells and its helper subsets results in the production of inflammatory mediators in RA synovium that further recruits and activates the pathogenic cells, leading to the development of the disease.[3] Activation of T-cells is modulated through the interactions between the inhibitory CTLA-4 and CD28, as a stimulatory signal transducer, with their ligands. Both of these receptors are activated by the same ligands, namely CD80 and CD86, which are expressed particularly on the antigen-presenting cells.[4] CD28 ligation to CD80 and CD86 molecules promotes the activation of T-cells, while the ligation of CTLA-4 culminates in the suppression of T-cell activation.[5]

The immune checkpoint molecule CTLA-4 is expressed on T-cells and plays an important role in suppressing T-cell activation as well as peripheral tolerance.[6] Numerous studies have established that CTLA-4 plays a marked role in modulating the self-tolerance by the immune system and consequently is connected to the pathogenesis of various autoimmune diseases like RA.[7] T-allele of rs5742909 single-nucleotide polymorphism (SNP) has been reported to be associated with autoimmune diseases through upregulating the expression of CTLA-4.[8] Moreover, the rs231775 SNP in the *CTLA4* gene has been reported to be associated with the susceptibility to several autoimmune disorders, including RA.[9] On the other hand, genome-wide association studies (GWAS) of 3393 cases and 12,462 controls reported the association of *CD28* gene rs1980422 SNP with the RA risk.[10]

Several association studies have evaluated the role of CTLA4-rs231775, CTLA4-rs5742909, and CD28-rs1980422 polymorphisms in susceptibility to RA. However, the results were inconclusive and reached conflicting findings, which may stem from a relatively small sample size in each study as well as the difference in the ethnicity of the different populations. Therefore, conducting an accumulative analysis of the available data is needed to elicit a more conclusive and comprehensive estimation by performing meta-analysis. Such analysis, via pooling of the available data from different individual studies, provide a tool to obtain a unique estimation of the main effect. With respect to these issues, the purpose of this meta-analysis was to assess the association between CTLA4 gene rs231775, CTLA4 gene rs5742909, and CD28 gene rs1980422 polymorphisms and RA risk.

MATERIALS AND METHODS

This study was performed in a stepwise process in accordance with the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-analyses statement.[11] This meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO). The current project did not include data involving human or animal participants conducted by any of the authors.

Searches and data sources

In this meta-analysis, we investigated main databases, including ISI Web of Science, MEDLINE/PubMed, and Scopus (from the beginning until August 2020) to find any related case–control studies about the *CTLA4* and *CD28* gene polymorphisms and RA risk up to August 2020. The following keywords were used to search the databases: "rs231775 OR + 49A > G CTLA-4 polymorphism" AND "Rheumatoid arthritis OR RA," "rs5742909 or − 319C/T" AND "Rheumatoid arthritis OR RA," and rs1980422 AND "Rheumatoid arthritis OR RA." Moreover, related references in the obtained studies from the searches above were also assessed. In the search procedure, we evaluated studies conducted on only human population studies. To ensure the relevancy if the studies found, the title and abstracts of all the studies were assessed. The reference list of the eligible studies was also assessed to prevent exclusion of any potential study.

Inclusion and exclusion criteria

The following inclusion criteria were considered in this meta-analysis. (1) Case–control studies with an evaluation of CTLA4-rs231775, CTLA4-rs5742909, or CD28-rs1980422 polymorphisms and RA risk. (2) Only studies that contained allele or genotype frequencies were included, which permitted the calculation of odds ratio (OR) with a 95% confidence interval (CI). The exclusion criteria were (1) duplicated studies and (2) studies other than original research works, including Letter, Comment, and Review [Table 1 and Figure 1].

Data extraction and quality assessment

Data were extracted by two researchers (MJM and SA) based on the inclusion and exclusion criteria mentioned above. The extracted information included the first author's last name, publication year, detection method, country and ethnicity of the study population, number of cases and controls, and the frequency of the alleles and genotypes for all of the polymorphisms. In case of ambiguous data, the corresponding authors were contacted for clarification. Furthermore, we contacted the authors of non-English papers for data acquisition, if applicable. Two independent authors

