SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2016; 33; 102-111

The association between ANXA11 gene Polymorphisms and sarcoidosis: a meta-analysis and systematic review

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Abstract. *Background and Objective:* The associations of ANXA11 gene polymorphisms and susceptibility to sarcoidosis have been evaluated in recent years. However, the results remain controversial, especially in different ethnicity. To assess the associations between ANXA11 and sarcoidosis, we conducted this meta-analysis. *Method:* Articles were searched in MEDLINE, EMBASE and PubMed from their establishment date to August of 2014, and 4,567 sarcoidosis patients and 4,278 controls from 6 studies were included. The strength of associations was determined by ORs with 95% CIs. The associations between ANXA11 SNP rs1049550, rs2573346, rs2789679 polymorphisms and sarcoidosis risk were assessed using additive, recessive and dominant models. *Results:* ANXA11 SNP rs2573346 and rs2789679 T allele conferred protection against sarcoidosis (OR: 0.664, 95% CI: 0.607-0.726 for rs2573346, and OR: 0.698, 95% CI: 0.640-0.762 for rs2789679). For SNP rs1049550, individuals carrying the "T" allele (TT+CT) had a nearly 46% increased risk for the development of sarcoidosis, when compared with CC homozygotes (OR: 1.461, 95% CI: 1.183-1.803) in overall population. A significant association was also found in additive model (OR: 1.477, 95% CI: 1.328-1.642 for CC vs. CT; OR: 0.610, 95% CI: 0.412-0.905 for TT vs. CC). In addition, ethnicity factors may contribute to the disease risk. *Conclusion:* The meta-analysis revealed that "T" allele of ANXA11 SNP rs2573346 and rs2789679 conferred protection against sarcoidosis. "C" allele of SNP rs1049550 may be a risk factor for sarcoidosis in overall population. Our study shows that ANXA11 closely associated with the development of sarcoidosis but further studies in different ethnicity were needed. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 102-111)*

Key words: sarcoidosis, meta-analysis, anxa11, gene polymorphisms

Introduction

Sarcoidosis is a systemic inflammatory disease characterised by the presence of destructive, non-caseating epithelioid granulomatous lesions, with accumulated monocytes, macrophages, and activated T-lymphocytes, which can affect the lungs, skin, heart, eyes, brain and nervous system, and various other tissues and organs (1, 2), and the clinical symptoms were widely different due to the difference of the lesions located (3). As pulmonary damages ranged from spontaneous resolution which was no impairment to fibrotic lung disease which might lead to respiratory failure, the clinical course, treatment and prognosis of sarcoidosis is variable. It is reported that sarcoidosis can occur in a pattern of family clustering, and the racial incidence is also different, suggesting genetic factors may contribute to disease risk (4).

ANXA11(annexin A11), located on chromosome 10q22.3, is a member of annexin family of calcium-dependent, phospholipids-binding proteins, ubiquitously expressed in many cell types, involved in

Received: 3 November 2014
Accepted after revision: 13 August 2015
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growth control, cell division, cell construction, vesicle trafficking, calcium signaling and apoptosis (5), which linked to immune disease and other chronic disorders (6). At the same time, ANXA11 also plays an indispensable role in the terminal phase of cell cytokinesis (7).

Previous study has showed that ANXA11 may plays a role in the occurrence of sarcoidosis. A genome-wide association study (GWAS) in sarcoidosis conducted in the German population firstly revealed an association of the variants in the ANXA11 (annexin A11) gene with susceptibility to sarcoidosis (8). In addition, this association has recently been supported by a number of case-control studies from the same or different population in different single-nucleotide polymorphism (SNP). However, in another GWAS study of sarcoidosis conducted in the African Americans, the association did not reach genome-wide significance (9), which suggests the presence of ethnic specific genetic effects. A lot of works have been done in previous studies about the involvement of ANXA11 gene in sarcoidosis, however, results from these studies were inconclusive or inconsistent due to small sample sizes. To further assess the associations between ANXA11 Gene Polymorphisms and sarcoidosis, we conducted this meta-analysis.

