Systematic Review and Meta-Analysis

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The *BTNL2 G16071A* gene polymorphism increases granulomatous disease susceptibility

A meta-analysis including FPRP test of 8710 participants

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Abstract

Objective: The butyrophilin-like 2 (*BTNL2*) *G16071A* gene polymorphism has been implicated in the susceptibility to granulomatous diseases, but the results were inconclusive. The objective of the current study was to precisely explore the relationship between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility by the meta-analysis including false-positive report probability (FPRP) test.

Methods: A systematic literature search in the PubMed, Embase, and Wanfang databases, China National Knowledge Internet, and commercial Internet search engines was conducted to identify studies published up to April 1, 2016. The odds ratio (OR) with 95% confidence interval (CI) was used to assess the effect size. Statistical analysis was conducted using the STATA 12.0 software and FPRP test sheet.

Results: In total, all 4324 cases and 4386 controls from 14 eligible studies were included in the current meta-analysis. By the overall meta-analysis, we found a significant association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility (A vs G: OR = 1.25, 95% CI = 1.07–1.45, $P = 0.005$). The meta-regression analyses showed that a large proportion of the between-study heterogeneity was significantly attributed to the ethnicity (A vs G, $P = 0.013$) and the types of granulomatous diseases (A vs G, $P = 0.002$). By the subgroup meta-analysis, the *BTNL2 G16071A* gene polymorphism was associated with granulomatous disease susceptibility in Caucasians (A vs G: OR = 1.37, 95% CI = 1.18–1.58, $P < 0.001$). Moreover, a significant relationship between the *BTNL2 G16071A* gene polymorphism and sarcoidosis susceptibility (A vs G: OR = 1.52, 95% CI = 1.39–1.66, $P < 0.001$) was found. However, to avoid the "false-positive report," we further investigated the significant associations observed in the present meta-analysis by the FPRP test. Interestingly, the results of FPRP test indicated that the *BTNL2 G16071A* gene polymorphism was truly associated with sarcoidosis susceptibility (A vs G, FPRP < 0.001). Additionally, the FPRP test confirmed that the *BTNL2 G16071A* gene polymorphism was associated only with granulomatous disease susceptibility among Caucasians (A vs G, FPRP < 0.001) at the level of a prior probability, which was 0.001.

Conclusion: The meta-analysis indicated that *BTNL2 G16071A* gene polymorphism may as a likelihood factor contributed to granulomatous disease susceptibility, especially increasing the sarcoidosis susceptibility. In addition, the polymorphism may be greatly associated with likelihood of granulomatous diseases among Caucasians.

Abbreviations: *BTNL2* = butyrophilin-like 2, CD = Crohn disease, CI = confidence interval, FPRP = false-positive report probability, HLA = human leukocyte antigen, OR = odds ratio, TB = tuberculosis, WG = Wegener granulomatosis.

Keywords: *BTNL2*, FPRP, granulomatous disease, meta-analysis, polymorphism

1. Introduction

Granuloma is defined as a small collection of lymphocytes and activated macrophages (including epithelioid cells, multinucleated giant cells).[1] In addition, the fibroblasts and granulocytes also can be counted. The aggregated abnormal granuloma always results in pseudotumorous lesions.[2] In fact, sarcoidosis, tuberculosis (TB), Crohn disease (CD), and Wegener granulomatosis (WG) are common diseases that are characterized by a similar histopathological feature, namely, immune-mediated granulomata.[3–6] However, the etiology of granulomatous diseases is unclear. According to the previous studies, the environmental exposures, immune disorder, and inflammatory reaction appear to play an essential role in the pathogenesis of granulomatous diseases.[7–9] Saboor et al found that there was a significant proportion of patients with sarcoidosis having mycobacteria in their lungs.[10] Additionally, the immunohistochemical staining of sarcoid granulomas shows majority of lymphocytes as $CD4^+$ T cells, whereas the periphery of the

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granuloma is made up of $CD4^+$ as well as $CD8^+$ T cells.[11] Currently, increasing evidence indicated that the host genetic susceptibility was also closely associated with granulomatous disease susceptibility.[12–14]

