Original Article
Association of IL-12A and IL-12B gene polymorphisms with rheumatoid arthritis: a meta-analysis

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Abstract: The aim of our study was to investigate the association between common polymorphisms in interleukin-12 (IL-12) and rheumatoid arthritis (RA) risk using meta-analysis. A search was conducted in the PubMed database, covering all papers published by October 14, 2015. Odds ratios (OR) and 95% confidence intervals (CI) were used to evaluate the strength of this association. Publication bias was assessed with Begg's test. Overall, 6 case-control studies with 3,436 RA patients and 4,644 healthy controls were retrieved. No significant association was observed between the *IL-12B rs3212227* polymorphism and RA risk in all genetic models. Similar negative observations were also detected for *IL-12B rs6887695* and *IL-12A rs2243115* polymorphisms. Interestingly, an increased association was found between the *rs3212227* polymorphism and RA risk in the hospital-based source of control (CC vs. AA: OR = 2.13, 95% CI = 1.11-4.09; CC+CA vs. AA: OR = 2.08, 95% CI = 1.06-4.09). Overall, no obvious evidence of publication bias was detected. There had a poor evidence to confirm that *IL-12B* gene *rs3212227* polymorphism was associated with susceptibility of RA.

Keywords: Interleukin-12, rheumatoid arthritis, polymorphism, risk, meta-analysis

Introduction

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory disorder, affecting 1% of the adult population worldwide [1]. Assessment of the contribution of a genome-wide association study (GWAS) of single nucleotide polymorphisms (SNPs) predicted that SNPs outside the RA loci contribute to a significant amount of RA heritability [2], which suggests that genetic variants contribute to the development of RA. Furthermore, evidence for a strong inherited component in RA is supported by data obtained in familial and twin studies, suggesting that approximately 60% of disease susceptibility is caused by genetic factors [3, 4].

Cytokines play an important role in the regulation of immune response in RA [5], and SNPs in cytokine genes may confer flexibility on the immune response [1], finally influencing the development or severity of the disease.

Interleukin (IL)-12, a pro-inflammatory cytokine, is composed of two disulfide-linked subunits of 35 kDa (IL-12A) and 40 kDa (IL-12B) [6]. IL-12 has an antagonistic effect on the T helper type 1 (Th1)/Th2 cytokine balance and provides a functional link between innate and acquired immune responses [5, 7, 8]. Petrovic-Rackov et al. [9] reported an increased level of IL-12 in both serum and synovial fluid from patients with RA, and that this increase was correlated with disease activity score. The above information suggested that the IL-12 gene is a candidate gene in the pathogenesis of RA.

Cargill et al. [10] first reported that the *IL-12B rs3212227* and *rs6887695* polymorphisms may play fundamental roles in psoriasis pathogenesis. Moreover, the *IL-12B rs3212227* polymorphism can decrease IL-12 production [11]. Another study [12] established that the *IL-12A rs2243115* polymorphism may be associated with an increased risk of developing Graves'
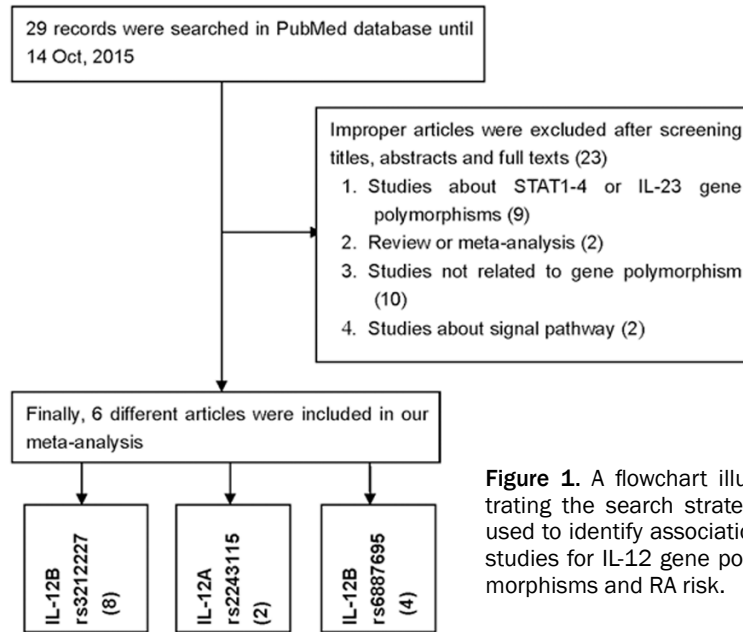
Figure 1. A flowchart illustrating the search strategy used to identify association studies for IL-12 gene polymorphisms and RA risk.

