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Protective effect of anthrax toxin receptor 2 polymorphism rs4333130 against the risk of ankylosing spondylitis

Haitao Xu, PhD, Yiming Qu, PhD*

Abstract

Background: The present study was performed to statistically explore the effect of anthrax toxin receptor 2 (ANTXR2) polymorphism rs4333130 on individual susceptibility to ankylosing spondylitis (AS) using the method of meta-analysis.

Methods: All of the eligible reports were retrieved from well-known electronic databases. The strength of the association between *ANTXR2* polymorphism rs4333130 and the susceptibility to AS was evaluated using pooled odds ratios (ORs) with 95% confidence intervals (95% CIs). In addition, subgroup analysis was also performed on the basis of ethnicity to further explore specific correlation between our studied polymorphism and the disease risk. Inter-study heterogeneity was detected with *Q* test, and $P < .05$ was considered statistically significant. Sensitivity analysis was implemented through removing each of eligible studies and then recalculating overall effects to test the reliability of final estimates. Publication bias among included studies was inspected with both Begg funnel plot and Egger regression test.

Results: A total of 6 eligible papers were finally incorporated into the present meta-analysis. In total analysis, *ANTXR2* polymorphism rs4333130 was significantly related to decreased risk of AS under CC versus TT, CC + TC versus TT, CC versus TT + TC, C versus T and TC versus TT contrasts (OR = 0.35, 95% CI = 0.20–0.64; OR = 0.81, 95% CI = 0.69–0.95; OR = 0.38, 95% CI = 0.21–0.68; OR = 0.89, 95% CI = 0.84–0.95; OR = 0.84, 95% CI = 0.72–0.99). Moreover, a similar effect was also observed in Asian and Caucasian subgroups under corresponding genetic models after stratification analysis based on ethnicity.

Conclusion: *ANTXR2* polymorphism rs4333130 may function as a protective factor against AS incidence.

Abbreviations: 95% CIs = 95% confidence intervals, ANTXR2 = anthrax toxin receptor 2, AS = ankylosing spondylitis, HLA = human leukocyte antigen, ORs = odds ratios, PA = protective antigen.

Keywords: ankylosing spondylitis, anthrax toxin, anthrax toxin receptor 2, meta-analysis, polymorphism

1. Introduction

Ankylosing spondylitis (AS) is a common disease with high disability rate. The prevalence of this disease is about 0.1% to 1.4% all over the world, which reaches approximately 0.23% in China.[1,2] With rapid progression, AS generally attacks young people, and exhibits poor prognosis when treatments are delayed or inappropriate.[3] In AS patients, the most common clinical symptom is back pain which is featured by morning stiffness and alleviation after exercises.[4,5] The sign of this disease is acute and chronic inflammations at sacroiliac joint and at the points of tendons and ligaments attaching to the bone.[6,7] In most cases, AS first attacks sacroiliac joint, and then involves axial skeleton. Besides, peripheral joints are also involved in different degrees for certain patients, especially the hip and knee.[8] Long-term chronic inflammation will eventually lead to complete fusion of vertebral column and/or peripheral joints as well as the loss of activity.[9] The exact pathogenesis of AS has not been totally understood, but it has been universally established that the disease susceptibility greatly depends on genetic factors.[10,11]

Anthrax toxin contains 3 protein subunits, namely protective antigen (PA), lethal factor, and edema factor.[12] These proteins are non-toxic when they exist in isolation, but they can aggregate on cells' surface to form a toxic complex, with PA as the central part. There are 2 known cell surface receptors for PA, namely anthrax toxin receptor 1 (ANTXR1) and ANTXR2, both of which can express in multiple human tissues.[13,14] ANTXR2, a capillary morphogenesis protein, is first expressed when capillary forms. This transmembrane protein can bind with laminins and type IV collagen,[15,16] thus expressing widely in heart, lung, liver, skeletal muscle, peripheral leucocytes, placenta, small intestine, kidney, colon, and spleen. An earlier genome-wide association study among Europeans has reported that the coding gene for this protein is closely related to AS onset.[17]