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

Author (reference)	Published year	Country/ethnicity	Detection technique	N (RA patients)	*n* (healthy controls)
CTLA4 - rs231775					
Barton *et al*.[28]	2004	UK/Caucasian	Fluorescence-based primer extension method	132	156
Muñoz-Valle *et al*.[29]	2010	Mexico/Latin American	PCR-RFLP	199	199
Liu *et al*.[30]	2013	China/Asian	PCR-RFLP	213	303
Elshazli *et al*.[31]	2015	Egypt/Caucasian	PCR-RFLP	112	122
Luterek-Puszyńska *et al*.[26]	2017	Poland/Caucasian	TaqMan genotyping	422	338
CTLA4- rs5742909					
Gonzalez-Escribano *et al*.[32]	1999	Spain/Caucasian	PCR-ARMS	138	305
Lee *et al*.[33]	2002	Korea/Asian		86	86
Liu *et al*.[34]	2004	Taiwan/Asian	PCR-RFLP	65	81
Barton *et al*.[28]	2004	UK/Caucasian	Fluorescence-based primer extension method	151	152
Takeuchi *et al*.[35]	2006	Japan/Asian	PCR-RFLP	100	104
Walker *et al*.[36]	2009	Canada/Caucasian	Mass spectrometric analysis (MassArray system)	1140	1248
Torres-Carrillo *et al*.[37]	2013	Mexico/Latin American	PCR-RFLP	200	200
Liu *et al*.[30]	2013	China/Asian	PCR-RFLP	213	304
Fattah *et al*.[38]	2017	Egypt/Egiptian	PCR-RFLP	100	100
CD28					
Hegab *et al*.[27]	2016	Egypt/Egyptian	TaqMan genotyping	385	394
Luterek-Puszyńska *et al*.[26]	2017	Poland/Caucasian	TaqMan genotyping	422	338

PCR-RFLP=Polymerase chain reaction-restriction fragment length polymorphism, ARMS=Amplification refractory mutation system, RA=Rheumatoid arthritis, CTLA4=Cytotoxic T-lymphocyte-associated protein 4

the search procedure for eligibility with respect to the inclusion/exclusion criteria and any conflicting study was discussed by the third author to reach a consensus.

Statistical methods

Pooled OR and their corresponding 95% CI for all of the comparisons, including minor alleles, genotypes, dominant, and recessive models, were calculated to measure the association of polymorphisms and RA risk. The risks were calculated for the following comparisons: G versus A (allelic), GG versus AA (genotypic), GA versus AA (genotypic), GG versus GA + AA (dominant), and GG + GA versus AA (recessive) for CTLA4-rs231775, T versus C (allelic), TT versus CC (genotypic), TC versus CC (genotypic), TT versus TC + CC (dominant), and TT + TC versus CC (recessive) for CTLA4-rs5742909, and C versus T (allelic), CC versus TT (genotypic), CT versus TT (genotypic), CC versus CT + TT (dominant), and CC + CT versus TT (recessive) for CD28-rs1980422 SNPs. To determine the possible between-study heterogeneity across the included data of studies, the Chi-square Q-test was used.[12] Furthermore, to quantitatively determine the heterogeneity level, I-squared (I^2) test was measured. Significant heterogeneity was assigned when I^2 score exceeded 50% or the Q test had a $P \leq 0.1$. In the current meta-analysis, in case of a significant level of heterogeneity ($P_{\text{Q-statistic}} < 0.10$ or $I^2 > 50\%$), the random-effects model (REM; DerSimonian–Laird method) was implemented. Conversely, a lack of significant level of heterogeneity ($P_{\text{Q-statistic}} > 0.10$ or $I^2 < 50\%$) led us to conduct the fixed-effects model (FEM; Mantel–Haenszel method) for pooling the data.[13,14] The forest plot demonstrates ORs from different studies as the central values and their CIs to measure the pooled OR and corresponding 95% CI. In fact, each study as well as the summary effect (OR) in the forest plot is depicted as a point estimate that is bounded by the related CI. The funnel plot is a graphical illustration to check for the publication bias and an approach for indicating the relationship between the study size and the effect size (OR). The funnel plot is usually plotted through the variance on the Y-axis and ORs on the X-axis. To determine the publication bias, the Begg's test, Egger's regression test, and visual evaluation of the funnel plots were implemented.[15,16] The purpose of the sensitivity analysis is to measure the robustness of the overall OR to the assumptions in conducting the analysis. The influence plots were depicted by omitting individual studies to evaluate the robustness of the overall OR against a given omitted individual study. Statistical analysis was conducted through MetaGenyo (http://bioinfo.genyo.es/metagenyo/): a web tool for meta-analysis of genetic association studies.[17]

RESULTS

Characteristics of eligible studies

Based on the inclusion/exclusion criteria, 16 population studies comprising 1078 cases and 1118 controls for CTLA4-rs231775,