included sufficient genotype numbers for cases and controls. Accordingly, the following exclusion criteria were also used: (a) the control population was absent from the study; (b) the genotype frequency was not specified in the study; (c) the study was a duplicate of previous publications.

Data extraction

Two of the authors extracted all data independently, complying with the selection criteria. The following items were collected: author's first and last name, year of publication, country of origin, ethnicity, total case/control number, all genotype frequencies of case and control groups, source of control, Hardy-Weinberg equilibrium (HWE) of controls, mutant-allele frequency and genotyping methods.

Statistical analysis

Odds ratios (OR) with 95% confidence intervals (CI) were used to measure the level of association of *IL-12B rs3212227, IL-12B rs6887695*, and *IL-12A rs2243115* polymorphisms with RA. Subgroup analysis of control sources was performed on two different groupings, where necessary: population-based (PB) and hospital-based (HB).

The summary OR was determined with the *Z*-test. Heterogeneity assumption was evaluated with the chi-square-based *Q*-test. A *P* value < 0.10 for the *Q*-test indicated that heterogeneity may exist. In case significant heterogeneity was detected, the random effects model (DerSimonian-Laird method) was used. Otherwise, the fixed effects model (Mantel-Haenszel method) was chosen [19, 20]. For each IL-12 gene polymorphism, we investigated the relationship between genetic variants and RA risk in allelic contrast (M-allele vs. W-allele), homozygote comparison (MM vs. MW), dominant genetic model (MM+MW vs. WW), heterozygote comparison (MW vs. WW) and recessive genetic model (MM vs. MW+WW).

disease. In the past 7 years, the three *IL-12* gene polymorphisms mentioned above have been studied extensively. Considering the vital role of these polymorphisms in the development of immune diseases such as RA, we performed meta-analysis of all eligible case-control studies [13-18] to derive a more precise estimation of the association of *IL-12A* and *IL-12B* gene polymorphisms with RA risk.

Materials and methods

Identification and eligibility of relevant studies

Information was obtained by searching the PubMed database (latest search updated on October 14, 2015), using keywords containing "IL-12" or "Interleukin-12", "polymorphism" or "variant", and "rheumatoid arthritis" or "RA", without any restriction on language or publication year. Using these terms, a total of 29 articles were retrieved, of which 6 matched the inclusion criteria. We also screened references of the retrieved articles and review articles by manual search.

Inclusion criteria and exclusion criteria

Studies that were included in our analysis had to meet all of the following criteria: (a) the study assessed the correlation between RA and *IL-12B rs3212227, IL-12B rs6887695*, and *IL-12A rs2243115* polymorphisms; (b) the study was a case-control study; (c) the study

Table 1. Study characteristics from published studies on the relationship between three IL-12 gene polymorphisms and RA risk

