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Review Article

Association between CALM1 gene polymorphisms and osteoarthritis risk: a systematic review and meta-analysis

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Abstract: Objective: The results of the association between CALM1 gene polymorphisms and osteoarthritis (OA) have been inconsistent. Our aim was to determine whether CALM1 rs12885713 and rs3213718 polymorphisms are associated with susceptibility to OA. Methods: PubMed, Embase, and ISI Web of Science databases were searched to identify relevant studies. Pooled odds ratios (ORs) with a 95% confidence interval (CI) were calculated to assess the association between CALM1 polymorphisms and OA susceptibility. We also conducted subgroup analysis stratified by ethnicity, OA site, and gender. Results: Five studies with 5051 participants (2292 OA patients and 2759 controls) were enrolled in this study. The combined results revealed no significant association between CALM1 rs12885713 polymorphism and OA risk (allele model: OR 1.09, 95% CI 0.98-1.21; dominant model: OR 1.10, 95% CI 0.93-1.30; recessive model: OR 1.41, 95% CI 0.88-2.26; homozygote model: OR 1.44, 95% CI 0.91-2.29; heterozygote model: OR 1.03, 95% CI 0.86-1.23). Subgroup analysis stratified by ethnicity suggested that TT genotype was associated with increased risk of OA in Asians (recessive model: OR 2.21, 95% CI 1.39-3.50; homozygote model: OR 2.20, 95% CI 1.37-3.54), but not in Caucasians. The pooled results revealed no significant association between CALM1 rs3213718 polymorphism and the risk of OA in the overall population or in each subgroup population. Conclusion: This meta-analysis suggested the TT genotype of CALM1 rs12885713 polymorphism significantly increased the risk of OA in Asians. In contrast, CALM1 rs3213718 polymorphism was not associated with OA risk. Due to the limitations of our study, further well-designed studies are required.

Keywords: CALM1, polymorphism, osteoarthritis, meta-analysis

Introduction

Osteoarthritis (OA; MIM165720) is a common musculoskeletal disease and a leading cause of disability among the elderly populations [1]. The hallmark of OA is progressive degradation of articular cartilage, accompanied with subsequent joint space narrowing and osteophyte formation at the joint margin, leading to chronic joint pain, deformity and restricted motion [2, 3]. It was estimated that over 26 million adults in the US suffered from clinical OA of their hand, knee, or hip joint [4, 5]. OA is regarded as a multifactorial disorder, and multiple risk factors have been correlated with its onset and progression, including age, gender, obesity, joint trauma, environmental factors, and genetic factors [6-9].

OA results from an imbalance between the synthesis and degradation of extracellular cartilage matrix through mechanisms controlled by chondrocytes [2, 10, 11]. Ca^{2+}-calmodulin signaling plays a crucial role in chondrogenesis and normal cartilage phenotype maintaining and thus may be involved in the pathogenesis of OA [12]. The human CALM1 gene is located on chromosome 14q32.11 and encodes calmodulin, a ubiquitous eukaryotic Ca^{2+} binding protein and is a principal mediator of the calcium signal [13]. In chondroprogenitor cells, the Ca^{2+} signal regulates chondrogenic differentiation. During differentiation, the expression of COL2A1 and AGC1, which encode principal cartilage proteins, were up-regulated. Addition of calmodulin inhibitor suppressed expression of these genes, indicating that calmodu-

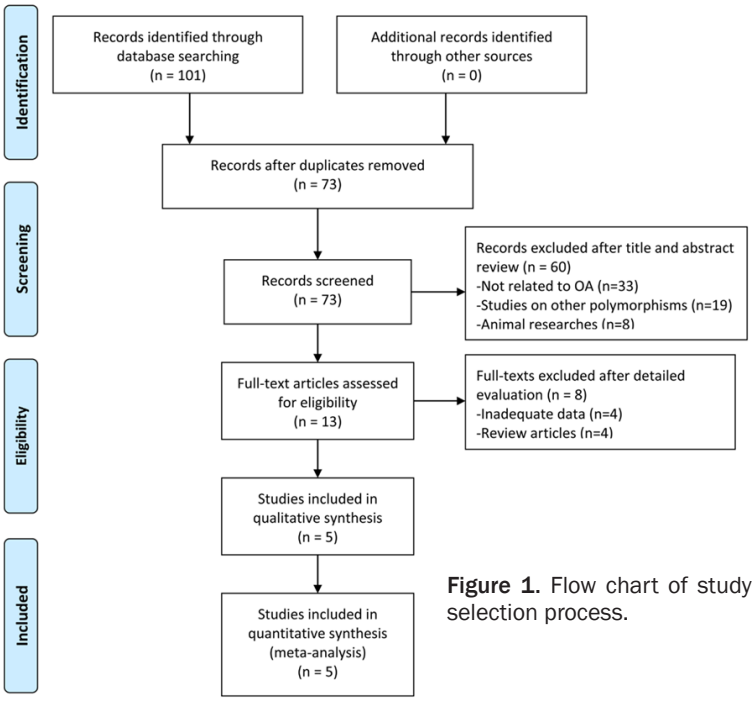


Figure 1. Flow chart of study selection process.

lin may be an important chondrogenic factor [14, 15]. Mechanical stimuli are essential in maintaining normal cartilage phenotype and function by triggering changes in aggrecan expression in mature chondrocytes, and such changes are dependent on Ca^{2+}-calmodulin signaling [16, 17]. Additionally, Ca^{2+}-calmodulin signaling also mediates cartilage repairing process by modulating adhesion of chondrocytes [18].