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The authors report no conflicts of interest.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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In the past several years, some scholars have focused on the relationships of AS with *ANTXR2* gene polymorphisms. Thereinto, rs4333130 is the one studied most commonly. Although the number of previous studies on AS susceptibility and *ANTXR2* polymorphism rs4333130 is not too much, they still fail to reach uniform findings on this relationship. Therefore, we performed the present meta-analysis based on available publications to pool relevant findings for a clearer perspective on this issue.

2. Materials and methods

2.1. Literature search strategy

We systemically searched the electronic databases of PubMed, EMBASE, Cochrane Library, Google Scholar Web, ISI Web of Science, Chinese National Knowledge Infrastructure, and Wanfang, adopting the combination of the following key terms: "ankylosing spondylitis or Bechterew's disease," "anthrax toxin receptor 2 or ANTXR2 or capillary morphogenesis gene-2 or CMG2," and "polymorphism or variation or variant or mutation." No restrictions were imposed on publication year, ethnic descent, or sample size. In addition, the references of all relevant articles were also manually screened for additional publications.

2.2. Selection criteria

In advance, we formulated a series of criteria for selecting eligible studies:

(1) published in English or Chinese language;
(2) enrolling human beings as study subjects;
(3) focusing on *ANTXR2* polymorphism rs4333130 and the susceptibility to AS;
(4) containing both case and control groups; and
(5) providing enough information about genotype and allele frequencies in the 2 groups.

Therefore, publications not fulfilling any one of those standards were deleted. Besides, letters, duplicates, comments, and case reports were all removed from the present meta-analysis.

2.3. Data extraction

Using a standardized data sheet, 2 reviewers independently extracted primary information from all eligible studies, and implemented cross-check over these data to ensure their accuracy. Any discrepancies occurred over these data would be settled through discussion between the 2 reviewers to reach a consensus. Recorded information on the sheet included the name of the first author, publication year, ethnic line, genotyping method, numbers of cases and controls, genotype and allele distribution in the 2 groups, *P*-value for Hardy–Weinberg equilibrium in controls, and human leukocyte antigen (HLA)-B27 status in 2 groups.

2.4. Statistical analysis

STATA 12.0 software (Stata Corporation, College Station, TX) was employed for all data syntheses in the present meta-analysis, and the significance level was set at $P < .05$ for all tests. The intensity of the relationship between *ANTXR2* polymorphism rs4333130 and individual susceptibility to AS was appraised through pooling summarized odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs) under all 5 genetic models: CC versus TT, CC+TC versus TT, CC versus TT+TC, C versus T and TC versus TT. Hardy–Weinberg equilibrium in the controls was determined by Chi-square test. Besides, subgroup analysis based on ethnicity was also performed to further explore potential specific relationship. Heterogeneity among included studies was detected using Chi-square-based *Q* test, with *P*-value less than .05 as significant level. When inter-study heterogeneity was significant, random-effects model would be selected for assessing overall estimates; otherwise, fixed-effects model would be applied. Sensitivity analysis was implemented via removing each of included studies sequentially and recalculating overall ORs so as to detect the stability of final effects. Begg funnel plot and Egger regression test were utilized to investigate potential publication bias between included studies.

3. Results

3.1. Searching outcomes and study characteristics

Literature searching in the electronic databases initially identified 65 potentially relevant articles, and 18 of them were first deleted for duplicates (Fig. 1). Next, 28 more reports were removed for obvious irrelevancy. And additional 13 papers were eliminated in further evaluation due to involving other diseases (3), reviews (2), concerning other polymorphisms (6), and exploring AS treatment (2). Consequently, 6 qualified publications from 2010 to 2016 were ultimately embraced in the current meta-analysis, containing a total of 5589 case and 10,742 controls.[18–23] Among these studies, 5 focused on Asian populations while only 1 referred to Caucasians. As for HLA-B27 status, only 2 articles offered exact numbers of cases and controls, while others only displayed incomplete information or did not provide at all. Table 1 lists more detailed information about the included studies.

