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RESEARCH ARTICLE

Association of rs4552569 and rs17095830 single-nucleotide polymorphisms with susceptibility to ankylosing spondylitis in east Asian population: a meta-analysis

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Abstract. Several studies have been conducted in east Asian population to evaluate the association between rs4552569 and rs17095830 single-nucleotide polymorphisms (SNPs) with susceptibility to ankylosing spondylitis (AS), but the outcomes are inconsistent. A summary evaluation of the evidence supporting the associations has not been performed. Therefore, we performed this meta-analysis to access whether the two SNPs are related to ankylosing spondylitis. We systematically searched PubMed, EMBASE, Web of Science and Cochrane Library for papers published up until 3 February 2017, to obtain relevant studies using our research strategy. The allele/genotype frequencies were extracted from each study. We calculated the summary odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the associations between the two SNPs and AS risk. Four papers including five studies were obtained for this meta-analysis. The included studies suggested that there was no significant association between rs4552569 SNP and AS (C vs T, OR = 1.08, 95% CI: 0.96–1.22, $P = 0.20$). With regard to rs17095830 SNP, significant association was observed (G vs A, OR = 1.19, 95% CI: 1.06–1.33, $P = 0.002$). Based on a comprehensive analysis of the currently available evidence, rs4552569 SNP is not significantly associated with the predisposition of AS, while rs17095830 SNP is likely a susceptibility variant for AS in east Asian population. Further studies with different population groups are needed to confirm these potential associations.

Keywords. ankylosing spondylitis; single-nucleotide polymorphism; rs4552569; rs17095830; meta-analysis.

Introduction

Ankylosing spondylitis (AS) is a common chronic inflammation disease, which usually affects the sacroiliac joints, lumbar spine, and peripheral joints; leads to functional and structural impairments (Braun and Sieper 2007). The estimated prevalence of this disease ranges from 0.2 to 0.54% in Chinese population (Zeng *et al.* 2008), similar to white population (Braun *et al.* 1998). Genetic factors have been involved in the aetiology of AS (Robinson and Brown 2012), though precise pathogenesis has not been completely clarified. It is well known that AS is firmly linked to human leukocyte antigen (HLA)-B27 gene (Brewerton *et al.* 1973). However, studies of dizygotic and monozygotic twins suggest that HLA-B27 accounts for no more than 16% of overall AS risk (Khan and Ball 2002), which indicates that nonHLA-B27 genes also contribute to the risk of AS (Laval *et al.* 2001). Accumulating evidence suggests that other genetic factors such as IL-23R, GRP-35, ERAP1 and ERAP2 also play an important role in AS pathogenesis (Brown *et al.* 2016).

Recent studies have shown that new bone formation, together with inflammation, contribute greatly to AS (Sieper *et al.* 2008). Hyaluronan and proteoglycan link protein 1 (HAPLN1), also known as cartilage link protein 1 (CRTL1), is a pivotal constituent of the cartilage extracellular matrix (Dudhia *et al.* 1994; Watanabe and Yamada 1999; Matsumoto *et al.* 2006). Urano *et al.* (2011) found that HAPLN1 polymorphisms were linked to the pathogenesis of osteoarthritis with spinal degeneration. Epidermal growth factor-like repeats and discoidin-I-like domains 3 (EDIL3), a secreted protein localized to the basement membrane and the extracellular membrane, was reported to be regulating bone formation (Aoka *et al.* 2002;

Glantschnig *et al.* 2010; Takai *et al.* 2010). Anoctamin 6 (ANO6) is a membrane protein that has been associated with phosphatidylserine exposure, which is involved in osteoclast formation in bone tissues (Hartzell *et al.* 2009; Suzuki *et al.* 2010).

Two novel susceptibility loci, one between *HAPLN1* and *EDIL3* gene (rs4552569), and the other within *ANO6* gene (rs17095830), were first identified to be associated with the risk of AS in Han Chinese by Lin *et al.* (2012), through a two-stage genomewide association study (GWAS). However, the results were not replicated in the following studies among the east Asian population (Cortes *et al.* 2013; Wei *et al.* 2013; Wang *et al.* 2016). Due to limited sample size of individual studies and conflicting outcomes, we performed this meta-analysis to provide a comprehensive estimation of the associations of rs4552569 and rs17095830 single-nucleotide polymorphisms (SNPs) with the risk of AS, so as to find out precise associations between them.