Several studies have been performed to investigate the association between CALM1 polymorphisms and OA susceptibility. Through a large-scale association study, Mototani et al. identified a functional single nucleotide polymorphism (SNP) (rs12885713) in the core promoter region of the CALM1 gene associated with a markedly increased prevalence of hip OA in a Japanese population [14]. However, subsequent replication studies failed to provide consistent results [19-21]. Several other SNPs (rs3213718, rs2300496, rs2300500, rs3179089) were also studied, but the results remained inconsistent and inconclusive [14, 22]. In the present study, we therefore performed a meta-analysis to investigate whether or not the CALM1 polymorphisms are associated with the risk of OA.

Materials and methods

Search strategy

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Table S1) [23]. We systematically searched PubMed, Embase, and ISI Web of Science databases to identify relevant studies published in English. The literature search strategy involved the combined use of the following keywords: ("CALM1" OR "calmodulin") AND ("polymorphism" OR "variant" OR "SNP") and ("osteoarthritis" OR "OA"). References of clinical trials and review articles were also hand-searched for additional eligible studies. The literature search was last updated on August 22, 2017.

Inclusion and exclusion criteria

The eligible studies met the following inclusion criteria: (1) Well-defined case-control study; (2) Patients with OA were diagnosed according to clinical and radiographic findings, or ascertained by total joint arthroplasty due to primary OA; (3) Evaluating the potential association between CALM1 polymorphisms and OA risk; (4) Sufficient data on genotype or allele frequency to calculate odds ratio (OR) and corresponding 95% confidence interval (CI). The criteria of exclusion were as follows: (1) Review articles or case reports; (2) Containing incomplete data; (3) Duplicate or overlapping publications. If several articles reported findings for overlapping study populations, only the most recent study or the one with the largest sample size was selected. All retrieved studies were reviewed independently by two researchers to determine eligibility for inclusion, and a third reviewer was introduced to resolve the discrepancies.

Data extraction

For each eligible study, the following data were extracted by two independent reviewers: (1)

Table 1. Characteristics of included studies

Author	Year	Country	Ethnicity	Study design	OA site	Studied SNPs	Genotyping method	Sample size (F/M)		Age		HWE	NOS
								OA	Control	OA	Control		
Mototani	2005	Japan	Asian	Case-control	Hip	rs12885713 rs2300496 rs2300500 rs3213718 rs3179089	TaqMan	428 (404/24)	1008 (491/517)	Mean 53.7	Mean 46.7	Y	7
Loughlin	2006	UK	Caucasian	Case-control	Hip	rs12885713	PCR-RFLP	920 (547/373)	752 (393/359)	64 (56-85)	69 (55-89)	Y	7
Valdes	2007	UK	Caucasian	Case-control	Knee	rs3213718	PCR-RFLP	603 (305/298)	596 (296/300)	F: 73.5±7.2 M: 72.1±6.9	F: 72.1±8.5 M: 71.8±7.8	Y	8
Poulou	2007	Greece	Caucasian	Case-control	Knee	rs12885713	PCR-RFLP	158 (138/20)	193 (137/56)	F: 68.1±8.2 M: 72.4±5.8	F: 68.0±10.9 M: 70.2±9.0	Y	8
Shi	2008	China	Asian	Case-control	Knee	rs12885713	PCR-RFLP	183 (124/59)	210 (142/68)	58.6±13.5	57.7±11.7	Y	9

F: Female; M: Male; HWE: Hardy-Weinberg equilibrium; NOS: Newcastle-Ottawa Quality Assessment Scale.

Table 2. Genotype distributions of CALM1 polymorphisms in the included studies

Author	Allele		Genotype distribution						Association findings
			OA			Control			
	1	2	11	12	22	11	12	22	
rs12885713 (promotor)									
Mototani	T	C	46	128	160	22	154	199	TT↑ in recessive model
Loughlin			296	478	146	245	381	126	NS
Poulou			38	80	36	37	103	46	NS
Shi			9	57	117	8	70	132	NS
rs3213718 (intron 3)									
Mototani	T	C	65	163	198	79	435	492	TT↑ in recessive model
Valdes			T vs. C, OR (95% CI): 0.87 (0.74-1.03)						NS
rs2300496 (intron 1)									
Mototani	C	A	46	129	159	23	155	197	CC↑ in recessive model
rs2300500 (intron 1)									
Mototani	G	C	47	128	159	23	156	196	GG↑ in recessive model
rs3179089 (exon 7)									
Mototani	G	C	45	131	158	20	160	195	GG↑ in recessive model

NS: Not significant; ↑: Increase the risk of OA.

Name of the first author; (2) Publication year; (3) Country and ethnicity of study population; (4) Study design; (5) OA sites; (6) Numbers of cases and controls; (7) Demographics of enrolled subjects; (8) Genotyping method; (9) Studied polymorphisms and genotype distributions.

Quality assessment

Two independent investigators assessed quality of the included studies using Newcastle-Ottawa Quality Assessment Scale (NOS) [24]. Each study scored from 0 point (worst) to 9 points (best), based on three categories: selection (4 items, 1 point each), comparability (1 item, up to 2 points), and exposure/outcome (3 items, 1 point each). Studies with a score of 7 or higher were defined as high quality, whereas studies scored 6 or less were classified as low quality.

Statistical analysis

The association between CALM1 polymorphisms with OA susceptibility was assessed by calculating pooled ORs with 95% confidence interval (CI). Five genetic models were analyzed: the allele model, dominant model, recessive model, homozygous model, and heterozygous model. Heterogeneity between studies was measured using Q and I^2 statistics [25]. If I^2 < 50% and *p*-value of Q statistic > 0.10, a fixed effect model was used to calculate pooled ORs and 95% CIs [26]. Otherwise, a random effects model was applied as the pooling method [27]. We also conducted subgroup analysis stratified by ethnicity, OA site, and gender. Sensitivity analysis was performed to evaluate the stability of pooled results by sequentially removing individual studies. Publication bias was evaluated using funnel plot and Egger's regression test. All statistical analyses were conducted using STATA version 12.0 (STATA Corporation, College Station, TX, USA), and *p*-value < 0.05 was considered significant except for the I^2 statistic.