Methods

Search strategy

This meta-analysis was conducted according to the guidelines of Meta-analysis of Observational Studies in Epidemiology (MOOSE) (10). Two investigators (HFZ and MYD) searched electronic databases including EMBASE, PubMed and MEDLINE (updated to 31 August 2014) independently. No language or geographic area restrictions were applied. We conducted Medical Subject Headings (MeSH) terms and keywords search using the following terms: "annexin A11", "ANXA11", "sarcoidosis", "polymorphism", and "genotype".

Study selection

Studies fulfilled the following criteria were included: (1) A case-control study design; (2) The diagnoses of sarcoidosis patients should be in accordance with internationally accepted criteria; (3) Sufficient published data should be contained for the evaluation of an odds ratio with a 95% confidence interval in the study; (4) The study should be published as an article.

Studies had one of the following criteria were excluded: (1) No control group; (2) A study based on family; (3) Insufficient data for extraction; (4) The study published as a review or abstract.

Data extraction

Two authors (HFZ and MYD) independently extracted data for each eligible study using the same standard protocol, regarding: (1) authors, (2) year of publication, (3) single-nucleotide polymorphisms (SNPs) studied in each article, (4) sample size, including number of patients and controls, (5) ethnicity of the studied population, (6) genotype and allele frequency information (Table 1).

Assessment of study quality

Newcastle-Ottawa scale (NOS) was chosen to assess the quality of the studies (11). NOS included eight aspects, which were divided into three categories including comparability, exposure and selection. The assessment was determined by stars given in each aspect culmulated. The studies given 0–3 stars, 4–6 stars and 7–9 stars were classified as low, moderate and high quality, respectively.

Statistical analysis

We performed the meta-analysis with allelic contrast, recessive, dominant, and additive models using STATA 12.0 (StataCorp, College Station TX, USA) to provide the ORs with 95% confidence intervals (95% CI) to measure the strength of the associations between ANXA11 gene polymorphisms and sarcoidosis risk. The Z-test was performed to determine the significance of the pooled OR. The heterogeneity among the studies was quantified by Q test and I^2 test ($I^2 = 100\% \times (Q-df)/Q$) (12, 13). If there was no significant heterogeneity ($P>0.10$ for Q test, and $I^2<50\%$ for I^2 test) among the studies, a fixed-effects model was used. Otherwise ($P<0.10$ for Q test, or $I^2>50\%$ for I^2 test), we used random-effects model. Publication bias was investigated by Begg's test. The sensitivity analysis was also performed by

Table 1. Characteristics of 6 publications included in the meta-analysis of ANXA11 and sarcoidosis susceptibility

SNP	Author	Year	Country	Ethnicity	Sample size	Case/ Control	TT case	CT case	TT control	CT control
rs1049550	Li Y et al.	2010	Germany	Caucasian	662	349/313	48	148	65	153
	Feng XJ et al.	2014	China	asian	830	412/418	34	170	74	190
	Mrazek F et al.	2011	Czech	Caucasian	499	245/254	25	123	39	133
	Levin AM et al.	2013	USA	European American	796	446/350	189	194	115	173
				African American	2125	1232/893	885	315	593	278
	Hofmann S et al.	2008	Germany	Caucasian	3447	1636/1811	188	706	302	895
	Morais A	2013	Portugal	Caucasian	405	208/197	29	80	41	95
rs2573346	Li Y et al.	2010	Germany	Caucasians	678	356/322	50	160	71	161
	Hofmann S et al.	2008	Germany	Caucasians	3313	1587/1726	198	728	337	883
rs2789679							TT case	AT case	TT control	AT control
	Feng XJ et al.	2014	China	asian	830	412/418	94	186	114	225
	Hofmann S et al.	2008	Germany	Caucasians	3453	1640/1813	214	736	337	925

leave-one-out method. Differences were considered to be significant when P<0.05.

