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Original Article

Association between CCR6 and rheumatoid arthritis: a meta-analysis

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Abstract: Objective: Chemokine (C-C motif) receptor 6 gene (CCR6) polymorphism has been reported to be associated with rheumatoid arthritis (RA) in different ethnic populations. Moreover, its inhibition by monoclonal antibody in mouse model has suppressed arthritis. However, few replication studies have reported conflicting results about this association. Therefore, to establish that CCR6 indeed is a risk factor associated with RA among different ethnic populations, a comprehensive meta-analysis study was conducted. Methods: PubMed and MEDLINE databases were searched using the term 'CCR6' for all articles published before May 2014. All the replication studies examining the association between CCR6 and RA were reviewed for meta-analysis. Data were summarized using random-effects meta-analysis. The heterogeneity and publication bias among studies were examined by χ^2 -based Q statistic test and Egger's test, respectively. Results: A total of 24955 RA patients and 56129 controls from seven articles were included in the meta-analysis. While CCR6 was a risk factor in Asian (OR = 1.19, 95% CI: 1.14-1.24) and European (OR = 1.14, 95% CI: 1.08-1.21) populations, it was indicated as a protective factor in African Americans (OR = 0.79, 95% CI: 0.62-0.96). Conclusions: Our meta-analysis study concludes that there is a significant association between CCR6 and RA in all racial groups except African-American subgroup, which require a large sample size for concrete prediction.

Keywords: Rheumatoid arthritis, CCR6, population genetics, susceptibility, meta-analysis

Introduction

Rheumatoid arthritis (RA) is one of the most common chronic systemic inflammatory diseases that cause joint destruction. The clinical presentation of RA is usually symmetrical invasion in the small joints, especially in the hands and feet [1]. Although the definite etiology of RA is not clear, more and more research have been suggested that it likely involve an interaction between genetic and environmental factors.

Recently, three independent GWAS studies identified the chemokine (C-C motif) receptor 6 gene (CCR6) as a susceptibility locus for RA [2-4], and this finding was validated in several independent replication cohorts [5-8]. In Japanese population it was observed that there was a strong association between CCR6 and RA and the evidence supported it play an important pathogenic role in the disease. However, an inconsistent result was reported in later studies using African American populations [8, 9]. Due to this discrepancy, the role of CCR6 polymorphisms in RA remains controversial and is necessary to be assessed.

To our knowledge there has been no published systematic review of the literature that has characterized the magnitude of these associations. Therefore, expecting to investigate these conflicting results and reveal the role of CCR6 in RA, we conducted the meta-analysis of all published studies on the association between CCR6 polymorphisms and RA risk.

Materials and methods

Identification of eligible studies

The first association study of CCR6 with RA was published in May 2010. We systematically searched PubMed and MEDLINE for all articles

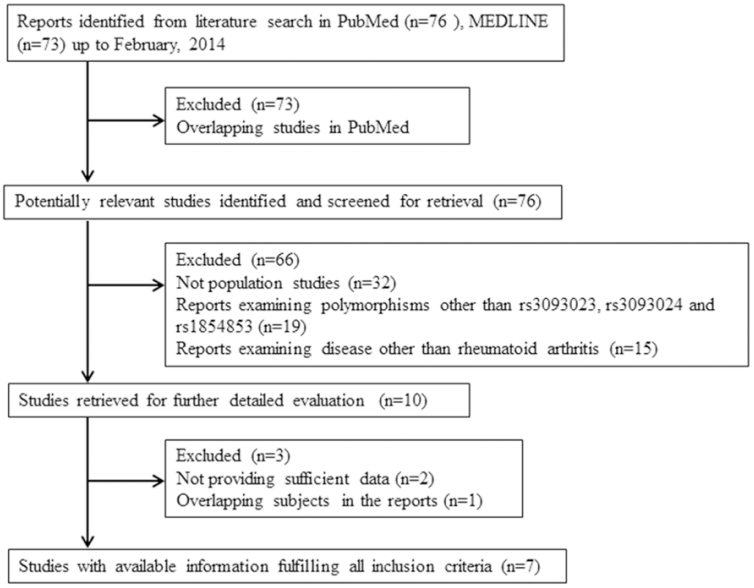

Figure 1. Flow chart for the selection of studies and specific reasons for exclusion of studies from the meta-analysis.

published between May 2010 and May 2014. The gene name 'CCR6' and disease name ('rheumatoid arthritis' or 'RA') was respectively used to retrieve the appropriate articles. Moreover, additional hand searching was performed to identify potentially relevant studies.