Results

Study selection

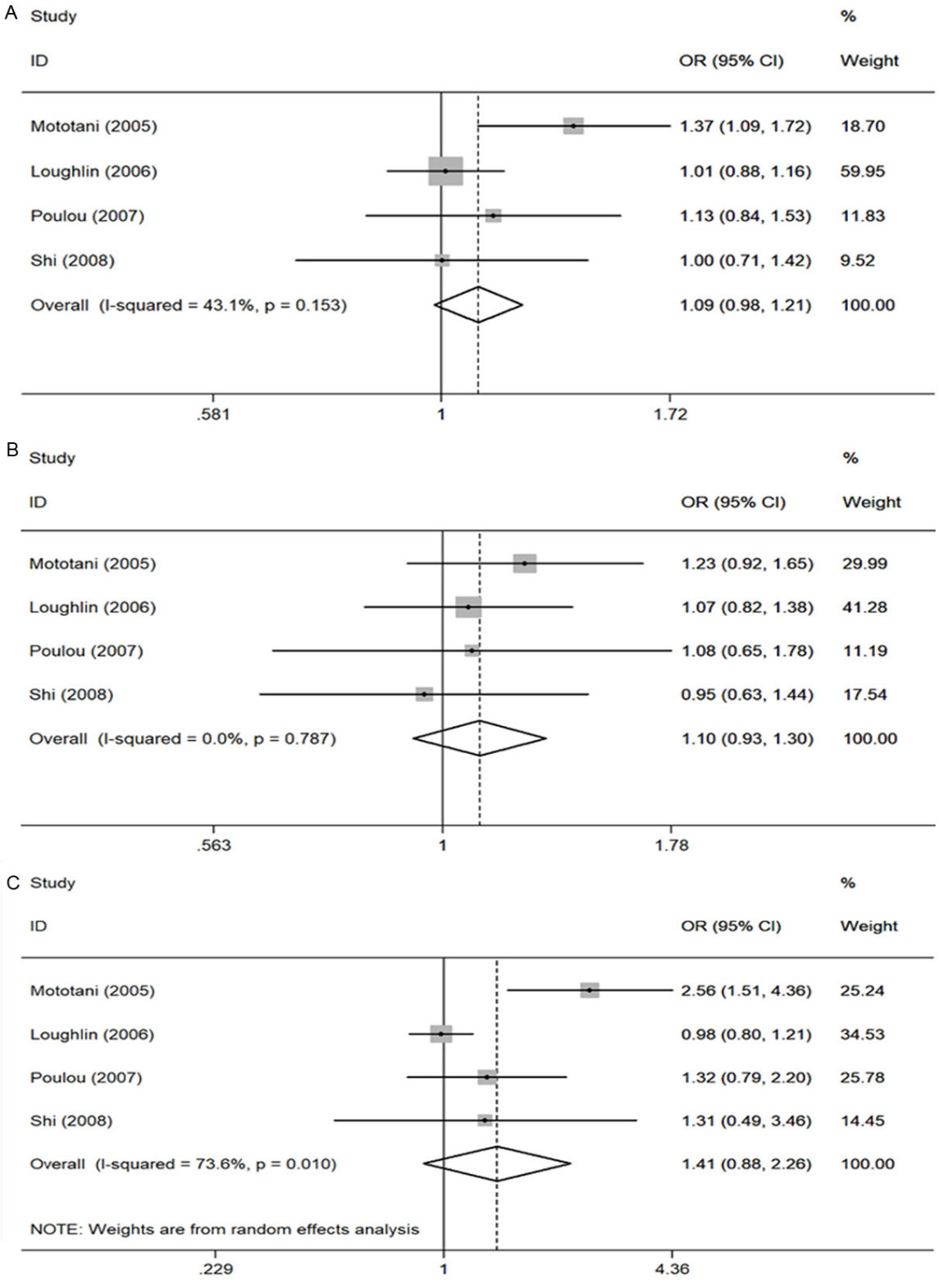
A flow diagram for the study selection process and reasons for exclusion is presented in **Figure 1**. A total of 101 articles were retrieved from a systematic literature search, and 73 articles remained for further screening after removing duplications. Sixty articles were excluded after title and abstract review, and 13 full-text articles remained for detailed evaluation. Afterwards, 4 articles were excluded due to inadequate data, and 4 review articles were also removed. Finally, 5 articles met the inclusion criteria [14, 19-22].

Table 3. Summary of pooled results on the association between CALM1 rs12885713 polymorphism and OA risk