Results

Studies included in the meta-analysis

The flowchart of selecting studies included in the meta-analysis was shown in Figure 1. Six relevant studies about the association between ANXA11 gene polymorphisms and sarcoidosis were finally included in this meta-analyses. Six articles were identified to assess the SNP rs1049550 within ANXA11 gene and sarcoidosis (8, 14-18), two of which contained the data about SNP rs2573346 (8, 15), and two of which contained the data about SNP rs2789679 (8, 14). All the articles published in peer-review journals in English.

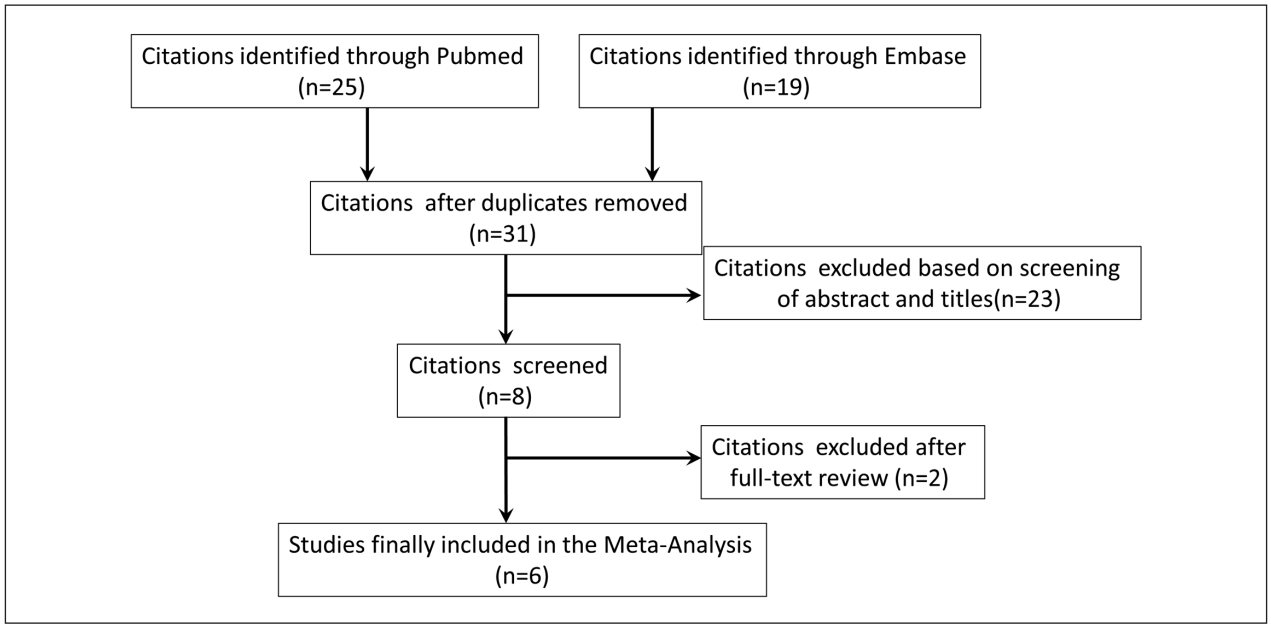

Fig. 1. Flowchart of the present meta-analysis

Characteristics of included studies

The six included studies were published between 2008 and 2014, with data for 4,567 sarcoidosis patients and 4,278 controls. All the studies fulfilled the inclusion criteria and the characteristics of the six studies are shown in Table 1.

Assessment of study quality

To evaluate the studies quality, we used the NOS scoring system. One study scored eight stars, two scored seven stars, two scored six stars and one scored five stars (Table S1). No one was considered to be low quality.