First author	Year	Origin	Ethnicity	Design	Case	Control	Method	Case			Control				
								CC	CA	AA	CC	CA	AA	C %	HWE
IL-12B rs3212227															
Orozco	2005	Spain	Caucasian	PB	545	393	SSCP	20	161	364	19	125	249	0.21	0.52
Shen	2015	China	Asian	HB	597	831	SNPscan	112	309	176	166	391	274	0.43	0.22
Alvarez-Rodriguez	2009	Spain	Caucasian	PB	72	465	SSCP	2	23	47	17	132	316	0.18	0.49
Chang	2008	USA	Caucasian	PB	470	474	Multiplex	27	142	301	23	145	306	0.20	0.28
Chang	2008	USA	Caucasian	PB	659	1317	Multiplex	27	195	437	61	433	823	0.21	0.68
Chang	2008	USA	Caucasian	PB	591	699	Multiplex	24	169	398	30	191	478	0.18	0.06
Wang	2015	China	Asian	HB	412	279	PCR-RFLP	4	107	301	1	37	241	0.07	0.74
Angelo	2015	Brazil	Mixed	HB	90	186	PCR-RFLP	7	68	15	14	92	80	0.32	0.07
IL-12A rs2243115								GG	GT	TT	GG	GT	TT	G%	
Shen	2015	China	Asian	HB	604	832	SNPscan	5	109	490	6	148	678	0.10	0.50
Wang	2015	China	Asian	HB	412	279	PCR-RFLP	69	189	154	36	132	111	0.36	0.74
IL-12B rs6887695								CC	CG	GG	CC	CG	GG	C%	
Wang	2015	China	Asian	HB	412	279	PCR-RFLP	11	168	233	7	65	207	0.14	0.49
Chang	2008	USA	Caucasian	PB	469	473	Multiplex	52	194	223	51	194	228	0.31	0.31
Chang	2008	USA	Caucasian	PB	659	1316	Multiplex	58	278	323	137	578	601	0.32	0.91
Chang	2008	USA	Caucasian	PB	591	699	Multiplex	58	237	296	66	262	371	0.28	0.05

HB: Hospital-based; PB: Population-based; SSCP: Single-stranded conformational polymorphism; SNPscan: A custom-by-design 48-Plex SNPscan™ Kit; Multiplex: A multiplexed conventional polymerase chain reaction followed by a flow cytometry-based oligonucleotide ligation assay or kinetic; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; HWE: Hardy-Weinberg equilibrium of control group.

Funnel plot asymmetry was assessed with Egger's test. Publication bias was assessed with Begg's test. $P < 0.05$ was considered statistically significant [21, 22]. The departure of frequencies of the EGF polymorphism from expectation under HWE was assessed by the chi-square test in controls using Pearson's chi-square test, and $P < 0.05$ was considered significant. All statistical tests for the meta-analysis were performed with STATA software (version 10.0; StataCorp LP, College Station, TX).

Results

Eligible studies

A total of 29 articles were retrieved based on the search criteria. Among them, 23 articles were excluded because they did not provide information on IL-12 gene polymorphisms (**Figure 1**). Thus, a total of 6 articles [13-18] accounting for a total of 3,436 RA patients and 4,644 healthy controls were included in our meta-analysis. All RA patients were then screened to select individuals who fulfilled the American College of Rheumatology (formerly the American Rheumatism Association) 1987 revised criteria for RA [23]. The controls were age- and ethnicity-matched unrelated healthy individuals. Characteristics of studies that

investigated *IL-12B rs3212227, IL-12B rs6887695*, and *IL-12A rs2243115* polymorphisms are summarized in **Table 1**. The distribution of genotypes in all controls was consistent with the HWE. Genotyping of the SNPs of *IL-12* gene polymorphism was conducted using polymerase chain reaction and restrictive fragment length polymorphism (PCR-RFLP), single-stranded conformational polymorphism (SSCP), a custom-by-design 48-Plex SNPscan™ Kit (SNPscan), and a multiplexed conventional polymerase chain reaction, followed by a flow cytometry-based oligonucleotide ligation assay or kinetic (Multiplex).

Meta-analysis

IL-12B rs3212227: The results of the overall meta-analysis suggested negative associations between this polymorphism and RA susceptibility in all five genetic models (C-allele vs. A-allele: OR = 1.05, 95% CI = 0.90-1.24, $P_{heterogeneity}$ = 0.002; CC vs. AA: OR = 0.98, 95% CI = 0.80-1.20, $P_{heterogeneity}$ = 0.787; CA vs. AA: OR = 1.11, 95% CI = 0.90-1.37, $P_{heterogeneity}$ = 0.001; CC+CA vs. AA: OR = 1.10, 95% CI = 0.90-1.35, $P_{heterogeneity}$ = 0.001; CC vs. CA+AA: OR = 0.93, 95% CI = 0.77-1.13, $P_{heterogeneity}$ = 0.897) (**Table 2**). Analysis of ethnicity subgroups also showed no statistically significant association in Caucasians or Asians. To our sur-