Genetic model	Sub-group	No. of studies	Test of association		Statistical model	Test of heterogeneity		P_{Egger}
			OR (95% CI)	p		I^2 (%)	p	
Allele model (T vs. C)	Overall	4	1.09 (0.98-1.21)	0.105	Fixed	43.1	0.153	0.628
	Ethnicity							
	Asian	2	1.20 (0.89-1.63)	0.227	Random	54.1	0.140	
	Caucasian	2	1.03 (0.91-1.17)	0.647	Fixed	0.0	0.505	
	OA site							
	Hip OA	2	1.16 (0.86-1.56)	0.329	Random	79.9	0.026	
	Knee OA	2	1.07 (0.85-1.35)	0.545	Fixed	0.0	0.604	
Dominant model (TT+CT vs. CC)	Overall	4	1.10 (0.93-1.30)	0.274	Fixed	0.0	0.787	0.624
	Ethnicity							
	Asian	2	1.13 (0.89-1.43)	0.324	Fixed	0.0	0.327	
	Caucasian	2	1.07 (0.85-1.35)	0.571	Fixed	0.0	0.974	
	OA site							
	Hip OA	2	1.14 (0.93-1.38)	0.202	Fixed	0.0	0.480	
	Knee OA	2	1.00 (0.73-1.38)	0.989	Fixed	0.0	0.715	
Recessive model (TT vs. CT+CC)	Overall	4	1.41 (0.88-2.26)	0.159	Random	73.6	0.010	0.312
	Ethnicity							
	Asian	2	2.21 (1.39-3.50)	0.001	Fixed	29.5	0.234	
	Caucasian	2	1.02 (0.84-1.24)	0.819	Fixed	8.8	0.295	
	OA site							
	Hip OA	2	1.54 (0.60-3.93)	0.371	Random	90.8	0.001	
	Knee OA	2	1.32 (0.84-2.07)	0.236	Fixed	0.0	0.986	
Homozygote model (TT vs. CC)	Overall	4	1.44 (0.91-2.29)	0.124	Random	63.9	0.040	0.492
	Ethnicity							
	Asian	2	2.20 (1.37-3.54)	0.001	Fixed	35.7	0.212	
	Caucasian	2	1.09 (0.83-1.42)	0.540	Fixed	0.0	0.515	
	OA site							
	Hip OA	2	1.60 (0.65-3.90)	0.305	Random	88.0	0.004	
	Knee OA	2	1.30 (0.77-2.21)	0.332	Fixed	0.0	0.955	
Heterozygote model (CT vs. CC)	Overall	4	1.03 (0.86-1.23)	0.755	Fixed	0.0	0.936	0.214
	Ethnicity							
	Asian	2	0.99 (0.77-1.28)	0.951	Fixed	0.0	0.663	
	Caucasian	2	1.06 (0.83-1.36)	0.623	Fixed	0.0	0.773	
	OA site							
	Hip OA	2	1.06 (0.86-1.30)	0.572	Fixed	0.0	0.827	
	Knee OA	2	0.95 (0.68-1.32)	0.750	Fixed	0.0	0.823	

Characteristics of included studies

Table 1 presents the primary characteristics of included studies and **Table 2** lists the genotype distributions of each polymorphism. A total of 5051 participants with 2292 OA patients and 2759 controls were enrolled in this study, which involved 2 Asian and 3 Caucasian populations. OA patients were recruited according to clinical and radiographic findings, or ascertained by total joint arthroplasty. The genotype distribution of the control group in all the included studies conformed to Hardy-Weinberg equilibrium and quality of each study was fairly high (**Tables 1**, S2). Finally, a total of 4 studies [14, 19-21] and 2 studies [14, 22] were included in the meta-analyses for the associations between the CALM1 rs12885713 or rs3213718


A
Study
ID
OR (95% CI)
%
Weight
Mototani (2005)
1.37 (1.09, 1.72)
18.70
Loughlin (2006)
1.01 (0.88, 1.16)
59.95
Poulou (2007)
1.13 (0.84, 1.53)
11.83
Shi (2008)
1.00 (0.71, 1.42)
9.52
Overall (I-squared = 43.1%, p = 0.153)
1.09 (0.98, 1.21)
100.00
.581
1
1.72
B
Study
ID
OR (95% CI)
%
Weight
Mototani (2005)
1.23 (0.92, 1.65)
29.99
Loughlin (2006)
1.07 (0.82, 1.38)
41.28
Poulou (2007)
1.08 (0.65, 1.78)
11.19
Shi (2008)
0.95 (0.63, 1.44)
17.54
Overall (I-squared = 0.0%, p = 0.787)
1.10 (0.93, 1.30)
100.00
.563
1
1.78
C
Study
ID
OR (95% CI)
%
Weight
Mototani (2005)
2.56 (1.51, 4.36)
25.24
Loughlin (2006)
0.98 (0.80, 1.21)
34.53
Poulou (2007)
1.32 (0.79, 2.20)
25.78
Shi (2008)
1.31 (0.49, 3.46)
14.45
Overall (I-squared = 73.6%, p = 0.010)
1.41 (0.88, 2.26)
100.00
NOTE: Weights are from random effects analysis
.229
1
4.36