Selection

Manuscripts were selected if they met all of the following criteria: (1) The diagnosis of RA was determined according to the revised criteria of the American College of Rheumatology 1987 criteria for Rheumatoid Arthritis [11]; (2) the odds ratio (OR) with 95% confidence intervals (CIs) in patients and in controls were available or could be calculated; (3) the study was published as a full paper, not as a meeting abstract or review. The exclusion criteria included: (1) not a population study; (2) studied other SNPs; (3) the duplicate studies. A flow chart depicting the selection process for the studies and the reasons for exclusion is presented in **Figure 1**. The qualities of all included studies were assessed through a table (Table S1).

Data extraction

The following information was extracted from each study: first author, year of publication, region, and study population, the type of study design, the numbers and sex ratio of the patients and controls, estimated OR with 95% confidence interval. Data were extracted by two authors (P.C. and Y.Z.) independently and in duplicate. All disagreements and uncertainties were discussed and resolved by consensus, with the involvement of another author (F.G.) if necessary.

Data analysis

Based on the 1000 genomes project, the single nucleotide polymorphism (SNP) rs3093024 was in strong linkage disequilibrium (LD) with rs3093023 and rs1854853 (D' = 1.0, $r^2 > 0.9$). Therefore, the SNP rs3093024, which tags rs3093023 and rs1854853, is most likely the best proxy to evaluate the effect of this gene.

The population-wide impact of CCR6 polymorphism on susceptibility to RA was assessed by pooling together the per-allele ORs data weighted by their inverse variance from each independent study. The random-effects model was used to calculate the pooled OR. Heterogeneity was evaluated by χ^2-based Q statistic and I^2 statistic [12, 13]. To evaluate the reliability and stability of our results, publication bias was evaluated with Egger's linear regression and Begger's funnel plot [14-16], and the influence of each study on the pooled-OR was investigated in a sensitivity test by excluding one study each time. All analyses were carried out using Stata SE 12.0 data analysis and statistical software.

Results

Search results and study characteristics

Our search strategy resulted in 76 articles in PubMed and 73 articles in MEDLINE. Of these, 73 were excluded for overlapping. In these 76 articles, 32 were excluded because they were not population studies, 19 because of obvious irrelevant polymorphisms and 15 because of not involving RA. The full texts of the 10 remaining articles were obtained for detailed review.

Table 1. Information of all the studies included in the meta-analysis

	Study		Country/region	Population	Study design	GWAS	Cases		Controls		COHORT name
	First Author	Year				(YES/NO)	Total	FEMALE%	Total	FEMALE%	
1a	Kochi et al. 1 [3]	2010	Japan	Japanese	Population based case-control	YES	2301	81.40%	3368	44.40%	NA
1b	Kochi et al. 2 [3]	2010	Japan	Japanese	Population based case-control	NO	3662	81.50%	15873	34.10%	NA
1c	Kochi et al. 3 [3]	2010	Japan	Japanese	Population based case-control	NO	1106	79.20%	1486	60.60%	NA
2a	Stahl et al. 1 [4]	2010	USA/Canada/UK/North American/The Netherlands	European	Population based case-control	YES	5539	NA	20169	NA	NA
2b	Stahl et al. 2 [4]	2010	USA/Canada/UK/North American/The Netherlands	European	Population based case-control	NO	6768	NA	8806	NA	NA
3	Hughes et al. [8]	2010	USA	African Americans	Population based case-control	NO	556	83.88%	804	83.88%	CLEAR
4	Teng et al. [6]	2012	Japan	Asian	Hospital based case-control	NO	556	82%	440	45%	TTSH
5	Prasad et al. [7]	2012	India	North Indians	Population based case-control	NO	983	NA	1007	NA	AIIMS, R & R
6	Chang et al. [5]	2012	Taiwan	Han Chinese	Hospital based case-control	NO	400	81.79%	680	49.93%	NA
7a	Jiang et al. 1 [2]	2014	China	Han Chinese	Hospital based case-control	YES	952	NA	943	NA	NA
7b	Jiang et al. 2 [2]	2014	China	Han Chinese	Population based case-control	NO	2132	NA	2553	NA	NA

Case definition: Diagnosis was determined according to the revised criteria of the American College of Rheumatology 1987 criteria for Rheumatoid Arthritis.