Materials and methods

This meta-analysis was based on the preferred reporting items for systematic review and meta-analyses (PRISMA) criteria (Moher *et al.* 2009). An unpublished protocol was prepared for internal comment.

Search strategy

Two independent reviewers conducted a systematic search for relevant available articles published in PubMed, EMBASE, Web of Science and Cochrane Library using the following keyword search string: HAPLN1 or EDIL3 or ANO6; single-nucleotide polymorphism or polymorphism or variant; and ankylosing spondylitis or AS. Reference lists of included studies were reviewed for additional relevant studies. Literature search was performed without any restriction on language or publication date in our study. The last electronic search was conducted on 3 February 2017.

Inclusion/exclusion criteria

Qualified studies were those that met the following inclusion criteria in the current meta-analysis: (i) case–control study; (ii) evaluation of the associations between HAPLN1–EDIL3 (rs4552569) or ANO6 (rs17095830) polymorphism and the risk of AS; (iii) adequate data provided to calculate ratios (ORs) and 95% confidence intervals (CIs); (iv) AS diagnosis by the modified New York criteria (van der Linden *et al.* 1984). Correspondingly, the exclusion criteria were: (i) reviews, comments, or animal studies; (ii) family-based design studies; (iii) studies with no detailed genotype data. Study selection was achieved by two reviewers independently with the inclusion and exclusion criteria. Any disagreement between reviewers was settled by discussion until consensus was achieved.

Data extraction

The following data were extracted from the included studies: (i) author; (ii) publication year; (iii) country or region where the study was carried out; (iv) study design; (v) case and control selection criteria; (vi) allele/genotype frequencies; (vii) genotyping method. Any discrepancy in data retrieval was solved by discussion.

Methodological quality assessment

Two independent authors assessed the quality of the included studies using a modified version of the Newcastle–Ottawa scale (NOS). The NOS score ranged from 0 to 9 stars, and studies with a score of five or more stars were deemed of high methodological quality. Any discrepancy between two reviewers was settled by discussion until consensus was achieved.

Statistical analysis

Meta-analysis was performed using Review Manager 5.3 software (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark). The associations of rs4552569 and rs17095830 SNPs with AS were estimated by calculating pooled ORs and 95% CIs. Heterogeneity among studies was assessed using chi-square and I^2 statistic. The fixed-effect model was adopted to evaluate the pooled OR when the value of I^2 was less than 50% (Higgins *et al.* 2003). While, $I^2 \geq 50\%$ indicated a high degree of between-study heterogeneity (Higgins and Thompson 2002) and the random-effect model was adopted (DerSimonian and Laird 1986). Hardy–Weinberg equilibrium (HWE) was examined in control groups using the goodness-of-fit chi-square test. Evidence of publication bias was detected by funnel plots (Ioannidis and Trikalinos 2007). Each study was removed in turn to test the effect of the respective data, and then erased the study of high heterogeneity. To adjust for multiple testing, we utilized the Benjamini–Hochberg (BH) method (Benjamini and Hochberg 1995) and step-down Bonferroni method (Holm 1979), which mainly control the false discovery rate (FDR).

Results

Study characteristics

Based on the combined search strategy, 19 articles were obtained. Following deduplication, nine articles were

Table 1. Quality assessment of studies included in the meta-analysis on the associations of the HAPLN1-EDIL3 (rs4552569) and ANO6 (rs17095830) SNP with AS using a modified Newcastle–Ottawa scale.