Table 2. Total and stratified analysis of three IL-12 gene polymorphisms and RA risk

Variables	N	Case/Control	M-allele vs. W-allele		MW vs. WW		MM vs. MW+WW		MM vs. WW		MM+MW vs. WW	
			OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h
rs3212227A/C												
Total	8	3436/4644	1.05 (0.90-1.24)	0.002	1.11 (0.90-1.37)	0.001	0.93 (0.77-1.13)	0.897	0.98 (0.80-1.20)	0.787	1.10 (0.90-1.35)	0.001
Ethnicity												
Caucasian	5	2337/3348	0.95 (0.86-1.04)	0.533	0.94 (0.84-1.06)	0.571	0.93 (0.71-1.21)	0.860	0.91 (0.69-1.19)	0.818	0.94 (0.84-1.05)	0.525
Asian	2	1009/1110	1.47 (0.72-2.99)	0.001	1.65 (0.89-3.06)	0.009	0.94 (0.72-1.23)	0.338	1.08 (0.80-1.46)	0.324	1.62 (0.83-3.18)	0.004
Mixed	1	90/186	-		-		-		-		-	
Source of control												
HB	3	1099/1296	1.55 (0.95-2.52)	0.000	2.13 (1.11-4.09)	0.000	0.95 (0.74-1.23)	0.620	1.15 (0.86-1.53)	0.167	2.08 (1.06-4.09)	0.000
PB	5	2337/3348	0.95 (0.86-1.04)	0.533	0.94 (0.84-1.06)	0.571	0.93 (0.71-1.21)	0.860	0.91 (0.69-1.19)	0.818	0.94 (0.84-1.05)	0.525
rs2243115T/G												
Total	2	1016/1111	1.09 (0.92-1.29)	0.535	1.02 (0.83-1.26)	0.954	1.33 (0.89-2.00)	0.796	1.35 (0.87-2.09)	0.782	1.06 (0.86-1.30)	0.712
rs6887695G/C												
Total	4	2131/2767	1.13 (0.89-1.43)	0.000	1.22 (0.86-1.72)	0.000	0.95 (0.77-1.16)	0.771	0.98 (0.78-1.18)	0.467	1.20 (0.86-1.67)	0.000

HB: Hospital-based; PB: Population-based; W allele: Wild-type allele; M allele: Mutant allele; P_h: P-value of Q-test for heterogeneity test.
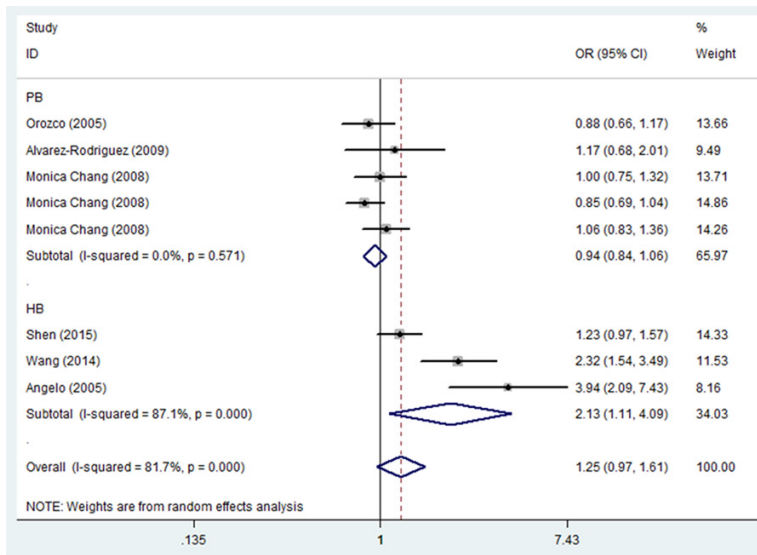
Figure 2. Forest plot of RA risk associated with IL-12B rs3212227 polymorphism (CA vs. AA) in HB subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.
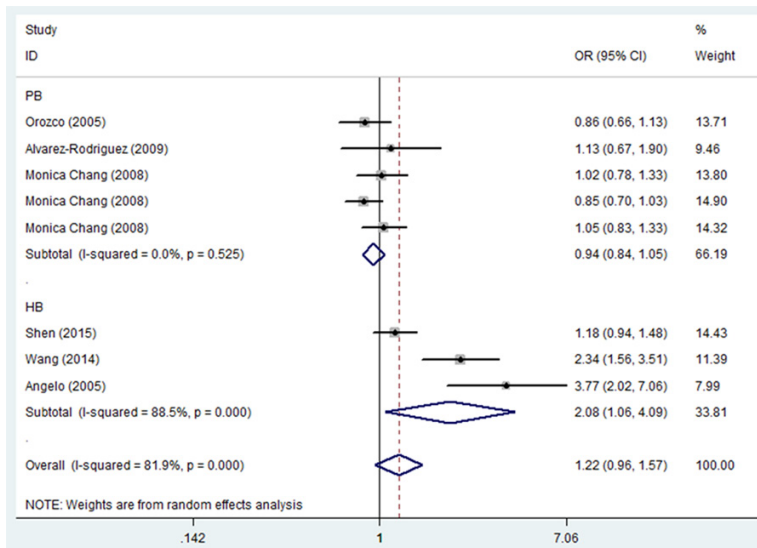