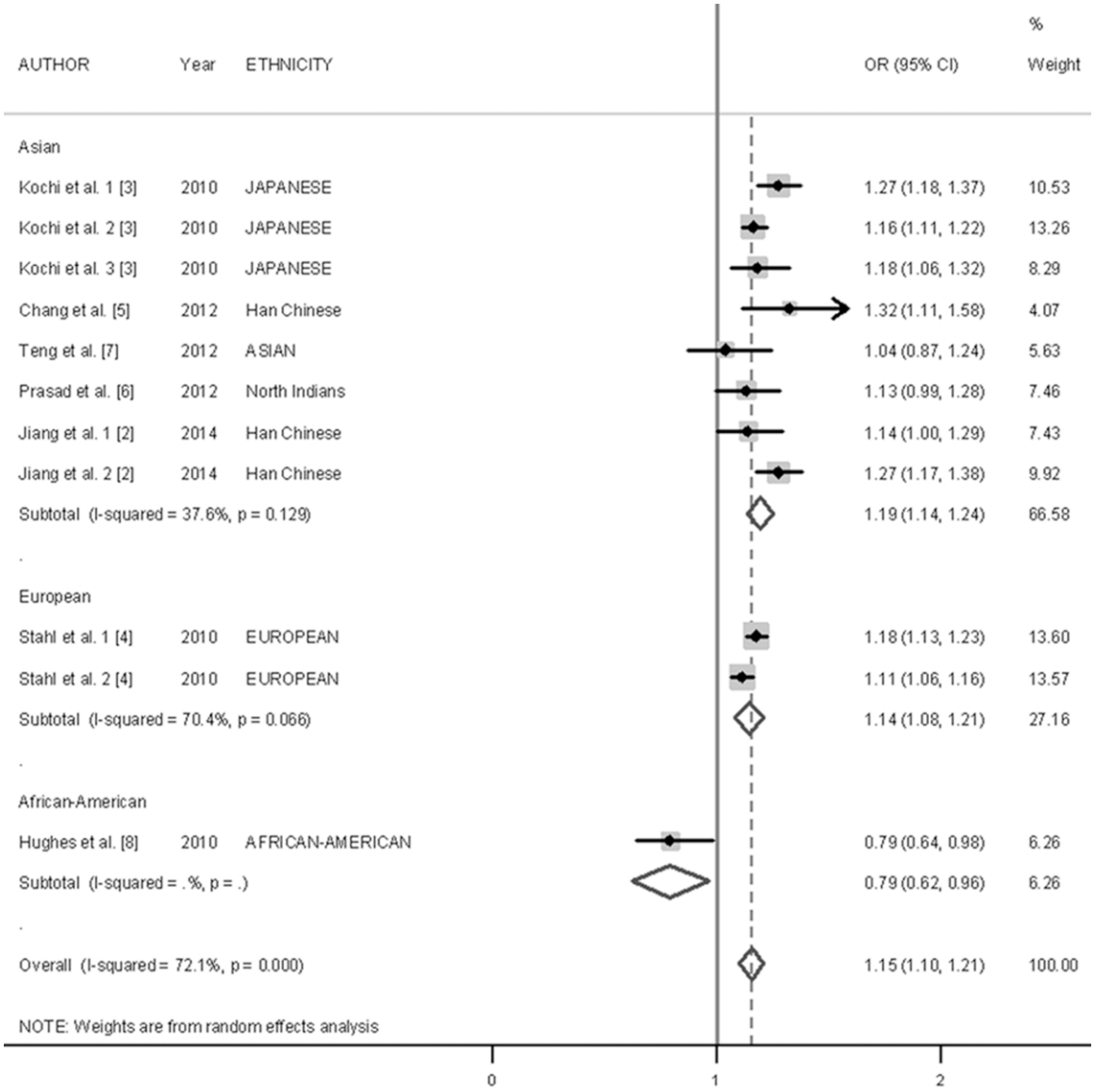

Figure 2. Forest plot describing the association between the CCP6 rs3093024 variant and RA risk. Squares indicate the OR in each study, with square sizes inversely proportional to the standard error of the OR. Horizontal lines represent 95% CI. Analysis was stratified according to ethnicity.