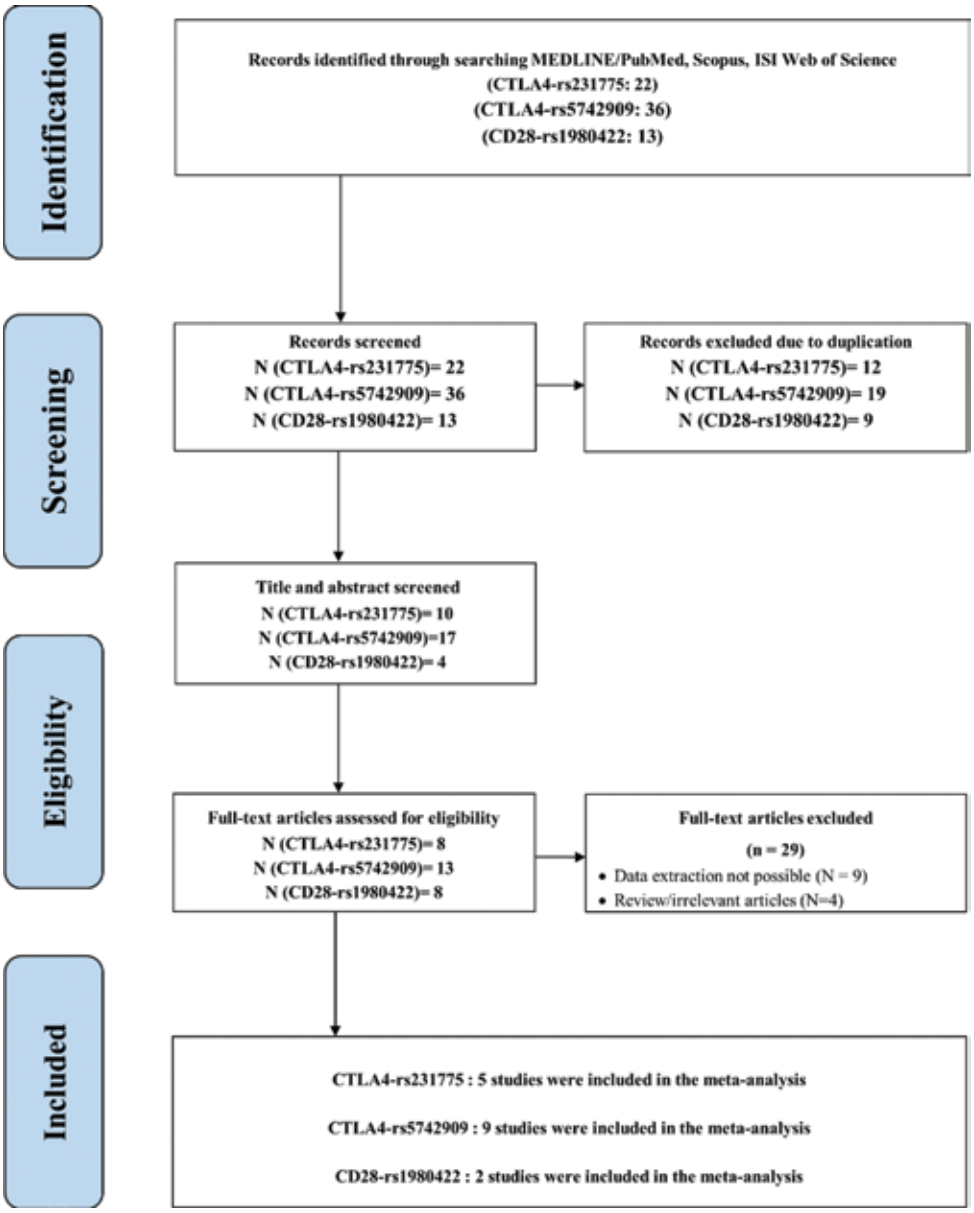

Figure 1: Flowchart for the procedure of the literature search. In this meta-analysis, 16 population studies comprising 1078 cases and 1118 controls for CTLA4-rs231775, 2193 cases and 2580 controls for CTLA4-rs5742909, and 807 cases and 732 controls for CD28-

2193 cases and 2580 controls for CTLA4-rs5742909, and 807 cases and 732 controls for CD28-rs1980422 variation were included in the final meta-analysis. Among the 16 evaluated studies, five case–control studies were performed in European people, five studies in Asians, three studies in Africans, and the remaining three studies were conducted in Mexico and Canada. The publication year of these studies was ranged from 1999 to 2017. The main characteristics of the included studies in this meta-analysis are shown in Table 1.