As the above-described immune and inflammatory reactions are important to granulomatous diseases pathogenesis, the genes encoding antigen presentation and recognition molecules including human leukocyte antigen (HLA), cytokines, receptors, etc., are among those that are mostly implicated as the genetic factors of granulomatous diseases.[15] Recent candidate gene studies further identified a number of susceptibility loci with the *HLA II* alleles (e.g., *HLA-DR* and *HLA-DQ*) representing the main contributor to granulomatous disease susceptibility across patients of different ethnicity.[16–18] The butyrophilin-like 2 (*BTNL2*) gene was first identified by comparing the mouse and human genomic sequences at the major histocompatibility complex class II and class III regions.[19] It is located at the border of the human HLA class II and class III regions of chromosome 6p21.3, and is in strong linkage disequilibrium with *HLA-DRB1* and *-DQB1* genes.[19] However, the important role of *BTNL2* gene has yet to be fully investigated.

As a member of immunoglobulin superfamily, *BTNL2* shares significant sequence homology with B7 family members that are crucial regulators of T-cell activation and tolerance.[20–23] Furthermore, a recent study in a mouse model has shown that *BTNL2* binds to a putative receptor on activated T cells and inhibits T-cell proliferation.[24] As a consequence, dysfunction of the *BTNL2* could impair the normal T-cell regulation and response to antigens. In recent years, 1 important polymorphism named *G16071A* (rs2076530) in *BTNL2* gene has been wildly investigated, and it involves a G→A substitution leading to an alternative splice site that causes an early stop codon and a truncated protein.[25] Earlier studies found that the *BTNL2* gene might be responsible for the pathogenesis of *Dermatophagoides farinae*–specific immunoglobulin E responsiveness and Kawasaki disease.[26,27] Interestingly, many recent studies have indicated that there was a relationship between *BTNL2 G16071A* gene polymorphism and granulomatous diseases including sarcoidosis, TB, etc.[28,29] Rybicki et al found a strong association between *BTNL2 G16071A* gene polymorphism and sarcoidosis susceptibility in white population.[30] A similar result was reported by Milman et al and Spagnolo et al.[31,32] However, the study conducted by Lian et al suggested that there was no association between *BTNL2 G16071A* polymorphism and TB susceptibility in Chinese population,[28] similar to the result of Johnson et al. Furthermore, Mochida et al showed that there is no significant association between *BTNL2 G16071A* gene polymorphism and CD susceptibility.[33,34]

The results of those genetic association studies were inconclusive. Moreover, a single study may be too underpowered to detect a possible slight effect of the *BTNL2 G16071A* gene polymorphism on granulomatous disease susceptibility; especially the sample size is relatively small. Considering the critical role of *BTNL2 G16071A* gene polymorphism in the pathogenesis of granulomatous disorders, we performed a meta-analysis to precisely investigate the association of *BTNL2 G16071A* gene polymorphism with the likelihood of granulomatous diseases. Furthermore, to avoid the "false-positive report," we further assessed the significant associations that were observed in the current meta-analysis by the false-positive report probability (FPRP) test. To our knowledge, this is the most recent and accurate meta-analysis performed to explore the effect of *BTNL2 G16071A* gene polymorphism on susceptibility to granulomatous diseases.

2. Materials and methods

2.1. Study selection

We performed a systematic literature search in the PubMed, Embase, and Wanfang databases, and the China National Knowledge Internet, to identify studies involving the association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility up to April 1, 2016. The key words were as follows: "granulomatous" or "granulomatous diseases" or "granulomatous inflammation" or "granulomatous lesions" or "granuloma" or "sarcoidosis" or "tuberculosis" or "TB" or "Crohn's disease" or "CD" or "Wegener's granulomatosis" or "WG" or "leprosy," "*BTNL*" or "*BTNL2 G16071A*" or "*BTNL2* rs2076530," and "polymorphism" or "variant" or "mutation." Additionally, we also carried out a web-based search using many commercial Internet search engines (e.g., Google and Baidu), using the same keywords. Furthermore, the reference lists of the obtained articles were also reviewed. The language was restricted to English or Chinese. All analyses were based on previously published studies; thus, no ethical approval and patient consent are required.