Study	Lin *et al.* (2012)	Wei *et al.* (2013)	Cortes *et al.* (2013)	Wang *et al.* (2016)
Selection				
Adequacy of case definition	With independent validation (a) ⋆	With independent validation (a) ⋆	With independent validation (a) ⋆	With independent validation (a) ⋆
Representativeness of the cases	Consecutive or obviously representative series of cases (a) ⋆	Consecutive or obviously representative series of cases (a) ⋆	Consecutive or obviously representative series of cases (a) ⋆	Consecutive or obviously representative series of cases (a) ⋆
Selection of controls	Not hospital or community controls and genetic polymorphism of interest in HWE (c)	Hospital controls and genetic polymorphism of interest in HWE (b)	Not hospital or community controls and genetic polymorphism of interest in HWE (c)	Community controls and genetic polymorphism of interest in HWE (a) ⋆
Definition of controls	No history of AS (a) ⋆	No history of AS (a) ⋆	No history of AS (a) ⋆	No history of AS (a) ⋆
Comparability				
Case and control of homogeneous ethnic descent	Homogeneous ethnic descent (a) ⋆	Homogeneous ethnic descent (a) ⋆	Homogeneous ethnic descent (a) ⋆	Homogeneous ethnic descent (a) ⋆
Population stratification	Han Chinese (a) ⋆	Taiwanese (a) ⋆	Not reported (c)	Han Chinese (a) ⋆
Exposure				
Ascertainment of exposure	Quality control procedures; no report of blinding (b)	Quality control procedures; no report of blinding (b)	Not reported (c)	Not reported (c)
Same method of ascertainment for cases and controls	Yes (a) ⋆	Yes (a) ⋆	Yes (a) ⋆	Yes (a) ⋆
Genotyping call rate	>99% (a) ⋆	Not reported (b)	Not reported (b)	Not reported (b)
Total	⋆⋆⋆⋆⋆⋆⋆ (n = 7)	⋆⋆⋆⋆⋆⋆ (n = 6)	⋆⋆⋆⋆⋆(n = 5)	⋆⋆⋆⋆⋆⋆⋆(n = 7)

AS, ankylosing spondylitis; HWE, Hardy–Weinberg equilibrium; (a), (b), (c) signify corresponding options. The explanation of each column of the Newcastle–Ottawa scale is available at http://www.ohri.ca/programs/clinical_epidemiology/nos_manual.pdf.

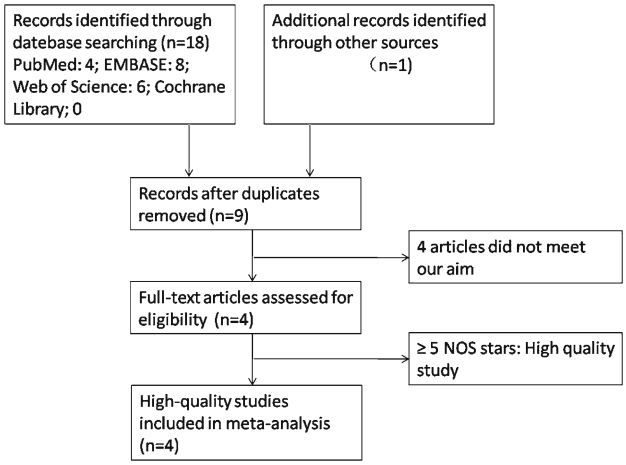


Figure 1. Flow diagram for study selection process in the meta-analysis.

reserved for screening. After reading the full-text, four articles (Lin *et al.* 2012; Cortes *et al.* 2013; Wei *et al.* 2013; Wang *et al.* 2016) of high methodological quality (≥5 NOS stars) including five studies were obtained for this meta-analysis. Quality assessment of the included studies is presented in table 1. The study selection process is summarized in figure 1. Five studies all reported both alleles and genotypes of rs4552569 and rs17095830 SNPs. For the rs4552569 SNP meta-analysis, 6829 AS cases and 11484 controls were included, whereas, there were 6836 AS cases and 11441 controls involved in the rs17095830 SNP meta-analysis. In all studies, the control groups were in HWE. Characteristics of included studies are presented in table 2. Allele/genotype frequencies are summarized in table 3.