3.2. Quantitative data synthesis

As shown in Table 2, *ANTXR2* polymorphism rs4333130 significantly reduced the risk of developing AS in total analysis under all 5 genetic models of CC versus TT, CC+TC versus TT, CC versus TT+TC, C versus T (Fig. 2) and TC versus TT (OR = 0.35, 95% CI = 0.20–0.64; OR = 0.81, 95% CI = 0.69–0.95; OR = 0.38, 95% CI = 0.21–0.68; OR = 0.89, 95% CI = 0.84–0.95; OR = 0.84, 95% CI = 0.72–0.99). Moreover, such a downward tendency was also detected in Asians and Caucasians under corresponding comparisons after subgroup analysis on the basis of ethnicity (Fig. 2).

3.3. Heterogeneity test and sensitivity analysis

P-values from *Q* test were more than .05 under all contrasts, demonstrating the lack of statistical significance for inter-study heterogeneity, so the fixed-effects model was chosen for OR calculations in this work.

According to the results from sensitivity analysis, none of recalculated ORs had substantial differences from original ones (Fig. 3), indicating that our findings were stable and reliable.

3.4. Publication bias examination

Funnel plots displayed fine symmetry (Fig. 4), and statistical data from Egger test further supported such judgment (C vs T: $P = .660$). Therefore, publication bias between included studies was negligible in the present meta-analysis.