2.2. Inclusion and exclusive criteria

The inclusive criteria were as follows: a study designed as case–control study, a study involving association between *BTNL2 G16071A* gene polymorphism and granulomatous disease (sarcoidosis, TB, CD, WG, or leprosy) susceptibility, a primary study providing available data for calculating the odds ratio (OR) and 95% confidence interval (CI), and the genotype distributions in the control group following the Hardy–Weinberg equilibrium (HWE). The excluded items were as follows: abstract or review and a study that did not provide available allelic or genotype frequency for counting OR and 95% CI. All analyses were based on previously published studies; thus, no ethical approval and patient consent are required.

2.3. Quality score evaluation

The qualities of included studies were assessed by the Newcastle–Ottawa Scale (case–control study), to estimate quality based on 3 aspects including selection, comparability, and exposure in the primary study. The total score ranged from 0 to 9, and 0 to 3, 4 to 6, and 7 to 9 were considered low, moderate, and high quality, respectively. In addition, we assessed the quality of the studies in a consensus meeting with all authors.

2.4. Date extraction

Two independent reviewers (Xiang Tong and Yao Ma) extracted the information from each study and used a predesigned data extraction Excel form. If there was a disagreement, a third reviewer (Xundong Niu) further assessed these articles. The following information was collected: first author, publication year, country, ethnicity, age of participant, genotype and allele distribution, sample size, granulomatous disease type, and genotyping method.

2.5. Statistical analysis

The current meta-analysis was performed using the STATA 12.0 software. Before performing the meta-analysis, the HWE was evaluated by χ^2 test in the control group of each study. We used OR and 95% CI to investigate the strength of association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility. The χ^2 statistics test and I^2 test were used to evaluate the heterogeneity. The OR would be assessed using a random-effect model if the heterogeneity was considered statistically significant ($P < 0.10$ and $I^2 > 50\%$). Otherwise, the pooled OR was calculated by the fixed-effect model. In addition, meta-regression was used to explore the sources of between-study heterogeneity. We investigated the association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility in the dominant model (AA + AG vs GG), recessive model (AA vs AG + GG), codominant models (AA vs GG, AG vs GG), and allele model (A vs G). To evaluate ethnicity and types of disease-specific effects, subgroup analyses were performed by ethnicity and types of diseases for *BTNL2 G16071A* gene polymorphism. Publication bias was assessed using several methods including funnel plot and Begg and Egger test. The sensitivity analysis was conducted to evaluate the stability of results by excluding individual study each time.

Moreover, to evaluate whether significant associations ($P < 0.05$) detected in the present study are "noteworthy," we further calculated the FPRP value at a probability level of 0.001 and an OR of 1.5. In the FPRP test, we set a FPRP cutoff value of 0.2 as suggested by a previous study,[35] and only the results with FPRP <0.2 were considered "noteworthy."

3. Results

3.1. Study characteristics

In total, all 56 articles were identified when we initially searched in PubMed, Embase, and Wanfang databases, China National Knowledge Internet, and commercial Internet search engines according to the search strategy (Fig. 1). Seventeen studies were excluded because they were duplicated studies. Fourteen articles were removed after initial screening of titles and abstracts. After further, full-view screening, 3 articles were excluded because they investigated the relationship between granulomatous disease susceptibility and other polymorphisms (CNV_ID 507 polymorphism, *BAT1-LTA-TNF-BTNL2* gene, etc.)[36–38] rather than *BTNL2 G16071A* polymorphism. Two review articles were eliminated[24,39]; 1 article was not included because it was not designed as a case–control study.[40] Two articles did not extract the available data to further count the pooled OR and 95% CI[41,42]; 1 article was excluded because it mainly included family members of patients with sarcoidosis rather than healthy people in the control group, and 1 article was repeated.[43] Thus, all 15 articles were identified. However, according to the results of HWE test, 1 of them[44] was excluded because it did not meet the HWE in the control group. Finally, all 14 eligible case–control studies were included in the current meta-analysis.[25,28–34,45–50] In addition, the quality score found that all studies were considered as moderate to high quality. The characteristics of selected studies are listed in Table 1. Genotype distributions for each case–control study are shown in Table 2.