Meta-analysis results

Overall, this meta-analysis suggested that there was no significant association between rs4552569 SNP and AS for the allelic and genotypic comparisons (C vs T, OR = 1.08, 95% CI: 0.96–1.22, P = 0.2000, figure 2; CT vs TT, OR = 1.12, 95% CI: 0.97–1.29, P = 0.13; CC vs TT, OR = 1.14, 95% CI: 0.91–1.43, P = 0.2700, figure 3; CT + CC vs TT, OR = 1.12, 95% CI: 0.96–1.30, P = 0.1600; CC vs CT + TT, OR = 1.10, 95% CI: 0.93–1.30, P = 0.2700, figure 4). With regard to rs17095830 SNP, significant associations under allele contrast, codominant and recessive models were observed (G vs A, OR = 1.19, 95% CI: 1.06–1.33, P = 0.0020, FDR = 0.0033 with P = 0.0060 in step down Bonferroni testing, figure 5; GA vs AA, OR = 1.17, 95% CI: 1.02–1.34, P = 0.02, FDR = 0.0250 with P = 0.0400 in step down Bonferroni testing; GG vs AA, OR = 1.67, 95% CI: 1.27–2.21, P = 0.0003, FDR = 0.0015, P = 0.0015 in step down Bonferroni testing, figure 6; GG vs GA + AA, OR = 1.62, 95% CI: 1.23–2.14, P = 0.0006, FDR = 0.0015, P = 0.0024 in step down Bonferroni testing, figure 7). However, no positive results under dominant model were detected (GG + GA vs AA, OR = 1.12, 95% CI: 0.89–1.43, P = 0.33; as indicated in table 4). The pooled ORs and 95% CIs are presented in table 4.

We conducted sensitivity analysis to evaluate the single study on the pooled ORs by omitting each study one by one from the pooled analysis. Although no HWE deviations were detected in Cortes *et al.* (2013) study, which including three different ethnicities, their study was likely to account for the between-study heterogeneity greatly. For the rs4552569 SNP, we found that the overall statistical significance of allele contrast reversed when Cortes *et al.* (2013) study was removed. Also, the between-study heterogeneity index I^2 changed from 83% to 49% after removal. For the rs17095830 SNP, the pooled effect did not change fiercely after excluding Cortes *et al.* (2013) study, but no overt between-study heterogeneity was observed for both allelic and genotypic comparisons. The insufficient number of studies ($n \leq 10$) did not allow for meaningful funnel plots to evaluate publication bias (Ioannidis and Trikalinos 2007) thus, we did not perform the funnel plots for publication assessment.

Discussion

Nowadays, the precise aetiology and pathogenesis of AS remain ambiguous. Several studies have suggested that genetic risk factors contribute greatly to AS. For rs4552569 and rs17095830 SNPs, a two-stage study in Han Chinese from Lin *et al.* (2012) firstly demonstrated that the genetic polymorphisms were likely to be associated with AS. In addition, Cortes *et al.* (2013) performed the SNP genotyping of the two loci among east Asians (Chinese, Taiwanese and Koreans) but found a negative correlation. The other two studies from Wei *et al.* (2013) and Wang *et al.* (2016), with relatively small sample size also indicated that there was no obvious relationship between the two loci and AS. Thus, we conducted this meta-analysis to make a more precise assessment of the associations between rs4552569 and rs17095830 SNPs and susceptibility to AS. The present meta-analysis showed a nonsignificant association between the rs4552569 SNP and AS. For rs17095830 SNP, nevertheless, the AS cases had a higher frequency of both G allele and GG genotype. Besides, both of Wei *et al.* and Wang *et al.*'s studies indicated that rs17095830 polymorphism was associated with the inflammatory bowel disease (IBD) in AS patients, but neither rs4552569 nor rs17095830 polymorphism had a relationship with AS severity (Wei *et al.* 2013; Wang *et al.* 2016).

It is noteworthy that Lin *et al.*'s study is in conflict with the other studies. We think the following factors may have contributed to this inconsistent. First, both Wang *et al.* and Wei *et al.*'s studies indicate that rs17095830 is associated with IBD. The enrichment of individuals with IBD in Lin *et al.*'s study provides a potential explanation

Table 2. Characteristics of studies included in the meta-analysis.