Main results, subgroup, and sensitivity analysis

Overall analysis demonstrated significant association of the CTLA4-rs231775 and CD28-rs1980422 variations [Table 2 and Figure 2]. The G-allele of the CTLA4-rs231775 SNP significantly increased the risk of RA (OR = 1.27, 95% CI = 1.03–1.57, $P = 0.021$). Moreover, both the GG (OR = 1.50, 95% CI = 1.15–1.95, $P = 0.0024$) and the GA (OR = 1.43, 95% CI = 1.15–1.79, $P = 0.0013$) genotypes were significantly associated with the increased susceptibility to RA. The dominant model of GG + GA significantly increased risk of the disease (OR = 1.49, 95% CI = 1.21–1.84, $P = 0.00017$).

In CTLA4-rs5742909 SNP, only the recessive model of TT versus TC + CC comparison was significantly associated with the increased risk of RA (OR = 1.97, 95% CI = 1.21–3.18, $P = 0.005$).

For CD28-rs1980422 SNP, the C-allele significantly was associated with increased RA risk (OR = 1.36, 95%

Table 2: Meta-analysis of the pooled association between cytotoxic T-lymphocyte associated protein 4-rs231775, cytotoxic T-lymphocyte associated protein *4*-rs5742909, and CD28-rs1980422 polymorphisms and rheumatoid arthritis disease

Comparison	Number of studies	Frequency (%)		Association test			Heterogeneity Test (I^2%, *P*)	Publication bias (Begg's test and Egger's test)	Effect model
		Case	Control	*P*	Pooled OR	(95 % CI)			
CTLA4-rs231775									
G	5	1025 (47.5)	1004 (44.9)						
A	5	1131 (52.5)	1232 (55.1)						
GG	5	343 (31.8)	318 (28.44)						
GA	5	516 (47.9)	494 (44.18)						
AA	5	219 (20.3)	306 (27.38)						
G versus A	5	-	-	0.021	1.27	1.03-1.57	62%, 0.03	*P*=0. 24, *P*=0.41	Random
GG versus AA	5	-	-	0.0024	1.50	1.15- 1.95	25%, 0.25	*P*=0. 17, *P*=0.56	Fixed
GA versus AA	5	-	-	0.0013	1.43	1.15- 1.79	47%, 0.11	*P*=0. 21, *P*=0.09	Fixed
GG versus GA+AA	5	-	-	0.19	1.24	0.88- 1.74	60%, 0.03	*P*=0. 36, *P*=0.10	Random
GG+GA versus AA	5	-	-	0.00017	1.49	1.21- 1.84	36%, 0.17	*P*=0.05, *P*=0.23	Fixed
CTLA4-rs5742909									
T	9	558 (23)	526 (10.19)						
C	9	2428 (77)	4634 (89.81)						
TT	9	40 (2.6)	34 (1.32)						
TC	9	478 (32)	458 (17.75)						
CC	9	975 (65.4)	2088 (80.93)						
T versus C	9	-	-	0.385	1.26	0.74- 2.15	92%, 0.0001	*P*=0. 03, *P*=0.09	Random
TT versus CC	9	-	-	0.060	2.23	0.96- 5.16	53%, 0.044	*P*=0. 38, *P*=0.44	Random
TC versus CC	9	-	-	0.344	1.37	0.71- 2.64	93%, 0.0001	*P*=0. 92, *P*=0.33	Random
TT versus TC+CC	9	-	-	0.005	1.97	1.21- 3.18	31%, 0.19	*P*=0. 16, *P*=0.28	Fixed
TT+TC versus CC	9	-	-	0.348	1.36	0.71- 2.62	93%, 0.0001	*P*=0. 31, *P*=0.14	Random
CD28-rs1980422									
C	2	512 (31.73)	386 (25.13)						
T	2	1102 (68.27)	1078 (74.86)						
CC	2	74 (9.16)	42 (5.7)						
CT	2	364 (45.10)	302 (41.25)						
TT	2	369 (45.74)	388 (53.05)						
C versus T	2	-	-	0.00014	1.36	1.16- 1.59	56%, 0.13	*P*=0. 26, *P*=0.37	Fixed
CC versus TT	2	-	-	0.00027	2.16	1.42- 3.27	0%, 0.38	*P*=0. 48, *P*=0.19	Fixed
CT versus TT	2	-	-	0.0057	1.35	1.09- 1.67	0%, 0.11	*P*=0. 99, *P*=0.20	Fixed
CC versus CT+TT	2	-	-	0.0044	1.77	1.19- 2.63	0%, 1.63	*P*=0. 08, *P*=0.29	Fixed
CC+CT versus TT	2	-	-	0.063	1.42	0.98- 2.07	69%, 0.068	*P*=0. 09, *P*=0.41	Random

CI=Confidence interval, OR=Odds ratio, CTLA4=Cytotoxic T-lymphocyte-associated protein 4

CI = 1.16–1.59, *P* = 0. 00014). Furthermore, both CC (OR = 2.16, 95% CI = 1.42–3.27, *P* = 0.00027) and CT (OR = 1.35, 95% CI = 1.09–1.67, *P* = 0.0057) genotypes were associated with increased risk of RA. As well, the recessive model of CC increased the proneness to RA (OR = 1.77, 95% CI = 1.19–2.63, *P* = 0.0044).