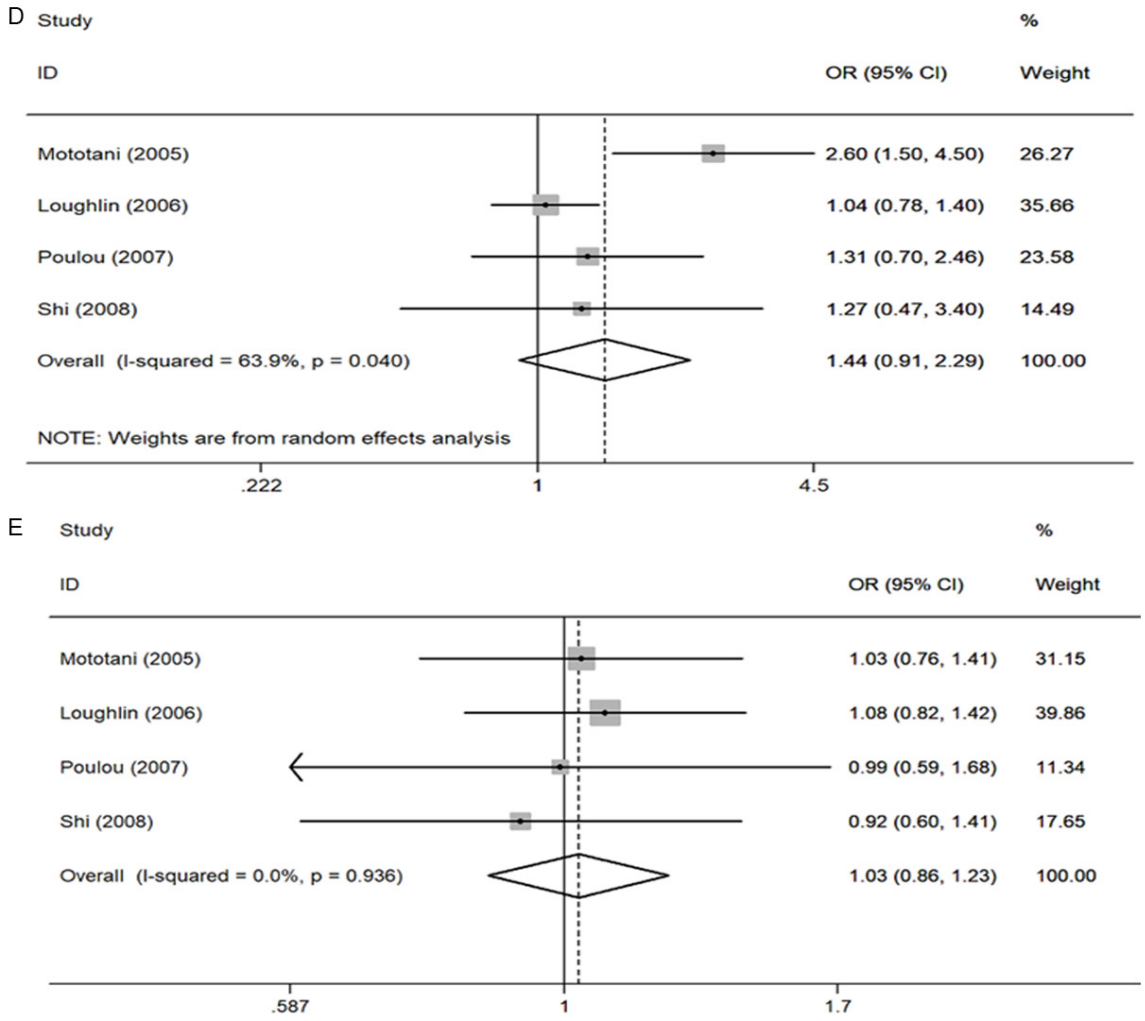


Figure 2. Meta-analysis for the association between CALM1 rs12885713 polymorphism and OA risk. (A) Allele model, (B) Dominant model, (C) Recessive model, (D) Homozygote model, (E) Heterozygote model. The squares and horizontal lines denote the ORs and 95% CIs of individual studies, and the size of the squares corresponds to the study-specific weight. The hollow diamond denotes the pooled OR and 95% CI.

polymorphisms and the risk of OA, respectively.

Association between CALM1 rs12885713 polymorphism and OA susceptibility

Table 3 and **Figure 2** summarize the meta-analysis results on the association between CALM1 rs12885713 polymorphism and risk of OA. Overall, the combined results revealed no significant association between CALM1 rs12885713 polymorphism and the risk of OA (allele model: OR 1.09, 95% CI 0.98-1.21; dominant model: OR 1.10, 95% CI 0.93-1.30; recessive model: OR 1.41, 95% CI 0.88-2.26; homozygote model: OR 1.44, 95% CI 0.91-2.29; heterozygote model: OR 1.03, 95% CI 0.86-1.23) (**Table 3**; **Figure 2**). When we divided the participants according to ethnicity, TT genotype was associated with increased risk of OA in Asians (recessive model: OR 2.21, 95% CI 1.39-3.50; homozygote model: OR 2.20, 95% CI 1.37-3.54), but not in Caucasians (**Table 3**; **Figure 3**). However, stratification by OA site showed no significant association between CALM1 rs12885713 polymorphism and knee OA risk or hip OA risk (**Table 3**).

With the aid of sensitivity analysis, we found that the combined effects remained stable when subsequently removing individual studies (**Figure 4**). Neither did we find any evidence of significant publication bias, by using the funnel plots and Egger's test (**Table 3**; Figure S1).