Study	Year	Genotyping method	Case characteristics		Control characteristics		Subject selection criteria
			rs4552569	rs17095830	rs4552569	rs17095830	
Lin *et al.*	2012	Illumina Human 610-Quad BeadChips, OmniExpress BeadChips	Stage I: *n* = 1837 AS cases Han Chinese Stage II: *n* = 2100 AS cases Han Chinese	Stage I: *n* = 1826 AS cases Han Chinese Stage II: *n* = 2080 AS cases Han Chinese	Stage I: *n* = 4230 AS cases Han Chinese Stage II: *n* = 3493 AS cases Han Chinese	Stage I: *n* = 4211 AS cases Han Chinese Stage II: *n* = 3469 AS cases Han Chinese	Cases: diagnosed with AS by at least two rheumatologists according to the 1984 modified New York criteria Controls: healthy normal people or patients without AS
Wei *et al.*	2013	PCR-TaqMan	*N* = 470 AS cases; mean age at 39 years; Taiwanese	*N* = 472 AS cases; mean age at 39 years; Taiwanese	*N* = 439 controls; Taiwanese	*N* = 444 controls; Taiwanese	Cases: (1) Patients aged 16–65 years; (2) cognitive performance not influence by other diseases such as dementia; (3) AS diagnosis by the modified New York criteria; (4) AS diagnosed by a qualified rheumatologist and sacroilitis was confirmed by a qualified radiologist; (5) AS patients with sacroilitis Controls: selected patients with no significant medical histories or abnormal laboratory results
Cortes *et al.*	2013	TaqMan probes	*N* = 1687 AS cases; East Asian	*N* = 1723 AS cases; East Asian	*N* = 2118 controls; East Asian	*N* = 2113 AS controls; East Asian	Cases: all case had definite AS according to the modified New York criteria; Controls: healthy individuals
Wang *et al.*	2016	PCR-HRMA	*N* = 735 AS cases (576 males and 159 females); Han Chinese		*N* = 1204 controls; (732 males and 472 females); Han Chinese		Cases: (1) diagnosis by 1984 modified New York criteria; (2) AS diagnosed by a qualified rheumatologist and sacroilitis was confirmed by a qualified radiologist; (3) AS patients with definite sacroilitis; Controls: healthy normal people

AS, ankylosing apondylitis; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction; HRMA, high resolution melting analysis.

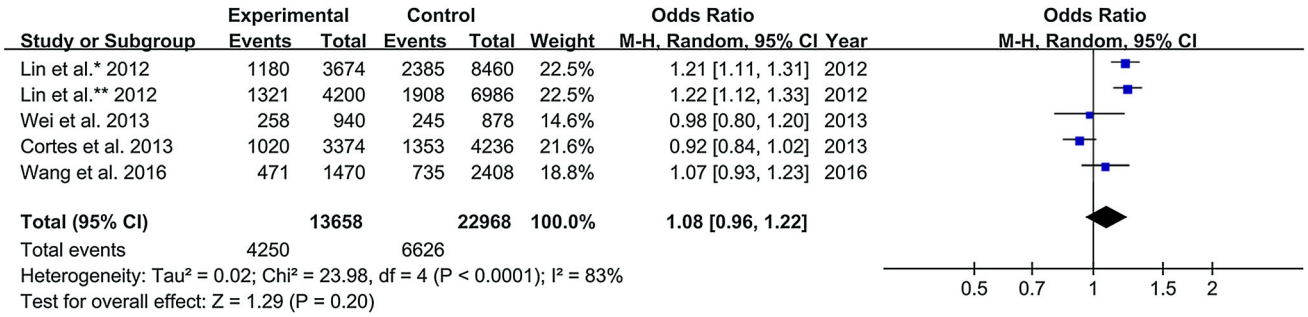


Figure 2. Forest plot of the association between rs4552569 SNP and AS risk for the allelic comparison (C vs T).