ANXA11 rs1049550 and susceptibility to sarcoidosis

Six studies evaluated the association between SNP rs1049550 T/C polymorphism and sarcoidosis risks. For the overall results, CC homozygotes had a nearly 46% increased risk for the development of sarcoidosis, compared with individuals who carried the "T" allele (CT+TT) (OR: 1.461, 95% CI: 1.183-1.803). And for the additive model, we also found significant associations (OR: 1.477, 95% CI: 1.328-1.642 for CC vs. CT; OR: 0.610, 95% CI: 0.412-0.905for TT vs. CC). Which means CC carriers were more susceptible to sarcoidosis compared with TT or CT carriers. (Table 2) (Figure 2). However, significant heterogeneity was found across the studies, considering caused by ethnicity. Then we performed the subgroup analyses by ethnicity. We can see the results of Asian and Caucasian populations were much similar, which showed that genotype "CC" carrier was a risk factor for sarcoidosis, and consistent with the results of the overall population. On the other hand, in American populations the result is opposite, especially in European Americans. This finding supported that ethnicity factors may contribute to the disease risk.

ANXA11 rs2573346 and Susceptibility to Sarcoidosis

Two studies evaluated the association between SNP rs2573346 T/C polymorphism and sarcoidosis. The results showed that the "T" allele protected people from sarcoidosis (OR: 0.664, 95% CI: 0.607-0.726). Which means individuals carried the "T" allele had a 33.6% lower risk of sarcoidosis than people with the "C" allele. Furthermore, the "TT" homozygotes had a 55.3% lower risk of sarcoidosis than "AA" genotypes (OR: 0.447, 95% CI: 0.369-0.541) (Table 2). There was no significant heterogeneity across the studies.

ANXA11 rs2789679 and susceptibility to sarcoidosis

Two studies evaluated the association between SNP rs2789679 T/A polymorphism and sarcoidosis. The dominant, additive and recessive models all showed that individuals carried the "T" allele had a significant decreased risk of sarcoidosis (Table 2). When individuals carried the "TT" genotype compared with "AA" genotype, the association was strongest, and the disease risk had a 49.6% decrease (OR: 0.504, 95% CI: 0.421-0.604). Significant heterogeneity was not found and the subgroup analysis could not be conducted because of the small sample size.

Sensitivity analysis

Sensitivity analyses were conducted by leave-one-out method. And there was no significant influence to the estimated ORs by removing any individual study.

Publication bias

No significant publication bias was found by Begg's test (Table 2), so as the funnel plots for the associations between ANXA11 and sarcoidosis (Data not shown).

Discussion

The great contribution of genetic factors in the happen of sarcoidosis was sustained by the increased risk of sarcoidosis in first-degree relatives of sarcoidosis patients. However, due to the inconsistent data of independent studies, the associations between ANXA11 and susceptibility to sarcoidosis remained poorly illuminated. In this study, we conducted a systematic review of published studies investigating the role of ANXA11 gene polymorphisms in sarcoidosis and performed a meta-analysis. To our knowledge, it is the first study to review and meta-analyze all