Figure 3. Forest plot of RA risk associated with IL-12B rs3212227 polymorphism (CC+CA vs. AA) in HB subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

prise, a marginally and poorly significant difference was found in the HB sources of control subgroup (CA vs. AA: OR = 2.13, 95% CI = 1.11-4.09, $P_{heterogeneity}$ = 0.000; CC+CA vs. AA: OR = 2.08, 95% CI = 1.06-4.09, $P_{heterogeneity}$ = 0.000) (**Table 2**; **Figures 2**, **3**).

IL-12B rs6887695 and IL-12A rs2243115: There was no significantly positive association between SNPs and RA susceptibility in both polymorphisms (*IL-12B rs6887695*: OR = 1.20, 95% CI = 0.86-1.67, $P_{heterogeneity}$ = 0.000 for CC+CG vs. GG, **Figure 4**; OR = 1.22, 95% CI = 0.86-1.72, $P_{heterogeneity}$ = 0.000 for CG vs. GG, OR = 1.13, 95% CI = 0.89-1.43, $P_{heterogeneity}$ = 0.000 for C-allele vs. G-allele, OR = 0.98, 95% CI = 0.78-1.18, $P_{heterogeneity}$ = 0.467 for CC vs. GG and OR = 0.95, 95% CI = 0.77-1.16, $P_{heterogeneity}$ = 0.771 for GG vs. CG+GG; *IL-12A rs2243115*: OR = 1.06, 95% CI = 0.86-1.30, $P_{heterogeneity}$ = 0.712 for GG+GT vs. TT; OR = 1.02, 95% CI = 0.83-1.26, $P_{heterogeneity}$ = 0.954 for GT vs. TT, **Figure 5**, OR = 1.09, 95% CI = 0.92-1.29, $P_{heterogeneity}$ = 0.535 for G-allele vs. T-allele, OR = 1.35, 95% CI = 0.87-2.09, $P_{heterogeneity}$ = 0.782 for GG vs. TT and OR = 1.33, 95% CI = 0.89-2.00, $P_{heterogeneity}$ = 0.796 for GG vs. GT+TT) (**Table 2**). Similar relationships were observed for sources including a control subgroup (data not shown) (**Table 2**).