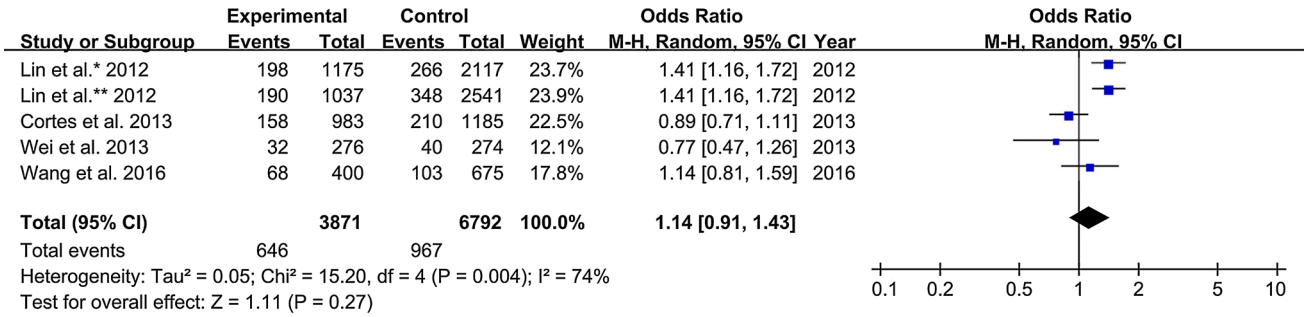


Figure 3. Forest plot of the association between rs4552569 SNP and AS risk for the genotypic comparison (CC vs TT).

Table 3. Genotype distributions of studies included in the meta-analysis

Study	Year	Country/region	Gender	Case			Control			HWE
				MM	MW	WW	MM	MW	WW	
rs4552569 (C/T)										
Lin *et al.**	2012	China	Both	190	800	847	348	1689	2193	0.369
Lin *et al.***	2012	China	Both	198	925	977	266	1376	1851	0.643
Wei *et al.*	2013	Taiwan	Both	32	194	244	40	165	234	0.168
Cortes *et al.*	2013	East Asia	NR	158	704	825	210	933	975	0.544
Wang *et al.*	2016	China	Both	68	335	332	103	529	572	0.213
rs17095830 (G/A)										
Lin *et al.**	2012	China	Both	28	402	1396	37	747	3427	0.597
Lin *et al.***	2012	China	Both	31	428	1621	31	600	2838	0.909
Wei *et al.*	2013	Taiwan	Both	6	84	382	5	67	372	0.319
Cortes *et al.*	2013	East Asia	NR	28	359	1336	21	462	1630	0.061
Wang *et al.*	2016	china	Both	9	163	563	11	241	952	0.319

*, Stage I study; **, stage II study; NA, not reported; M, mutant allele; W, wild allele; HWE, Hardy-Weinberg equilibrium.

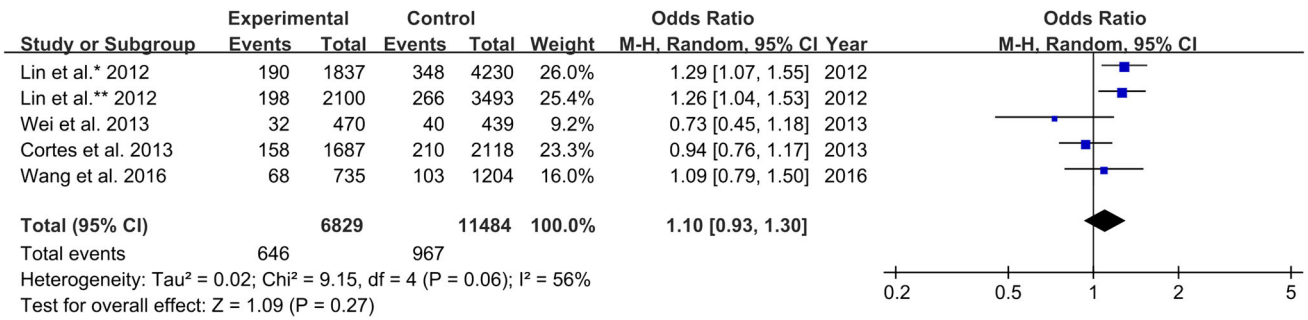


Figure 4. Forest plot of the association between rs4552569 SNP and AS risk for the genotypic comparison (CC vs CT + TT).