Table 2. Outcome, heterogeneity and publication bias tests for the meta-analyses

SNP	Comparison	Ethnicity	OR with 95%CI	Z	P	Heterogeneity				Publication Bias
						χ^2	P	I^2(%)	Tau-squared	Begg's test
rs1049550	T vs C	Caucasian	0.692(0.638-0.750)	8.89	0.000	1.76	0.624	0.0	0.0000	1.000
		Asian	0.598(0.488-0.734)	4.92	0.000	0.00			0.0000	
		EA	1.317(1.075-1.614)	2.66	0.008	0.00			0.0000	
		AA	1.210(1.028-1.424)	2.29	0.022	0.00			0.0000	
		overall	0.801(0.630-1.017)	1.82	0.069	69.79	0.00	91.4	0.0933	0.764
	TT vs CC	Caucasian	0.501(0.421-0.596)	7.82	0.000	0.51	0.916	0.0	0.0000	1.000
		Asian	0.340(0.216-0.537)	4.63	0.000	0.00			0.0000	
		EA	1.617(1.062-2.462)	2.24	0.025	0.00			0.0000	
		AA	1.026(0.590-1.783)	0.09	0.927	0.00			0.0000	
		overall	0.610(0.412-0.905)	2.46	0.014	35.47	0.000	83.1	0.2251	0.764
	CC vs CT	Caucasian	1.528(1.371-1.747)	7.06	0.000	2.12	0.548	0.0	0.0000	1.000
		Asian	1.510(1.125-2.025)	2.75	0.006	0.00			0.0000	
		EA	0.906(0.604-1.360)	0.48	0.634	0.00			0.0000	
		AA	1.284(0.729-2.261)	0.86	0.387	0.00			0.0000	
		overall	1.477(1.328-1.642)	7.21	0.000	8.50	0.203	29.4	0.0109	0.548
	TT vs CT	Caucasian	0.780(0.659-0.924)	2.87	0.004	0.26	0.967	0.0	0.0000	1.000
		Asian	0.514(0.326-0.810)	2.87	0.004	0.00			0.0000	
		EA	1.466(1.076-1.997)	2.42	0.015	0.00			0.0000	
		AA	1.317(1.087-1.596)	2.81	0.005	0.00			0.0000	
		overall	0.891(0.670-1.186)	0.79	0.430	30.50	0.000	80.3	0.1091	1.000
	TT vs TC+CC	Caucasian	0.637(0.543-0.748)	5.53	0.000	0.10	0.992	0.0	0.0000	1.000
		Asian	0.418(0.272-0.644)	3.96	0.000	0.00			0.0000	
		EA	1.503(1.123-2.012)	2.74	0.006	0.00			0.0000	
		AA	1.290(1.071-1.544)	2.68	0.007	0.00			0.0000	
		overall	0.761(0.530-1.094)	1.47	0.140	56.33	0.000	89.3	0.2024	1.000
	CC vs TC+TT	Caucasian	1.642(1.464-1.842)	8.48	0.000	2.25	0.523	0.0	0.0000	1.000
		Asian	1.748(1.325-2.306)	3.95	0.000	0.00			0.0000	
		EA	0.764(0.521-1.120)	1.38	0.168	0.00			0.0000	
		AA	1.056(0.609-1.830)	0.19	0.847	0.00			0.0000	
		overall	1.461(1.183-1.803)	3.53	0.000	18.96	0.004	68.4	0.0504	0.548
rs2573346	T vs C	Caucasians	0.664(0.607-0.726)	8.92	0.000	0.06	0.803	0.0	0.0000	1.000
	TT vs CC	Caucasians	0.447(0.369-0.541)	8.30	0.000	0.02	0.888	0.0	0.0000	1.000
	TT vs TC+CC	Caucasians	0.586(0.493-0.696)	6.10	0.000	0.01	0.940	0.0	0.0000	1.000
	CC vs TC+TT	Caucasians	1.733(1.519-1.976)	8.20	0.000	0.05	0.822	0.0	0.0000	1.000
rs2789679	T vs A	Caucasian and Asian	0.698(0.640-0.762)	8.11	0.000	0.00	0.945	0.0	0.0000	1.000
	TT vs AA	Caucasian and Asian	0.504(0.421-0.604)	7.41	0.000	0.01	0.904	0.0	0.0000	1.000
	TT vs TA+AA	Caucasian and Asian	0.689(0.587-0.808)	4.57	0.000	0.95	0.330	0.0	0.0000	1.000
	AA vs TA+TT	Caucasian and Asian	1.717(1.511-1.952)	8.26	0.000	1.20	0.273	16.7	0.0032	1.000

EA: European American AA: African American

available published data systemically to evaluate the association between ANXA11 and sarcoidosis risk.

In this meta-analysis, six studies were included (published from 2007 to 2014). Three of these were classified as high quality, three were medium, and no one was classified as low quality. Therefore, our meta-analysis should be reliable, and can clarify the associations between ANXA11gene polymorphisms and susceptibility to sarcoidosis.