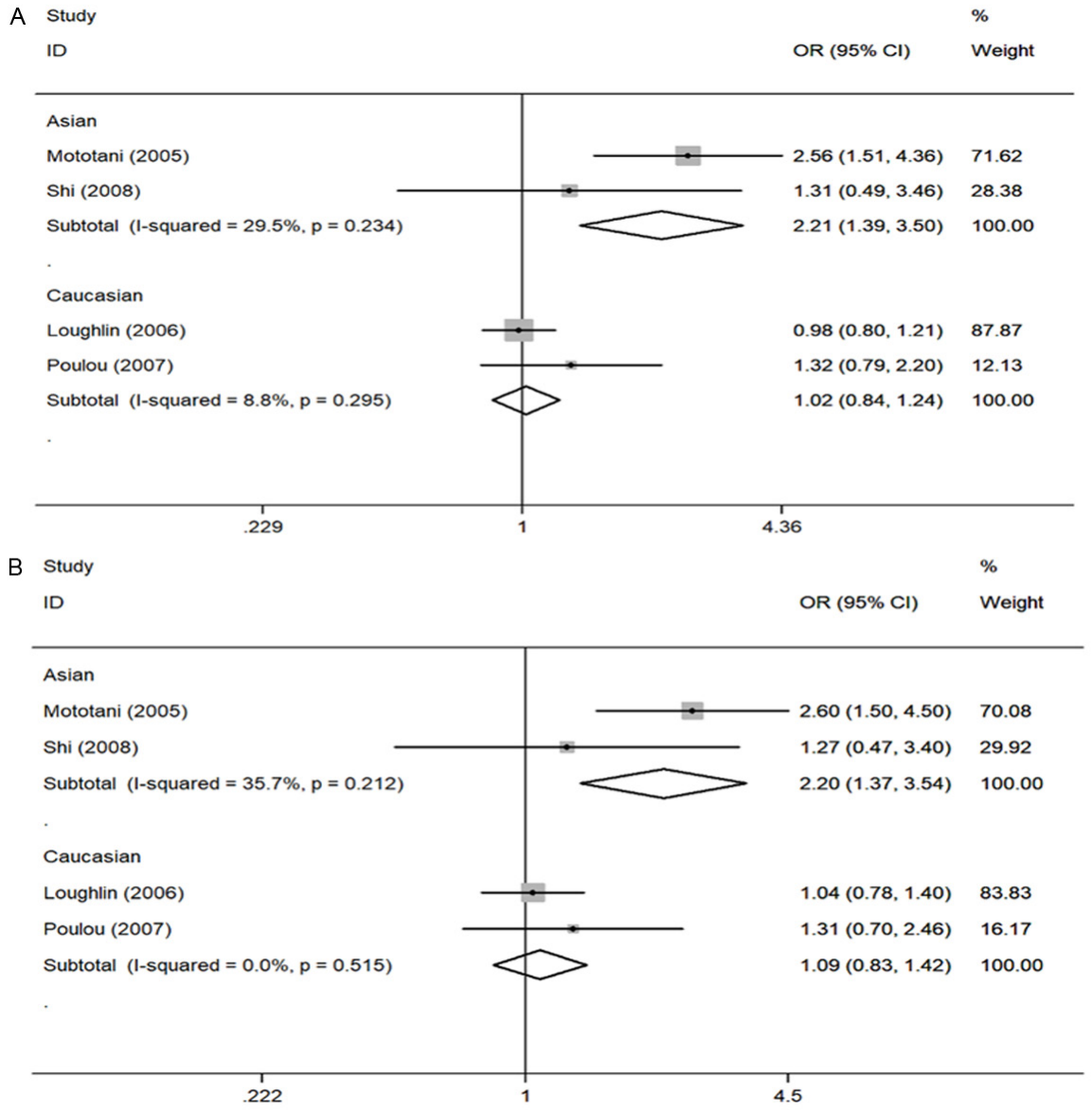


Figure 3. Meta-analysis for the association between CALM1 rs12885713 polymorphism and OA risk stratified by ethnicity. (A) Recessive model, (B) Homozygote model.

Association between CALM1 rs3213718 polymorphism and OA susceptibility

Results of pooled analysis on the association between CALM1 rs3213718 polymorphism and risk of OA are shown in **Table 4**. Because genotype distribution data was not reported by Valdes et al. [22], only allele model was analyzed to assess the association. Overall, the combined results revealed no significant association between CALM1 rs3213718 polymorphism and the risk of OA (allele model: OR 1.09, 95% CI 0.98-1.21) (**Table 4**). Furthermore, subgroup analysis stratified by gender suggested that no significant association existed between CALM1 rs3213718 polymorphism and OA risk in males (allele model: OR 1.20, 95% CI 0.89-1.63) or females (allele model: OR 1.03, 95% CI 0.91-1.17), further confirming the irrelevance between rs3213718 polymorphism and OA susceptibility. Stratification by ethnicity or OA site was not performed due to limited availability of data. CALM1 rs2300496, rs2300500 and rs3179089 polymorphisms were only investigated in one study [14], and significant associations were reported (**Tables 1**, **2**). Further replication studies were required to confirm the association.