After the exclusion of 2 articles for insufficient data and 1 article for overlapping subjects in the reports, a total of 7 independent articles with 11 studies were finally included in the current meta-analysis, which contained information for 56129 healthy subjects and 24955 RA patients. Of the included studies, 8 focused on Asian-descent populations, 2 focused on Caucasian- or European-descent populations, and 1 study reported data concerning populations of African-American descent. Of these studies, three are GWAS (Genome wide association study) studies (2 on Asian and 1 on European population) [2, 4] that are part of our meta-analysis. The detailed characteristics of the included studies are listed in **Table 1**. Additionally, after scoring for the studies, we found all included studies had a high level quality.

Quantitative assessment of all current evidence

The overall estimate suggested a significant association between the CCR6 rs3093024-A allele and RA risk (OR, 1.15; 95% CI, 1.10-1.21;

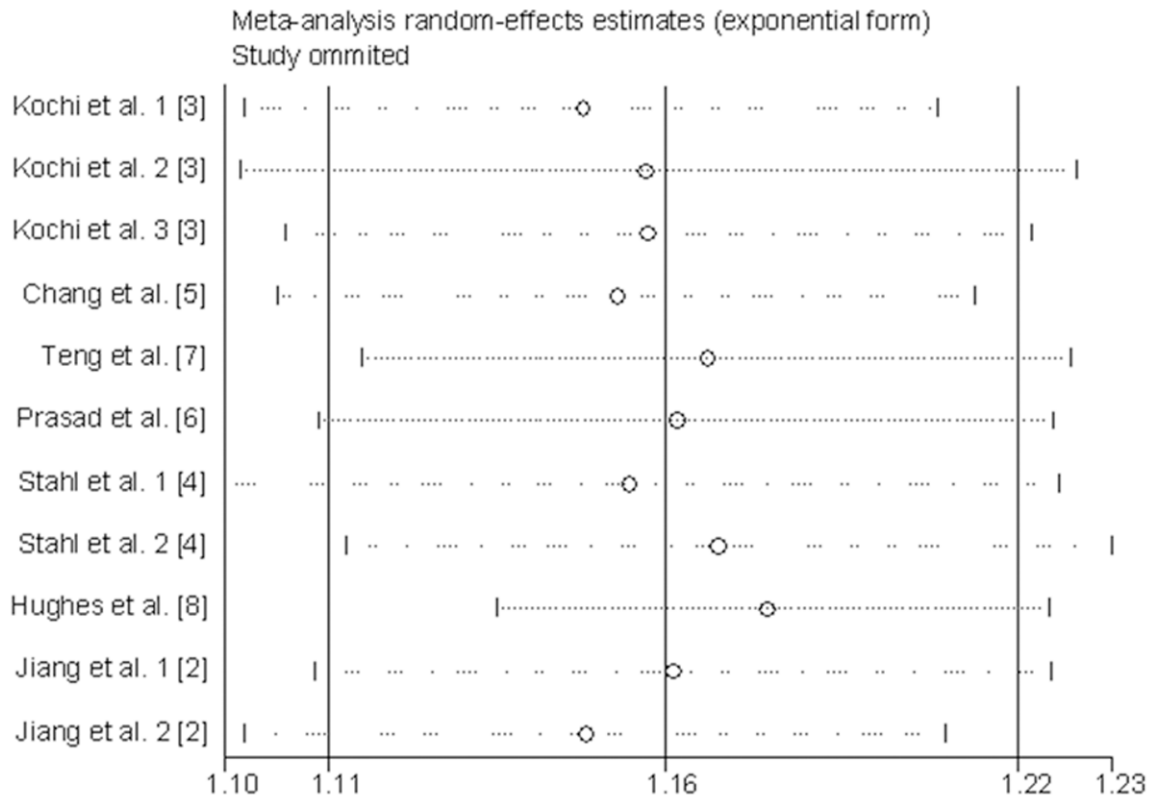

Figure 3. Sensitivity analyses of the CCP6 rs3093024 variant in an additive model by omitting one study at a time. The summary OR (95% CI) was indicated by each horizontal line when the labeled study was omitted, and the remaining studies were then reanalyzed.