As a result of this study, we can see that gene polymorphisms of ANXA11 were significantly associated with the happen of sarcoidosis. In this study, three SNPs were assessed to be associated significantly with sarcoidosis (Table **2**). ANXA11 rs1049550 "C", rs2573346 "C" and rs2789679 "A" alleles were genetic risk factors for sarcoidosis. And the association was strongest when individuals carried the genotype of "CC", "CC" or "AA" respectively, especially in Caucasians and Asians. However, for rs1049550, the association is very weak or even inverse in European Americans and African Americans. Sarcoidosis impacts people of all ages, genders and races, and

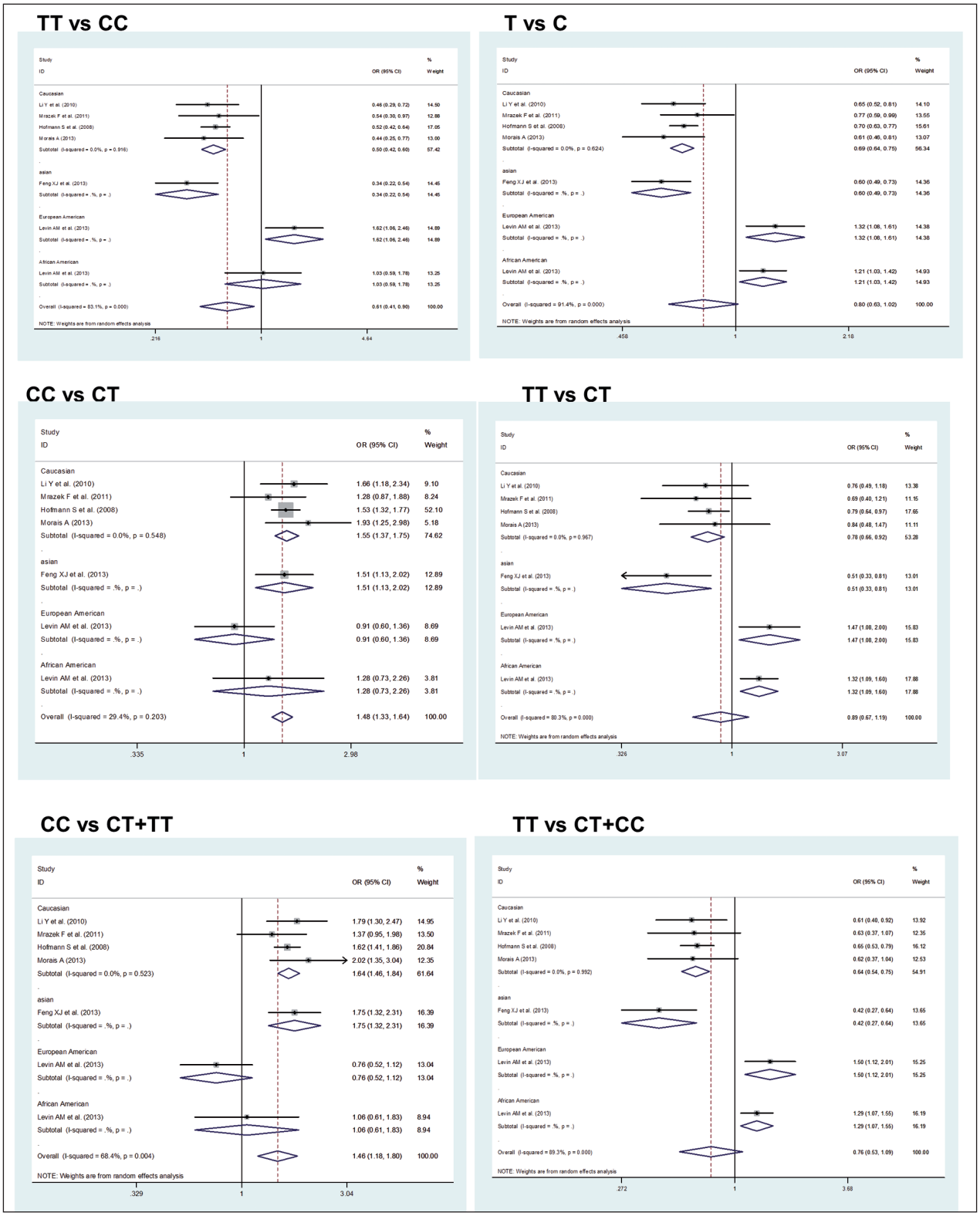

Fig. 2. Meta-analysis of the studies of ANXA11 SNP rs1049550 and sarcoidosis risk