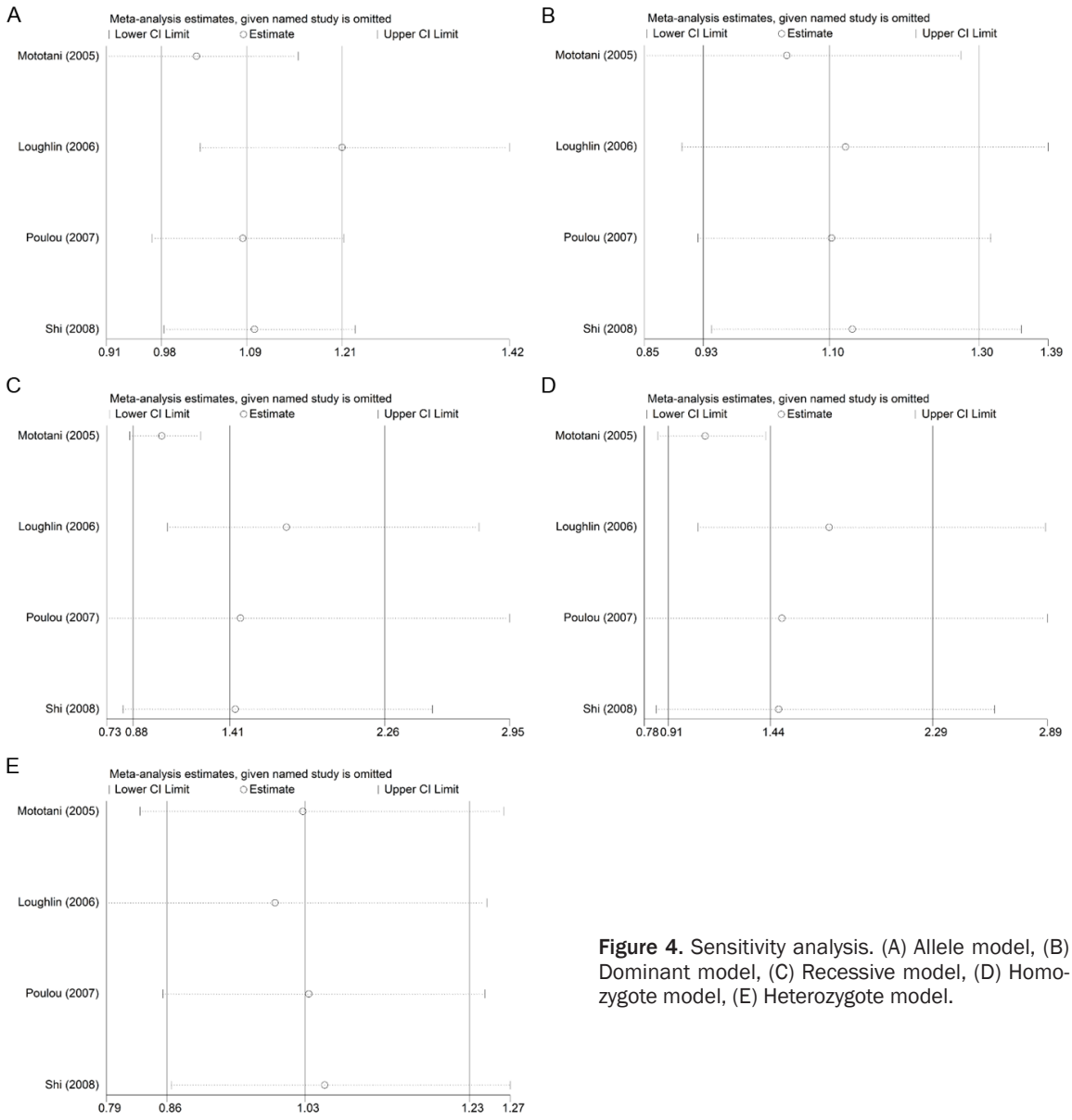


Figure 4. Sensitivity analysis. (A) Allele model, (B) Dominant model, (C) Recessive model, (D) Homozygote model, (E) Heterozygote model.

Table 4. Summary of pooled results on the association between CALM1 rs3213718 polymorphism and OA risk

Genetic model	Sub-group	No. of studies	Test of association		Statistical model	Test of heterogeneity	
			OR (95% CI)	p		I^2 (%)	p
Allele model (T vs. C)	Overall	2	1.09 (0.98-1.21)	0.105	Fixed	43.1	0.153
	Gender						
	Male	2	1.20 (0.89-1.63)	0.227	Random	54.1	0.140
	Female	2	1.03 (0.91-1.17)	0.647	Fixed	0.0	0.505

Discussion

Osteoarthritis (OA) is the most common cause of joint disease and physical disability after middle age [1]. Although the definite mechanism of OA remains unclarified, genetic factors are considered to be strong determinants in its pathogenesis [7-9]. The association between

SNPs and OA susceptibility has recently drawn enormous attention [28], and CALM1 SNPs were intensively studied. However, conflicting results ranging from no linkage to strong association were obtained from different studies [14, 19-22]. The controversial results may be partially attributable to the different populations, limited sample sizes, and other possible confounding factors. Therefore, we performed this systematic review and meta-analysis to draw a more definitive conclusion.

In the present meta-analysis, we investigated the association between CALM1 rs12885713 polymorphism and OA risk. The pooled results demonstrated no association between OA and rs12885713 polymorphism in the overall population, while subgroup analysis stratified by ethnicity revealed that TT genotype increased the risk of OA in Asians. Sensitivity analysis and publication bias estimation suggested that the results of our meta-analysis were stable. The results of our research are in good agreement with a previous meta-analysis performed by Zhang et al. [29]. Compared with the previous study, our meta-analysis enrolled 1199 additional participants (603 OA patients and 596 controls). Furthermore, previous meta-analysis only explored the association between rs12885713 polymorphism and OA risk using allele model, while we analyzed five genetic models. Several other SNPs (rs3213718, rs2300496, rs2300500, rs3179089) were also reviewed in our study.

CALM1 rs3213718 polymorphism located in intron 3 region was only investigated in two studies, but the combined results showed no significant association in the overall population or in each subgroup population. CALM1 rs2300496, rs2300500, and rs3179089 polymorphisms were reported to be in significant association with OA risk, but no further replication studies were conducted. Therefore, more studies are necessary to confirm whether these variants within CALM1 could influence the genetic risk of OA, as well as onset age and severity of OA.

Based on the present analysis, we propose that patients harboring the TT genotype of CALM1 rs12885713 polymorphism experience an increased susceptibility to OA in Asians. Our results coincide with previous functional analyses, which indicated that rs12885713 polymorphism was located in the promoter region of CALM1 gene, and TT genotype of this polymorphism decreased CALM1 transcriptional activity both *in vivo* and *in vitro* [14]. CALM1 gene encodes calmodulin, which probably plays a major part in articular cartilage in at least three ways: regulating chondrocyte differentiation [15], maintaining normal cartilage phenotype and function [16, 17], and mediating cartilage repairing process [18]. As a consequence, a deficiency of Ca^{2+}-calmodulin signaling due to impaired CALM1 transcription may be involved in the pathogenesis of OA.

The present meta-analysis may have some limitations. First, OA was considered as a multifactorial disease, but the effects of environmental and genetic interactions were not fully addressed in this meta-analysis. Therefore, the potential role of CALM1 polymorphisms may be undervalued or magnified. Second, due to limited availability of data, we were unable to perform subgroup analysis stratified by every potential confounding factor, including body mass index, and age. Third, although the funnel plot and Egger's test revealed no publication bias, selection bias could not be fully excluded because only studies published in English were searched.

In conclusion, the present meta-analysis demonstrated the TT genotype of CALM1 rs12885713 polymorphism significantly increased the risk of OA in Asians. In contrast, CALM1 rs3213718 polymorphism was not associated with OA risk. Due to the limitations of our study, further well-designed prospective studies with large sample size should be performed to confirm these results.

Acknowledgements

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Disclosure of conflict of interest

None.