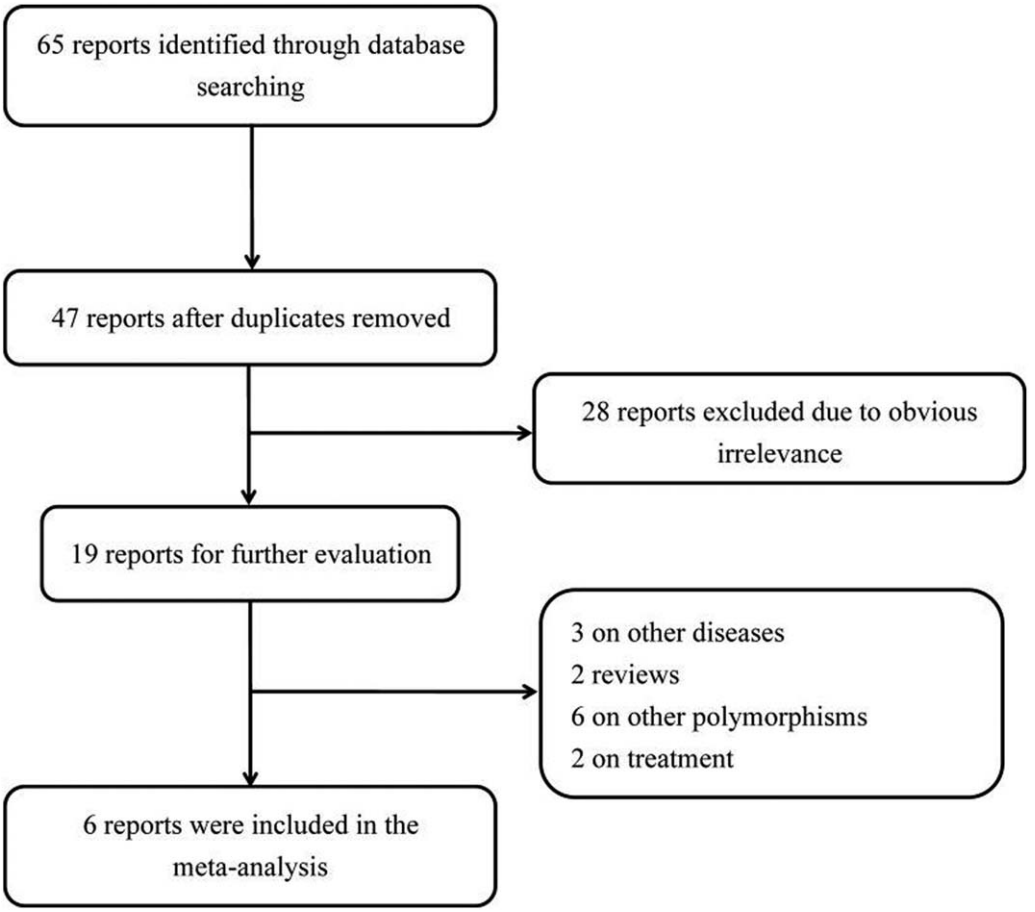

Figure 1. Flowchart for literature search and selection.

4. Discussion

AS is a chronic inflammatory disease mainly invading sacroiliac joint and vertebral column, with unclear pathogenic mechanism. Until now, no effective therapeutic measures have been developed for this disease yet. HLA-B27 belongs to major histocompatibility complex gene, and represents the most well known gene related to AS susceptibility.[24,25] According to relevant records, HLA-B27 is positive among the vast majority of AS patients, but only a small part of HLA-B27-positive individuals will eventually develop this disease, indicating that other genes than HLA may also play critical roles in the disease incidence.[26]

The gene *ANTXR2* has been proposed to be able to impact AS susceptibility, and a polymorphism in this gene, rs4333130, has been regarded as the target in such researches as well. However, studies on this topic obtain inconsistent findings. For example, Momenzadeh et al found a protective effect of the C allele and CC genotype of *ANTXR2* polymorphism rs4333130 against AS occurrence in their research ($P=.0328$, OR = 0.744, 95% CI = 0.598–0.927; $P=.0108$, OR = 0.273, 95% CI = 0.123–0.605).[22] Such a tendency was also observed in the study by Zhang, with an OR of 0.63 (95% CI = 0.43–0.91, $P=.012$).[23] Nonetheless, Guo et al detected no significant differences in genotype or allele frequencies of this polymorphism between AS cases and controls.[20] Moreover, Chen et al reported that this polymorphism had no significant impact on AS risk in Chinese Han population.[19] We hypothesized that the discrepancies in

Table 1

Essential information of eligible studies in the meta-analysis.

First author	Year	Country	Ethnicity	Genotyping method	Sample size (case/control)		Genotype and allele distribution (case/control)										HAL-B27 status (+/−)		
							TT		TC		CC		T		C		HWE	Case	Control
Momenzadeh	2016	Iran	Asian	TaqMan	349	469	180	209	161	226	8	34	521	644	177	294	0.01	262/87	21/448
Chen	2012	China	Asian	Sequenom MassARRAY	200	200	162	173	38	26	0	1	362	372	38	28	0.983	NA	NA
Zhang	2012	China	Asian	PCR-LDR	600	609	545	525	52	80	3	4	1142	1130	58	88	0.62	493/109	NA
Bang	2010	Korea	Asian	MassARRAY	1154	738	1039	656	113	77	2	5	2191	1389	117	87	0.106	NA	NA
Guo	2011	China	Asian	PCR-RFLP	308	361	277	314	29	45	2	2	583	673	33	49	0.779	308	0
Karaderi	2014	UK	Caucasian	KASP (case)/Illumina(conrol)	2978	8365	/	/	/	/	/	/	3931	10707	2025	6023	/	1935/358*	696/7996

HWE = Hardy–Weinberg equilibrium, NA = not available, PCR = polymerase chain reaction, PCR-LDR = PCR-ligation detection reaction, PCR-RFLP = PCR-restriction fragment length polymorphism, TaqMan = TaqManSNP.

* Part of information was missed.

Table 2

Relationship between *ANTXR2* polymorphism rs4333130 and the susceptibility to ankylosing spondylitis.