Heterogeneity and publication bias

Heterogeneity of the studies was analyzed using whit Cochran's Q test and I^2 test [Table 2]. The I^2% >50% and $P_{Heterogeneity}$ < 0.10 were considered as significant values. Among the significantly associated comparisons with the RA risk, a heterogeneity was observed in G versus A comparison (I^2% = 62%, *P* = 0.03). Then, the fixed- and random-effect models were used to pool the results. The funnel plot demonstrated the publication bias among the studies [Figure 3]. No publication bias was observed among the comparisons.

Sensitivity analysis

The stability of meta-analysis was determined by the sensitivity analysis. The pooled ORs were not changed when any individual study was omitted [Figure 4].

DISCUSSION

In this study, we carried out a meta-analysis to raise a clear and precise approximation of the associations between CTLA4-rs231775, CTLA4-rs5742909, and CD28-rs1980422 polymorphisms and susceptibility to RA. In fact, we biologically

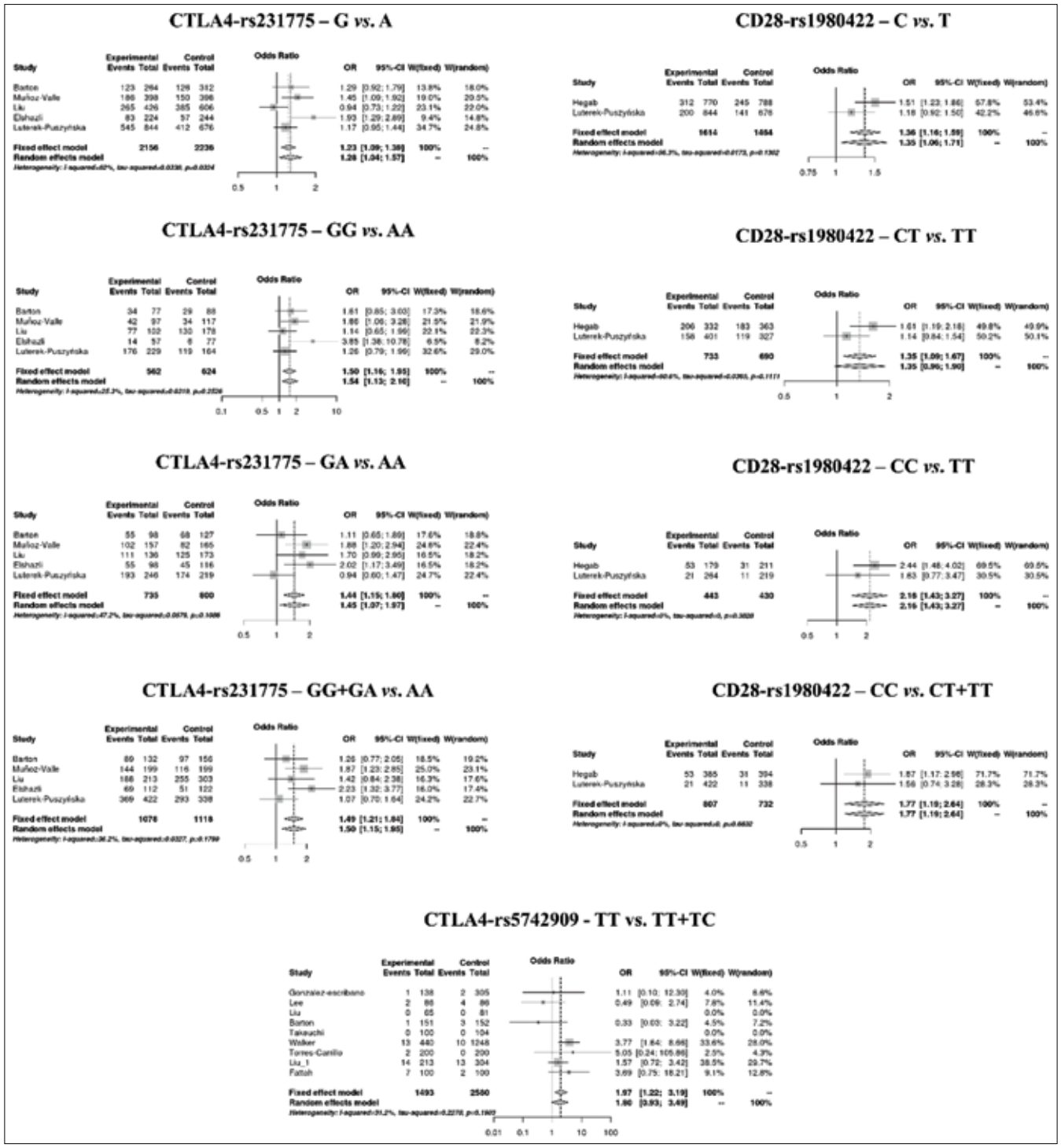

Figure 2: Forest plot. The plot shows the results of pooled odds ratio for significantly associated comparisons of between CTLA4-rs231775, CTLA4-rs5742909, and CD28-

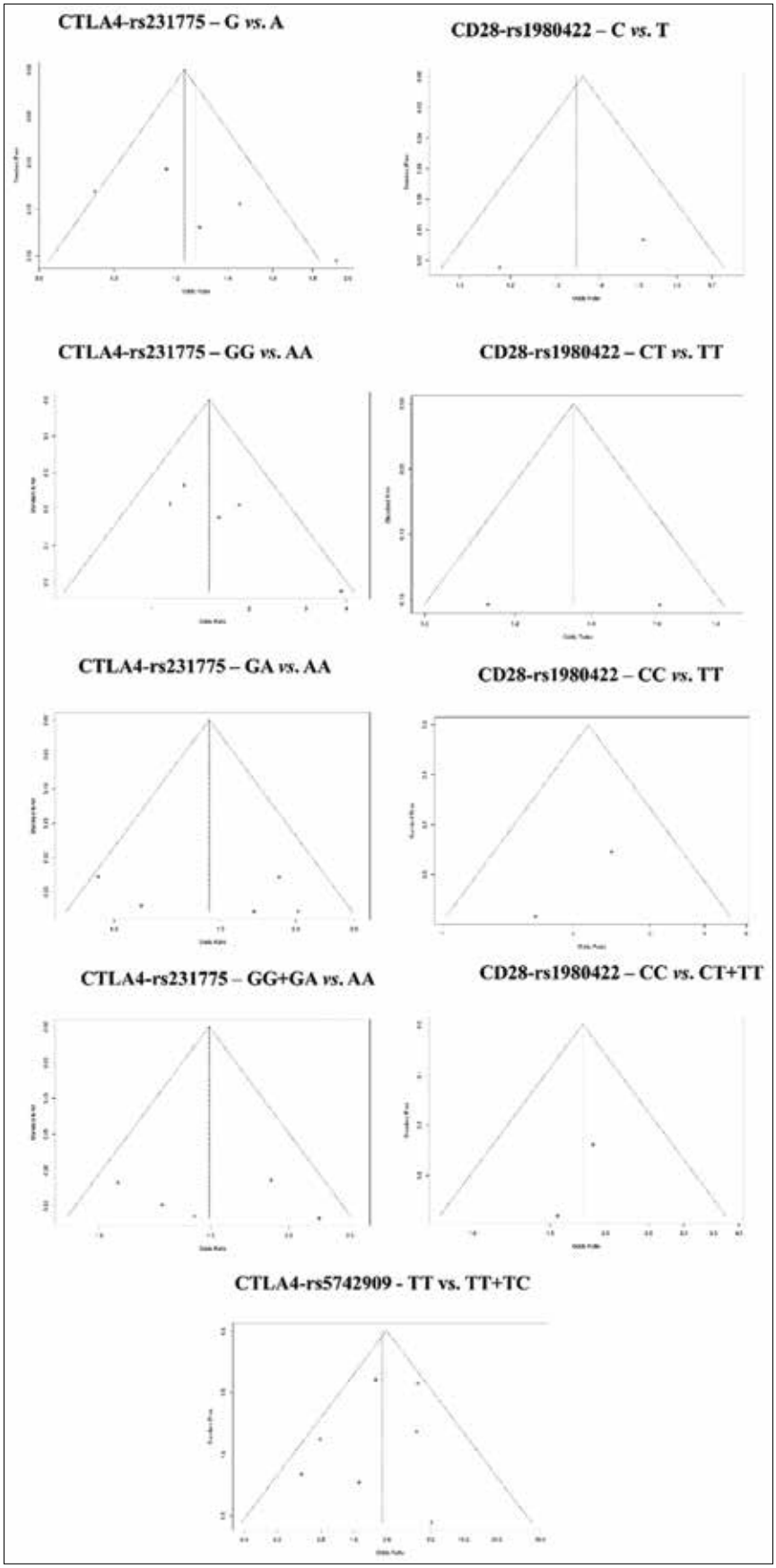

Figure 3: Funnel plot to show publication bias and heterogeneity. The plot depicts publication bias and heterogeneity between studies for significantly associated comparisons of between CTLA4-rs231775, CTLA4-rs5742909, and CD28-