P < 0.001) (**Figure 2**), but with a high level of heterogeneity (Q = 35.79; I^2 = 72.1%; *P* < 0.001). Interestingly, when considered separately by ethnicity, a contrasting effect of this variant on RA was observed (subgroup difference x^2 = 21.19; *P* < 0.001). The results from Asian studies indicated that the A-allele may be associated with an increased risk of RA (OR, 1.19; 95% CI, 1.14-1.24; *P* < 0.001), with moderate heterogeneity observed (Q = 11.22; I^2 = 37.6%; *P* = 0.129). The European studies also show similar trend (OR, 1.14; 95% CI, 1.08-1.21; *P* < 0.001), but have high level of significant heterogeneity (Q = 3. 83; I^2 = 70.4%; *P* = 0.066). Conversely, in African-Americans, the A-allele was associated with a decreased risk of RA (OR, 0.79; 95% CI, 0.62-0.96; *P* = 0.015). Given the limited sample size of African American studies, the current meta-analysis may be under-powered to draw conclusive insight into this discrepancy.

Sensitivity analysis and publication bias

Sensitivity analysis (exclusion of 1 study at a time) indicated that no single study changed the pooled ORs qualitatively (**Figure 3**), which suggested that the results of the meta-analysis were reliable. Egger's test suggested no publication bias in the current meta-analysis (*P* = 0.662), and the shape of the funnel plots appeared symmetrical (**Figure 4**). Thus, publication bias likely does not have a significant influence on the result of this meta-analysis.

Discussion

The present meta-analysis included 7 articles representing 11 studies on single-nucleotide polymorphisms of CCR6 (rs3093024, rs3099023 and rs1854853) among 81084 subjects. The overall data analysis demonstrated that CCR6 is a susceptibility gene for RA across populations, with an overall OR for the risk allele of 1.15. However, high heterogeneity (I^2 = 72.1%, *P* < 0.001) leads to an underpowered result. To make these conclusions more complete and reliable, subgroup analyses were conducted based on ethnic populations. While CCR6 was a risk factor in Asians (OR = 1.19, 95% CI: 1.14-1.24) and Europeans (OR = 1.14, 95% CI: 1.08-

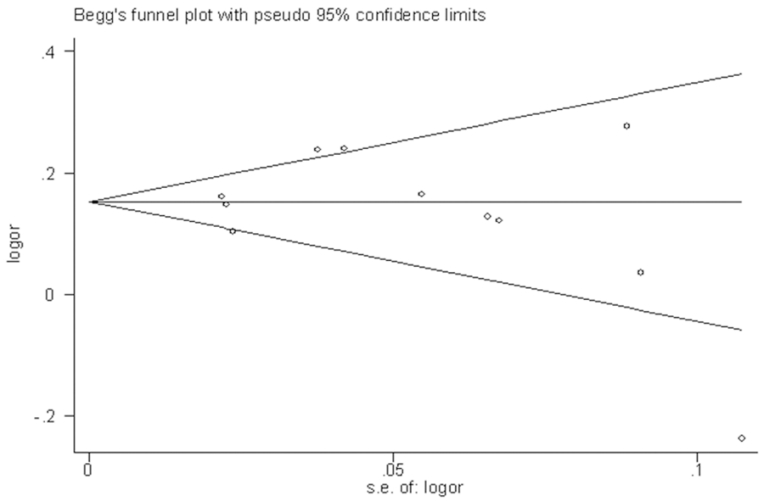

Figure 4. Begg's funnel plot of studies on the association between CCP6 rs3093024 variant and RA risk. Each point represents a separate study for the indicated association.

1.21), it was indicated as a protective factor in African Americans (OR = 0.79, 95% CI: 0.62-0.96). Therefore, population groups may be the main cause of the overall high heterogeneity.