Group	Comparison	OR (95% CI)	P_h
Total	CC versus TT	0.35 (0.20, 0.64)	.595
	CC+TC versus TT	0.81 (0.69, 0.95)	.107
	CC versus TT+TC	0.38 (0.21, 0.68)	.614
	C versus T	0.89 (0.84, 0.95)	.077
	TC versus TT	0.84 (0.72, 0.99)	.086
Asian	CC versus TT	0.35 (0.20, 0.64)	.595
	CC+TC versus TT	0.81 (0.69, 0.95)	.107
	CC versus TT+TC	0.38 (0.21, 0.68)	.614
	C versus T	0.79 (0.69, 0.91)	.161
	TC versus TT	0.84 (0.72, 0.99)	.086
Caucasian	C versus T	0.92 (0.86, 0.97)	/

ANTXR2 = anthrax toxin receptor 2, CI = confidence interval, OR = odds ratio, P_h = *P*-value for heterogeneity.

those findings between individual studies might be attributed to multiple aspects, including but not limited to different genetic backgrounds, participants' characteristics, and uneven sample sizes.

To strengthen the statistical power of conclusion on the relationship between *ANTXR2* polymorphism rs4333130 and AS risk, we carried out this meta-analysis through analyzing the findings from all available relevant papers. After literature search and selection, we totally obtained 6 eligible publications for our meta-analysis, enrolling 5589 case and 10,742 controls. According to synthesized data, *ANTXR2* polymorphism rs4333130 was related to reduced risk of developing AS in total analysis under CC versus TT, CC+TC versus TT, CC versus TT+TC, C versus T and TC versus TT genetic models; such an effect was also detected in Asian and Caucasian subgroups after stratification analysis by ethnicity. These findings indicated that the polymorphism rs4333130 might offer protection against AS incidence. *Q* test revealed no significant heterogeneity among included studies, so overall estimates in this work were pooled using the fixed-effects model. In sensitivity analysis, re-obtained ORs showed no substantial differences from original ones, providing evidence for the stability of final effects. In addition, both funnel plot and Egger test demonstrated that publication bias between eligible studies was negligible, possibly possessing little impact on our findings, which further guaranteed the robustness of our conclusion. That was in accordance with previous meta-analysis study.[27]

That being said, our findings still should be interpreted with prudence due to some inevitable restrictions in the present work. First, the number of included studies was relatively small, which reduced the comprehensiveness of our findings. Second, we only searched several electronic databases for eligible articles published in English or Chinese language, and some pertinent reports in other sources or languages might be missed, thus