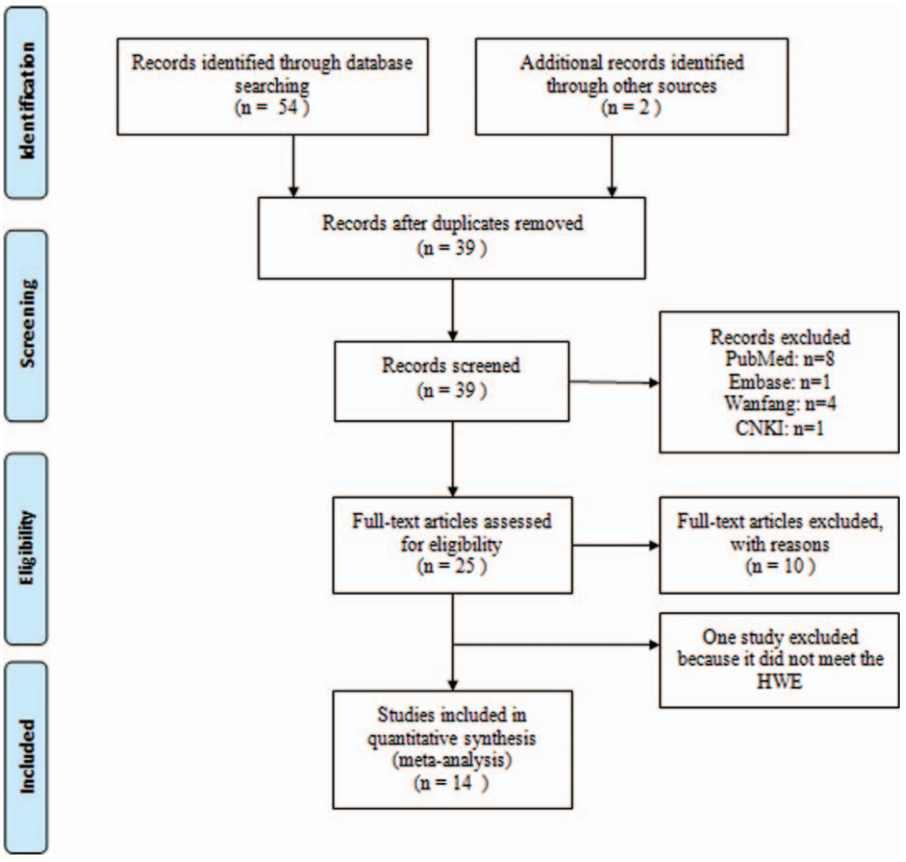

Figure 1. The flow diagram of included and excluded studies. CNKI = China National Knowledge Internet, HWE = Hardy–Weinberg equilibrium.

Table 1

Characteristics of case–control studies included in meta-analysis.

First authors	Years	Countries	Ethnicity	Ages, yr (case/control)	Sizes		Diseases
					Cases	Control	
Boutboul	2016	France	Caucasian	52.5/NR*	38	49	Sarcoidosis
Johnson	2007	England	Caucasian	26 ± 13.2/NR	476	760	Crohn disease
Li	2006	Germany	Caucasian	NR/38.32 ± 15.53	210	202	Sarcoidosis
Lian	2010	China	Asian	42/39	286	608	Tuberculosis
Milman	2011	Denmark	Caucasian	35/43	87	113	Sarcoidosis
Mochida	2007	Japan	Asian	23.6 ± 11.2/27.8 ± 10.9	290	282	Crohn disease
Moller	2007	South Africa	African	34 ± 14.8/27 ± 12.3	432	482	Tuberculosis
Morais	2012	Portugal	Caucasian	38.0 ± 4.2/NR	151	150	Sarcoidosis
Ozdemir	2014	Turkey	Caucasian	NR	53	52	Sarcoidosis
Rybicki	2005	American	Caucasian	43.6 ± 10.6/43.7 ± 10.5	366	366	Sarcoidosis
Spagnolo	2007	England + Netherlands	Caucasian	NR	225	446	Sarcoidosis
Szyld	2006	Germany	Caucasian	NR	180	261	Wegener granulomatosis
Valentonyte	2005	Germany	Caucasian	36.4 ± 11.1/NR	904	427	Sarcoidosis
Wijnen	2011	Netherlands	Caucasian	40.2 ± 11.7/48.8 ± 10.3	632	200	Sarcoidosis

* Not reported.