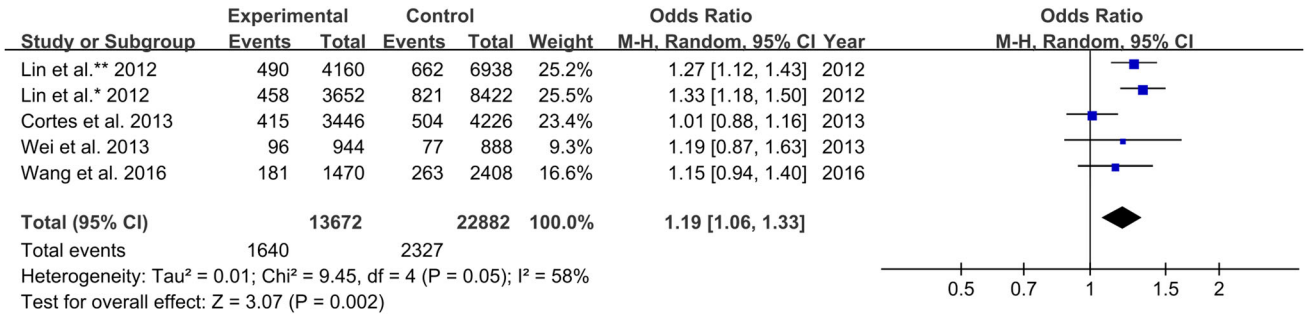


Figure 5. Forest plot of the association between rs17095830 SNP and AS risk for the allelic comparison (G vs A).

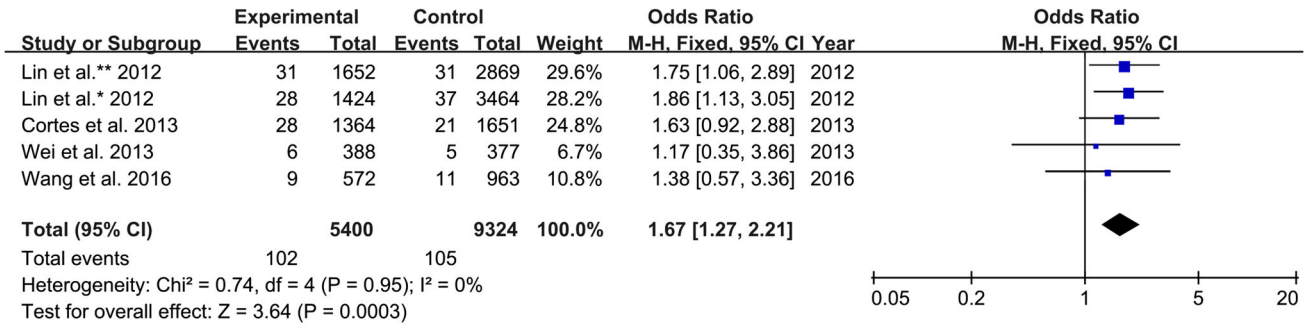


Figure 6. Forest plot of the association between rs17095830 SNP and AS risk for the genotypic comparison (GG vs AA).

Table 4. Results of genetic models on the association of rs4552569 and rs17095830 with AS.

Comparison	Analysis model	OR (95% CI)	*P* value	I^2 (%)	Bon	FDR
rs4552569						
C vs T	REM	1.08 (0.96,1.22)	0.2000	83	0.6000	0.2700
CT vs TT	REM	1.12 (0.97,1.29)	0.1300	78	0.6500	0.2700
CC vs TT	REM	1.14 (0.91,1.43)	0.2700	74	0.5400	0.2700
CT + CC vs TT	REM	1.12 (0.96,1.30)	0.1600	83	0.6400	0.2700
CC vs CT + TT	REM	1.10 (0.93,1.30)	0.2700	56	0.5400	0.2700
rs17095830						
G vs A	REM	1.19 (1.06,1.33)	0.0020	58	0.0060	0.0033
GA vs AA	REM	1.17 (1.02,1.34)	0.0200	64	0.0400	0.0250
GG vs AA	FEM	1.67 (1.27,2.21)	0.0003	0	0.0015	0.0015
GG + GA vs AA	REM	1.12 (0.89,1.43)	0.3300	89	0.3300	0.3300
GG vs GA + AA	FEM	1.62 (1.23,2.14)	0.0006	0	0.0024	0.0015

REM, random-effect model; FEM, fixed effect model; OR, odds ratio; CI, confidence interval; Bon *P* value in step-down Bonferroni testing; FDR *P* value from Benjamini–Hochberg method control for false discovery rate (FDR).