Publication bias

Begg's funnel plot was performed to assess publication bias. The shapes of the funnel plots did not reveal any obvious asymmetry in any of the comparison models. Next, Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias [for example: IL-12B rs3212227 polymorphism (CA vs. AA) (t = 1.36, P = 0.174 for Begg's test; IL-12B rs6887695 polymorphism (CC+CG vs. GG) (t = 1.02, P = 0.308 for Begg's test)] (**Figures 6**, **7**). However, for IL-12A
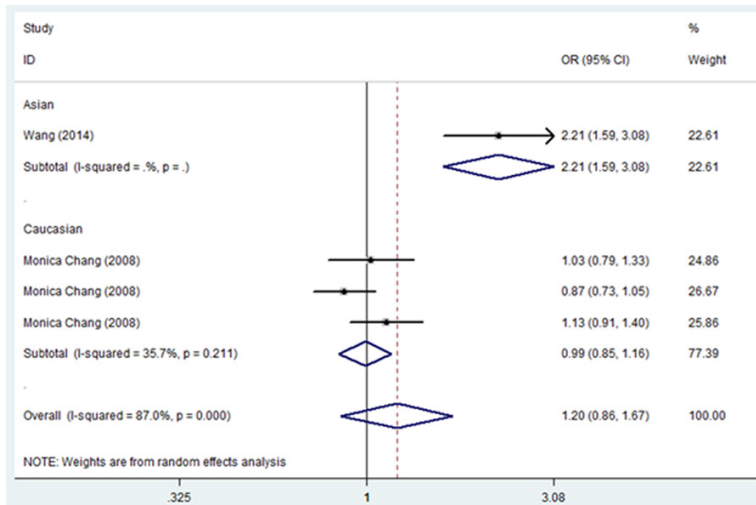
Figure 4. Forest plot of RA risk associated with IL-12B rs6887695 polymorphism (CC+CG vs. GG) in HB subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.
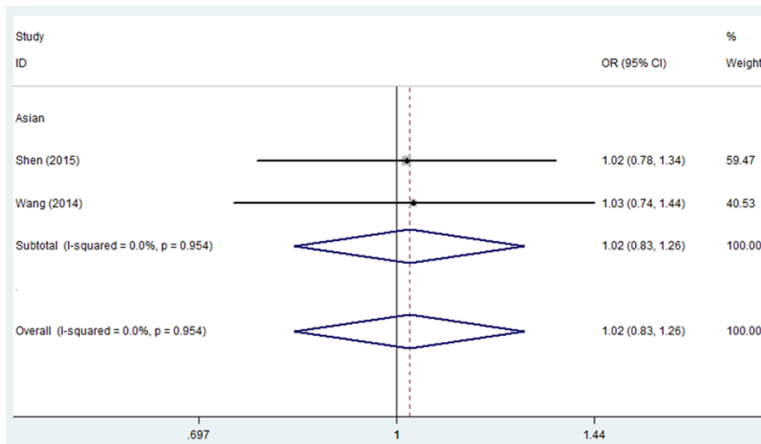

Figure 5. Forest plot of RA risk associated with IL-12A rs2243115 polymorphism (GT vs. TT) in HB subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

RA risk. It is well established that the main genetic determinant for RA susceptibility is correlated with specific alleles at the HLA-DRB1 gene [26]. However, genome-wide association studies (GWAS) have discovered more than 30 new RA susceptibility loci containing the IL-12 gene [27]. A US study first reported the association between IL-12 gene polymorphisms and RA risk [10], followed by similar studies that have showed inconsistent results [15-18]. Moreover, a GWAS revealed that the rs7574865 G/T polymorphism in the third intron of the signal transducer and activator of transcription 4 (STAT4) gene is significantly associated with RA [28]. The STAT4 gene is located on human chromosome 2q32.3 [29], and its protein product family member is uniquely activated by IL-12 through its receptor. Therefore, we speculated that the IL-12 gene polymorphism may also influence RA risk. To the best of our knowledge, this was the first analysis to combine all available publications in order to evaluate IL-12 gene polymorphisms and RA risk. We performed meta-analysis of these studies for a total 3,436 RA patients and 4,644 healthy controls. For each of IL-12 gene polymorphisms analyzed, the overall analysis showed no relationship in all genetic models. Furthermore, using stratified analysis by source of control, we found that individuals in the HB subgroup carrying *rs3212227* CA or CC genotype had increased risk of RA. These results suggested that *IL-12B rs3212227* polymorphism is a poor risk factor for RA.