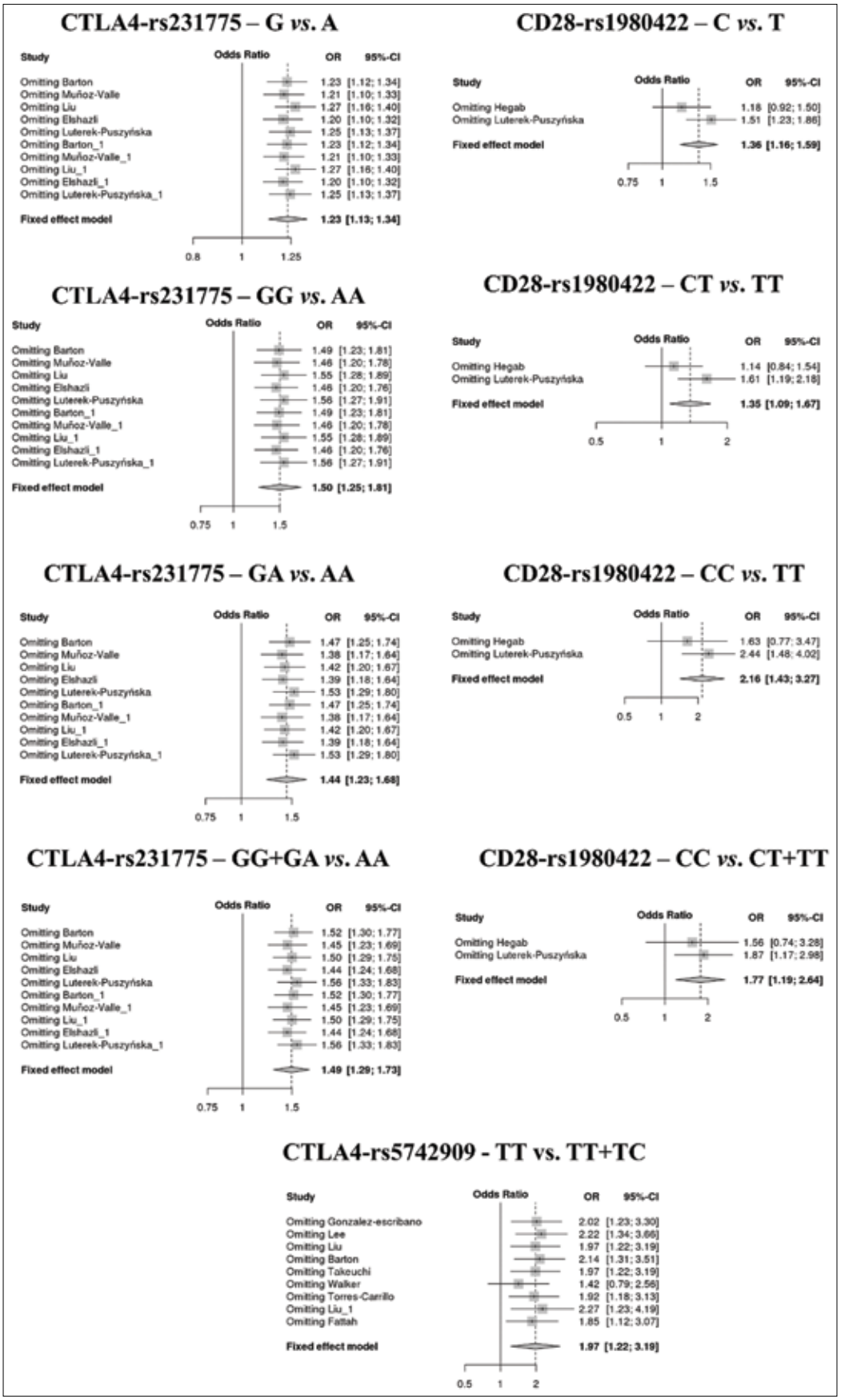

Figure 4: Influence plot. The graph demonstrates the sensitivity analysis for significantly associated comparisons of between CTLA4-rs231775, CTLA4-rs5742909, and CD28-