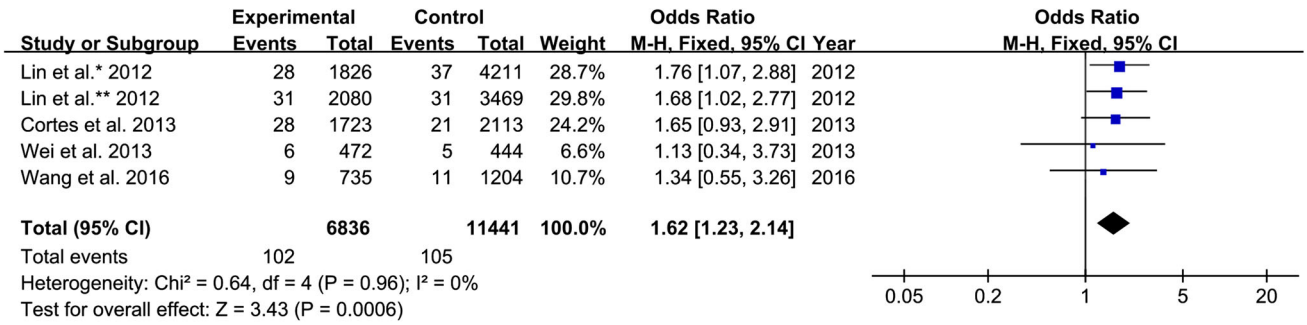


Figure 7. Forest plot of the association between rs17095830 SNP and AS risk for the genotypic comparison (GG vs GA + AA).

for the inconformity between various studies. Besides, geographical and environmental diversities might as well contribute to the variable results. What is more, diverse techniques and processes of genotyping together with the source of control subjects are also significant contributors to these controversial results.

Despite comprehensive and strict analyses with large sample size were performed to detect the association of rs4552569 and rs17095830 SNPs with AS, some limitations should be acknowledged. Initially, this meta-analysis included studies from only east Asian population, and no other population were involved. Besides, we could not assess the summary relationship between the two loci and AS activity or clinical features due to insufficient data. Further, because the included studies were less than 10, we could not evaluate the publication bias, with the result that publication bias may exist potentially. In addition, the high heterogeneity among different studies for rs4552569 cannot be ignored. Finally, because males are more susceptible to AS, but not all studies report the gender distribution in detail, the total consequences may be influenced.

The reporting quality among the included studies was relatively lacking, but adequate data was presented to support a calculation of the summary methodological quality. Four articles including five studies were evaluated by the modified NOS and judged with high methodological quality. HWE is a principle to examine whether observed allele/genotypic frequencies between parents and their offspring are in equilibrium. Population stratification, selection bias and genotyping errors may bring about the deviation from HWE in control subjects, and the deviation may affect the summary consequences. All studies were consistent with HWE in both case and control groups in our study. For rs4552569 SNP allelic contrast meta-analysis, Cortes *et al.* (2013) study caused the high between-study heterogeneity, and the total consequence reversed with the study exclusion. However, rs17095830 SNP meta-analysis results were relatively stable. Gender discrepancy existed in AS incidence was still with less understood, but it may cause a difference of AS susceptibility. The five studies included in our meta-analysis did not take gender discrepancy into account. Future studies should consider the problem.

In conclusion, the current meta-analysis illustrated that there is a lack of relationship between rs4552569 SNP and AS in east Asian population. But, there is a significant association between rs17095830 SNP and AS. Moreover, neither rs4552569 SNP nor rs17095830 SNP appears to be associated with the severity of AS. Populations beyond east Asians need to be explored in subsequent studies.

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