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Newcastle-ottawa qualiy assessment scale

For case control studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability

Selection

Q1) Is the case definition adequate?
- a) yes, with independent validation ❋
- b) yes, eg record linkage or based on self reports
- c) no description

Q2) Representativeness of the cases
- a) consecutive or obviously representative series of cases ❋
- b) potential for selection biases or not stated

Q3) Selection of Controls
- a) community controls ❋
- b) hospital controls
- c) no description

Q4) Definition of Controls
- a) no history of disease (endpoint) ❋
- b) no description of source

Comparability

Q5) Comparability of cases and controls on the basis of the design or analysis
- a) study controls for the most important factor ❋
- b) study controls for any additional factor ❋

Exposure

Q6) Ascertainment of exposure
- a) secure record (eg surgical records) ❋
- b) structured interview where blind to case/control status ❋
- c) interview not blinded to case/control status
- d) written self report or medical record only
- e) no description

Q7) Same method of ascertainment for cases and controls
- a) yes ❋
- b) no

Q8) Non-Response rate
- a) same rate for both groups ❋
- b) non respondents described
- c) rate different and no designation

Table S1. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3, 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4, 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4, 5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6, 7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7, 8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7, 8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8, 9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9, 10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10

Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

#: Number of checklist items. *From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi: 10.1371/journal.pmed1000097.

Table S2. Results of quality assessment for the included studies using the Newcastle-Ottawa Scale

Study	Selection				Comparability	Exposure/Outcome			Scores
	Q1	Q2	Q3	Q4	Q5[a]	Q6	Q7	Q8	
Mototani 2005	☆	☆	-	☆	☆	☆	☆	☆	7
Loughlin 2006	☆	☆	-	☆	☆	☆	☆	☆	7
Valdes 2007	☆	☆	☆	☆	☆	☆	☆	☆	8
Poulou 2007	☆	☆	-	☆	☆☆	☆	☆	☆	8
Shi 2008	☆	☆	☆	☆	☆☆	☆	☆	☆	9

[a]A maximum of 2 stars can be allotted in this category, one for age, the other for other controlled factors.

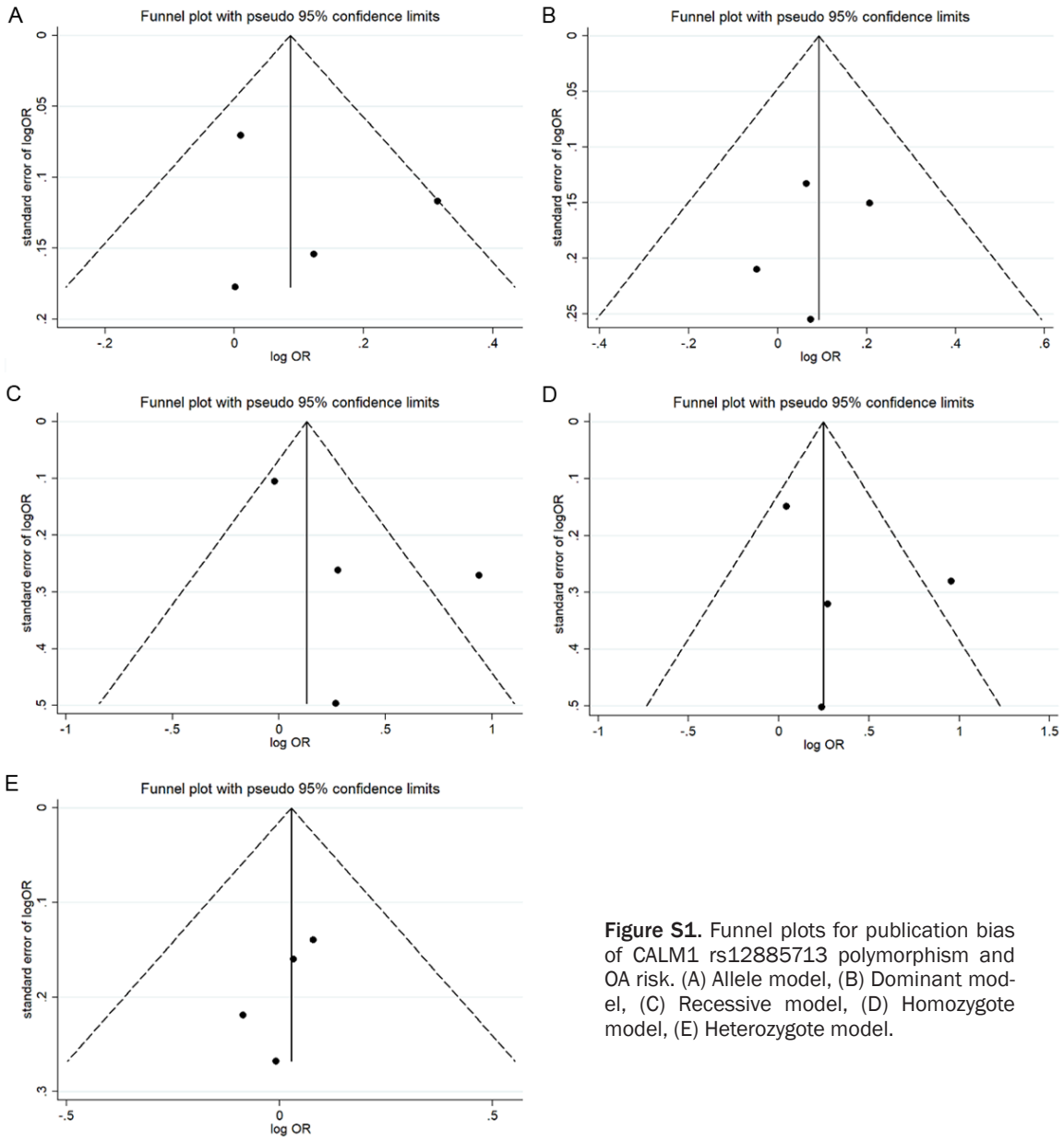

Figure S1. Funnel plots for publication bias of CALM1 rs12885713 polymorphism and OA risk. (A) Allele model, (B) Dominant model, (C) Recessive model, (D) Homozygote model, (E) Heterozygote model.