the incidence of sarcoidosis is varying in different ethnicity and area (19-21). The annual incidence proportion is 10–14/100,000 in central Europe (8), 10.9/100,000 in European American, 35.5/100,000 in African American, and less than 10/100,000 in China (22-25). As ANXA11 involved in growth control, cell division, cell construction, vesicle trafficking, calcium signaling and apoptosis, which linked to autoimmune and other chronic disorders, we infer that gene polymorphisms of ANXA11 in different ethnicity may result difference in incidence or clinical manifestations, or even response to treatment, and prognosis.

Despite the inspiring results of our study, there were limitations. The major limitation of our meta-analysis is the small number of included studies, which restricts the power of this study. Besides, although some studies have shown the associations of age, clinical manifestation, prognosis and ANXA11 gene polymorphisms, we could not perform subgroup meta-analysis by these factors due to insufficient of the data. At the same time, owning to the limited information of included studies, we could not evaluate gene–environment or gene-gene interactions. Meanwhile, the data unpublished which we could not reach, may contributed to publication bias which we could not assessed. Finally, despite subgroup analyses by ethnicity were conducted, other inherent confounding factors such as specific technique, country and sex could not be completely excluded.

In summary, our study revealed that ANXA11 gene polymorphisms were significantly associated with the development of sarcoidosis. The "CC" genotype of rs1049550 was independent risk factors for sarcoidosis. The "T" allele of rs2573346 and rs2789679 conferred protection against sarcoidosis. The results of our study might contribute to an understanding of pathogenesis in which it would be possible to lead sarcoidosis. However, the small sample size might affect the accuracy of our findings, and more study especially in different ethnicity is needed. The associations of ANXA11 and the clinical manifestation of sarcoidosis should be focused on in future studies.

Statements

All authors of this research paper have directly participated in the planning, execution, or analysis of the study. All authors of this paper have read and approved the final version submitted. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not now under consideration for publication elsewhere. The contents of this manuscript will not be copyrighted, submitted, or published elsewhere while acceptance by the Journal is under consideration. There are no directly related manuscripts or abstracts, published or unpublished, by any author(s) of this paper.

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Supplementary Information

Table S1. Methodological quality of included studies according to the NEWCASTLE-OTTAWA Quality Assessment Scale

Checklist S1. PRISMA checklist.

Table S1. Methodological quality of included studies according to the NEWCASTLE-OTTAWA Quality Assessment Scale

Study	Selection				Comparability	Exposure			Total
	Is the case definition adequate	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	
Li Y et al.	★	/	/	★	★	★	★	/	★★★★★
Feng XJ et al.	★	★	★	★	★	★	★	/	★★★★★★★
Mrazek F et al.	★	★	/	★	★	★	★	/	★★★★★★
Levin AM et al.	★	/	/	★	★	★	★	★	★★★★★★
Hofmann S et al.	★	★	★	★	★	★	★	★	★★★★★★★★
Morais A et al.	★	★	★	★	★	★	★	/	★★★★★★★★

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
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.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction
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.	No
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.	Study selection
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.	Search strategy
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Search strategy
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).	Study selection
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.	Data extraction
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Data extraction
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.	Data extraction
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Statistical analysis
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.	Statistical analysis

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
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).	Statistical analysis
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Statistical analysis
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.	Studies included in the meta-analysis
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.	Characteristics of included studies
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).	RESULTS
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.	RESULTS
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	RESULTS
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Publication bias
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Sensitively analysis and assessment of bias
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).	DISSCUSSION
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).	DISSCUSSION
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	DISSCUSSION
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.	online submission system

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(6): e1000097. doi:10.1371/journal.pmed1000097