The inconsistent OR of African Americans might involve several factors. First, this may be due to weak correlations between the causal allele and actual risk of RA, according to the International Haplotype Mapping Project (HapMap) [17]. What's more, Dickson.S.P et al [18] use simple computer simulations to show us that uncommon or rare genetic variants can easily create synthetic associations that are credited to common variants in some conditions, which needs to be considered seriously in GWAS. Second, genetic heterogeneity may account for the difference; the Asian and European risk allele can function differently in the effect on RA risk for African-Americans. For instance, Johanna Hadler et al [19] suggest that the association of the same SNP with the specific disease varies across ancestor groups, but association was seen in different groups with different SNPs at the same loci. The different associations may also be caused by genetically distinct subsets through some unknown ways, which are still not explored by researchers [19, 20]. Third, the inconsistency may simply be accidental. There is only one study included in the assay with limited cases and controls, so the weak association in African-Americans population needs to be further validated by including some additional studies. To sum up, the subgroup analysis conclusions are reliable to an extent, and further experiments with more individuals in different genetic background are necessary for a better understanding of possible explanations.

In recent studies, similar to the Th17 cell differentiation transcription factor RORγt, CCR6 is considered as a marker for Th17 cells, which is a novo subset of $CD4^+$ T cell [21, 22]. Upregulation of RORγt induces both IL-17 and CCR6 in naive T cells, blocking CCR6 with monoclonal antibody mainly suppressed arthritis in mouse model [22]. Haas, J. D and his colleagues also proved that only $CCR6^+$ δγ T cells produced IL-17A and $CCR6^+$ δγT cells are more responsive to TCR stimulation [23]. In innate immune responses to microbial stimulation, the CCR6/CCL20 chemokine loop increase B cells rapidly through a TNF-α dependent pathway [24]. Except for T cells and B cells, the chemoattraction of another important player in RA, dendritic cells, was also involved CCR6. All of these suggest CCR6 as a susceptibility gene for the aberrant immune environment in RA [24-26].

The relationship between CCR6 and RA has been discussed certainly, which would lead us to enrich the treatment for RA targeting CCR6. Despite the efficacy of specific blockade of CCR6 on RA has only been tested in an animal model so far [22, 27], the development of chronic IBD was found to be inhibited through regulating CCR6 biological function, and the alteration of CCR6 uses by viruses may influence the susceptibility of $CD4^+$ $CCR6^+$ T-cells and dendritic cell subsets in vivo [27, 28]. These findings all indicate a promising role for CCR6 in ameliorating RA. In humans, the majority of circulating Th17 cells expresses CCR6 [21, 22], and its ligand, CCL20, is also detected in inflamed synovial tissues [29, 30]. Therefore, the efficacy and safety of long-term blockade of CCR6 in treating RA are promising and warrant further investigation.

Several limitations of the current meta-analysis should be noted. First, although we included all articles on the susceptibility of CCR6 (rs3093024, rs3099023 and rs1854853) on RA, this was a total of only 11 studies. Second, the overall study was underpowered because of high heterogeneity, but the subgroup results show us a positive association. Future studies of large, well-characterized cohorts of different racial groups are necessary to better understand this association. We believe that detailed genetic studies of CCR6 with RA will lead to important insights into the pathogenesis of this disease. Additionally, the difference in associations between females and males is worthy of further discussion and analysis (data not shown).

In conclusion, pooled results for 81804 subjects demonstrated a significant association between CCR6 and RA. However, the subgroup analysis confirmed different effects of CCR6 on RA within different racial groups, and the meta-analysis results of associations of these SNPs with RA are required for further evaluation in larger samples from African American populations.

Acknowledgements

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Disclosure of conflict of interest

None.

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Table S1. Detailed criteria for assessing study quality of case-control studies

Criteria	Score
Selection	
Is the case definition adequate?	
Yes, with independent validation	2
Yes, eg medical record linkage or based on self-reports	1
No description	0
Representativeness of the Cases	
Consecutive or obviously representative series of cases	1
Potential for selection biases or not stated	0
Selection of Controls	
Community controls	2
Hospital controls	1
No description	0
Definition of Controls	
No history of RA	1
No description of source	0
Comparability	
Comparability of cases and controls on the basis of the design or analysis	
Study controls for any confounding risk factors	1
Study controls for no confounding risk factors	0
Exposure	
Same method of ascertainment for cases and controls	
Yes	1
No	0