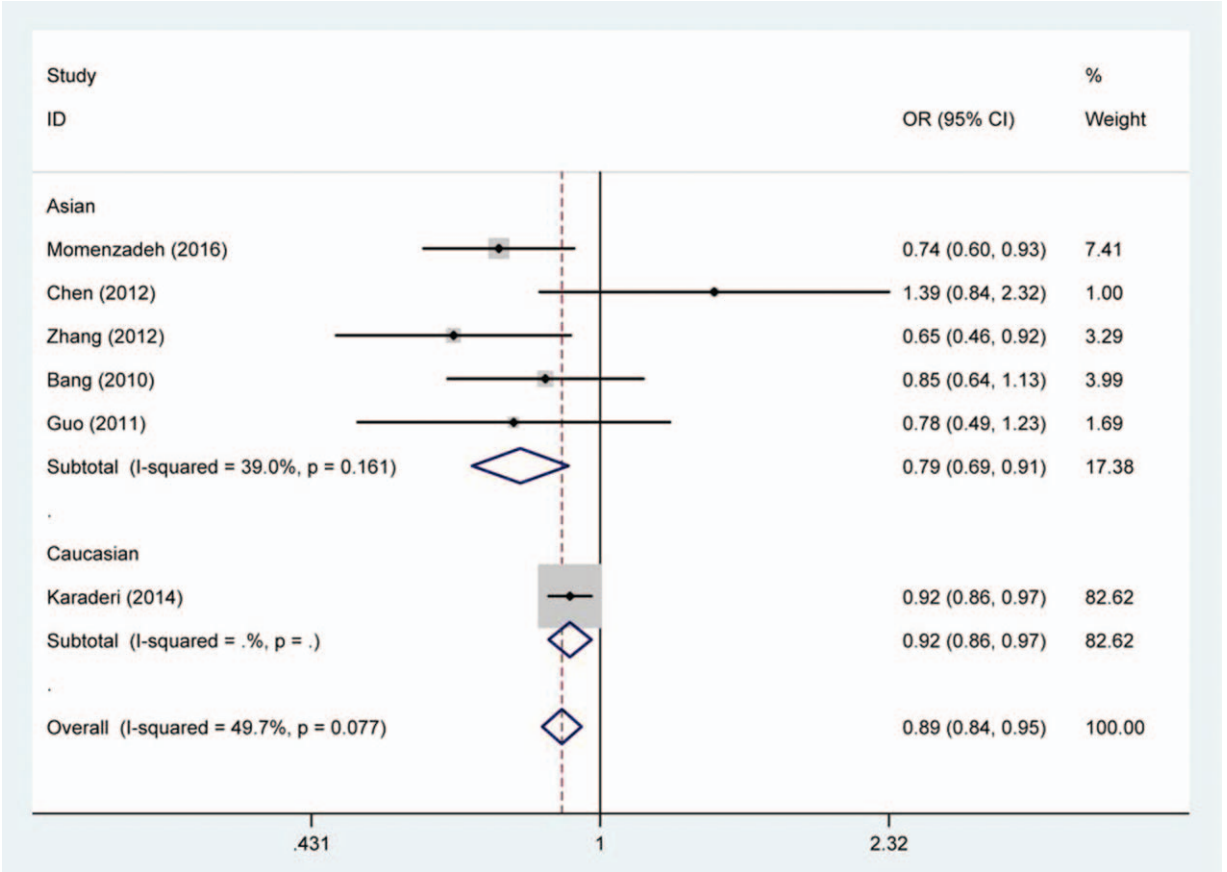

Figure 2. Forest plot for the association between *ANTXR2* polymorphism rs4333130 and the risk of ankylosing spondylitis under C versus T contrast by ethnicity. ANTXR2 = anthrax toxin receptor 2.