of CTLA-4 on the regulatory T (Treg) cells culminates in downmodulation and controlling of the immune system function.[21] Numerous studies have established that dysregulated expression of CTLA-4 on T-cells participates in predisposition to autoimmunity through conferring an imbalance in the homeostasis of the immune system. The rs231775 SNP in the *CTLA4* gene is a well-investigated genetic factor in the genetic association studies and has recently been suggested as a pathogenic biomarker in the context of autoimmune disorders.[22,23] Furthermore, the rs5742909 SNP is a functional polymorphism-318 C/T in the promoter region that has been reported to influence the cell surface expression of CTLA-4.[24] As a consequence, recognition of the RA risk by rs231775 and rs5742909 SNPs seems to be beneficial in the clinical diagnosis of the disease and devising new therapeutic approaches. Nonetheless, observations from various investigations have been conflicting because of limited sample size and the data have been incompatible. As a result, the current meta-analysis intended to pool the data from various studies to achieve a precise estimation of the involvement of *CTLA4* gene polymorphisms and *CD28* gene in conferring a genetic risk factor for RA risk.

The previous meta-analysis by Wang *et al*.[9] in 2016 included 4732 patients and 6270 healthy controls to analyze the association of *CTLA4* gene rs231775 SNP with autoimmune disorders, including RA, systemic lupus erythematosus, and type 1 diabetes. The results indicated that all genetic comparisons of rs231775, including homozygote comparison (GG vs. AA), heterozygote comparison (AG vs. AA), allelic model (T vs. G), dominant model (GG/AG vs. AA), and recessive model (GG vs. AA/AG) were strongly associated with increased risk of autoimmune disorders. On the other hand, our study focused on the association of *CTLA4* gene rs231775 SNP with only RA disease and included 1078 cases and 1118 controls for rs231775. It was recognized that the G-allele of the rs231775 SNP significantly promoted (OR = 1.27) the risk of RA susceptibility. Moreover, both the GG (OR = 1.50) and the GA (OR = 1.43) genotypes were significantly associated with the increased susceptibility to RA. The dominant model of GG + GA significantly increased risk of the disease (OR = 1.49). Even though the rs5742909 polymorphism did not show a significant association in allelic and genotypic levels, the recessive genetic model of TT versus TC + CC conferred a significantly increased risk (OR = 1.97) to RA development.

The association of *CD28* gene rs1980422 SNP was reported to be associated with RA susceptibility in a GWAS.[10] It was reported a higher prevalence of C-allele in RA patients in comparison to the controls and increased the risk of RA (OR = 1.16). rs1980422 is located 10 kb upstream of the *CD28* gene and is found in a region that is 129 kb upstream the rs3087243 polymorphism, a RA associated variant in the *CTLA4* gene.[25] It has been reported that the *CD28* gene rs1980422 variant is strongly associated with RA risk in Europeans.[10] On the other hand, a study performed in Poland did not show its association with RA.[26] On the contrary, *CD28* gene rs1980422 SNP was strongly associated with RA risk in Egyptians.[27] Furthermore, this strong association was also indicated in both anticitrullinated protein antibodies or rheumatoid factor positive RA subjects.[27] Although a little number of studies could meet our inclusion/exclusion criteria, the meta-analysis indicated that the allelic and genotypic comparisons of this SNP reached a significant association in increasing RA risk.

Although the meta-analysis demonstrated a statistically significant association with RA risk, there are some limitations and caveats. Insufficient original data about genetic distribution, clinical characteristics, treatment options, ethnicity, gene, and environment contribution confined the possibility of tracking more risk factors in RA susceptibility. Moreover, only English original studies may subject to a language bias. Nonetheless, only meta-analysis of case–control studies was carried out, they were directly applied for combining the studies with a similar design, conferring reliable results. Fortunately, no sparse data were identified in this study, as the sparse data are considered as the major limitation of such meta-analysis models.

CONCLUSION

Considering all the facts, the meta-analysis found that there was a positive association between alleles and genotypes of CTLA4-rs231775 and CD28-rs1980422 polymorphisms and RA susceptibility. Since CTLA4 as an inhibitory molecule and CD28 as an activating receptor were both involved in the susceptibility to RA, it seems that further combinational studies are still mandatory to reveal the pooled effect of immune regulatory molecules in predisposing a risk for RA. Further investigations might disclose the bona fide association of the genetic polymorphisms in the immune inhibitory and activating genes, which finally might be appreciative in the field of personalized medicine for the treatment of RA patients.

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Conflicts of interest

There are no conflicts of interest.

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