Meta-analysis has been recognized as an effective method to address a wide variety of clinical questions by summarizing and reviewing previously published quantitative research. However,

rs2243115 polymorphism, only two studies were included, so the Begg's and Egger's test could not be detected.

Discussion

RA is one of the most common chronic systemic autoimmune disorders. Multiple genetic and environmental factors are believed to be involved in the pathogenesis of RA [24, 25].

Several studies have addressed the association between IL-12 gene polymorphisms and
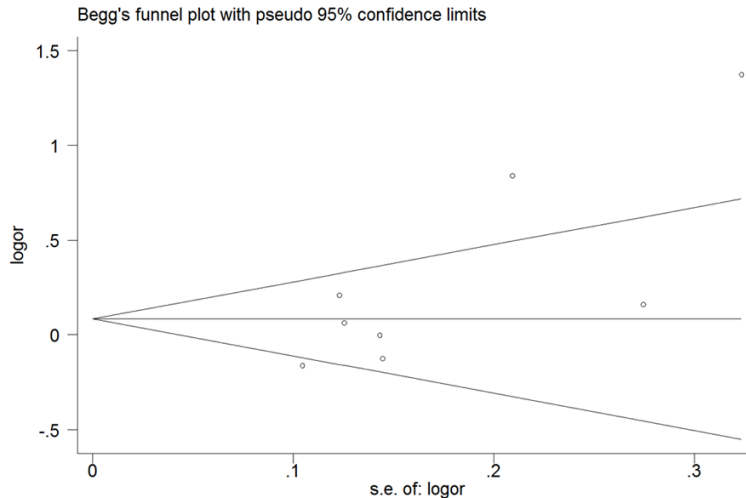
Figure 6. Begg's funnel plot for publication bias test (CA vs. AA in the IL-12B rs3212227).

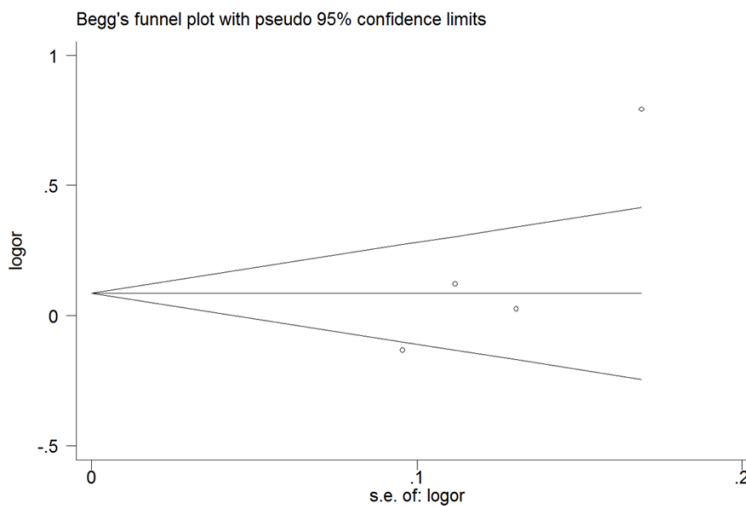**Figure 7.** Begg's funnel plot for publication bias test (CC+CA vs. AA in the IL-12B rs3212227).

In summary, our meta-analysis provided evidence that the *IL-12B rs3212227* polymorphism was poorly associated with significantly increased RA risk and may be a biomarker for the detection of RA susceptibility. Consequently, further well-designed large studies, particularly those related to gene-gene and gene-environment interactions, are warranted.

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Disclosure of conflict of interest

None.

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our study has some limitations. First, the number of published studies included was not sufficiently large for a comprehensive analysis. Second, gene-gene interactions, gene-environment interactions, and even interactions between different polymorphic loci of the same gene may modulate RA risk. Third, our meta-analysis was based on unadjusted estimates; if individual data were available, a more precise analysis should be performed, which would allow for adjustment for other covariates, including age, sex, family history, environmental factors, disease stage, and lifestyle.

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