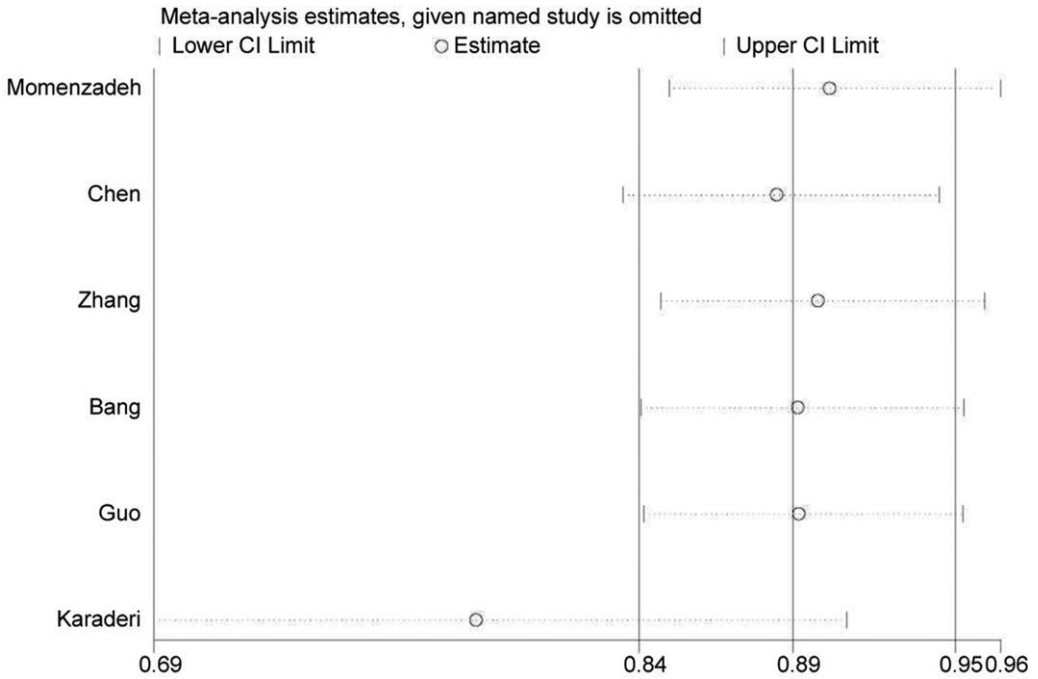

Figure 3. Sensitivity analysis testing the reliability of final estimate.

introducing certain publication bias, though not significant. Third, detailed subgroup analyses on other possibly relevant factors were not carried out in this work owing to limited information from original papers. For example, HLA-B27 status represents a key effector on AS risk, but our included studies did not precisely stated genotype and/or allele distribution in cases and controls stratified by this element, so we failed to complete stratification analysis according to this factor. Last but not the least, potential effects of gene-gene and gene-environment interactions on AS susceptibility were neglected in our study.

In conclusion, *ANTXR2* polymorphism rs4333130 may exert a protective effect against developing AS. Nevertheless, in view of the above mentioned shortcomings in the present meta-analysis, our findings need to be further verified by studies with larger sample sizes and more considerations of other factors possibly related to AS risk.

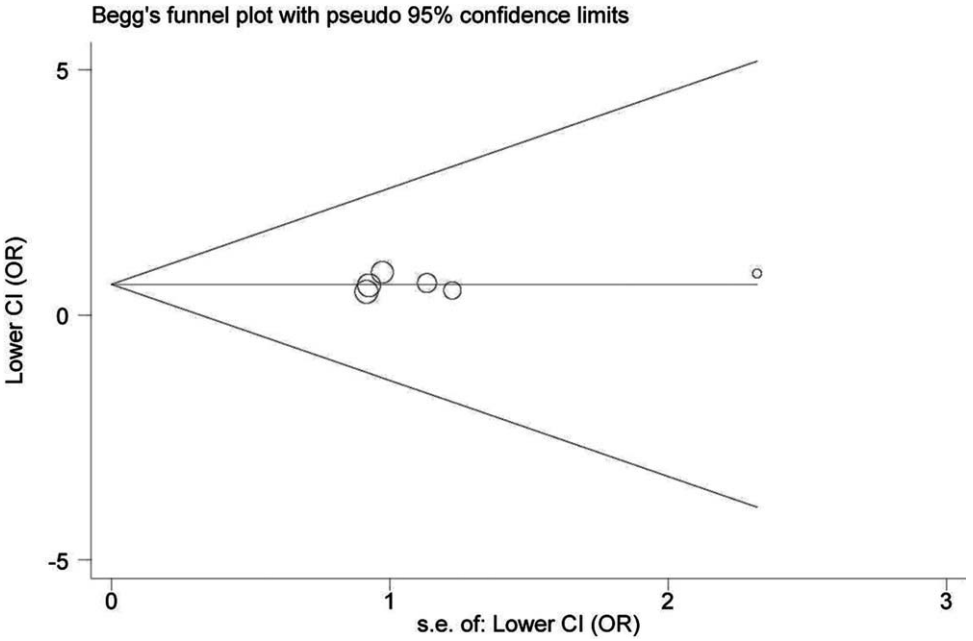

Figure 4. Begg funnel plot of potential publication bias (model: C vs T).

Author contributions

H.X. conceived and designed the experiments; Y.Q. analyzed the data, and wrote the paper; H.X., Y.Q. performed the experiments.
All authors read and approved the final manuscript.

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