3.2. Overall meta-analysis results

In final, all 4324 cases and 4386 controls from 14 studies were enrolled in the meta-analysis on the association between the *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility. We used the random-effect model to estimate the pooled ORs because the results of χ^2 and I^2 tests suggested a notable heterogeneity ($P < 0.001$, $I^2 = 72.7\%$). By total analysis, the results suggested that there is a significant association between the *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility in dominant genetic model (AA + AG vs GG: OR = 1.41, 95% CI = 1.09–1.84, $P = 0.010$), recessive genetic model (AA vs AG + GG: OR = 1.40, 95% CI = 1.15–1.72, $P = 0.001$), homozygote genetic model (AA vs GG: OR = 1.70, 95% CI = 1.23–2.33, $P = 0.001$), and allele model (A vs G: OR = 1.25, 95% CI = 1.07–1.45, $P = 0.005$) (Figs. 2 and 3). But there is no association between the *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility in heterozygote genetic model (AG vs GG: OR = 1.25, 95% CI = 0.98–1.59, $P = 0.068$) (Fig. 2). The results of overall meta-analysis are summarized in Table 3.

3.3. Meta-regression analysis and subgroup analysis

In view of a notable heterogeneity, we performed a series of univariate meta-regression analyses by adding single covariates including ethnicity, the types of granulomatous diseases, genotyping methods, and quality of the studies to assess the possible confounding factors. As listed in Table 4, the meta-regression analyses showed that a large proportion of the between-study heterogeneity was significantly attributed to the ethnicity (AA vs AG + GG, $P = 0.043$; AA vs GG, $P = 0.023$; A vs G, $P = 0.013$) and the types of granulomatous diseases (AA + AG vs GG, $P < 0.001$; AA vs AG + GG, $P = 0.027$; AA vs GG, $P = 0.002$; AG vs GG, $P = 0.002$, A vs G, $P = 0.002$). Hence, we carried out subgroup analyses on ethnicity and types of diseases.

Table 2

Distributions of *BTNL2 G16071A* allelic and genotype in cases and controls.

First authors	Years	Cases					Controls					HWE	Gene methods
		AA	AG	GG	A	G	AA	AG	GG	A	G		
Boutboul	2016	11	19	8	41	35	15	23	11	53	45	0.70	NR*
Johnson	2007	100	248	128	448	504	160	372	228	692	828	0.72	TaqMan SNP Genotyping Assay
Li	2006	99	93	18	291	129	84	82	36	250	154	0.05	TaqMan SNP Genotyping Assay
Lian	2010	137	122	21	396	164	260	277	59	797	395	0.23	SNaPshot kit
Milman	2011	47	35	5	129	45	31	64	18	126	100	0.12	TaqMan SNP Genotyping Assay
Mochida	2007	/	/	/	248	332	/	/	/	265	299	/	PCR-RFLP†
Moller	2007	212	172	48	596	268	265	183	34	713	251	0.10	SNPlex Genotyping System
Morais	2012	67	63	21	197	105	43	81	26	167	133	0.25	TaqMan SNP Genotyping Assay
Ozdemir	2014	23	13	17	59	47	12	24	16	48	56	0.61	Sequencing
Rybicki	2005	157	169	40	483	249	110	182	74	402	330	0.94	PCR-automated fluorescent DNA sequencers
Spagnolo	2007	93	113	19	299	151	142	228	76	512	380	0.34	PCR-SSPs‡
Szyld	2006	36	76	68	148	212	47	135	79	229	293	0.42	PCR-RFLP
Valentonyte	2005	419	403	82	1241	567	134	221	72	489	365	0.24	TaqMan SNP Genotyping Assay
Wijnen	2011	271	294	67	836	428	76	88	36	240	160	0.24	Real-time PCR-FRETS§

HWE = Hardy–Weinberg equilibrium.

* Not reported.

† Polymerase chain reaction-restriction fragment length polymorphism.

‡ Polymerase chain reaction-sequence-specific primer.

§ Polymerase chain reaction-fluorescence resonance energy transfer assay.

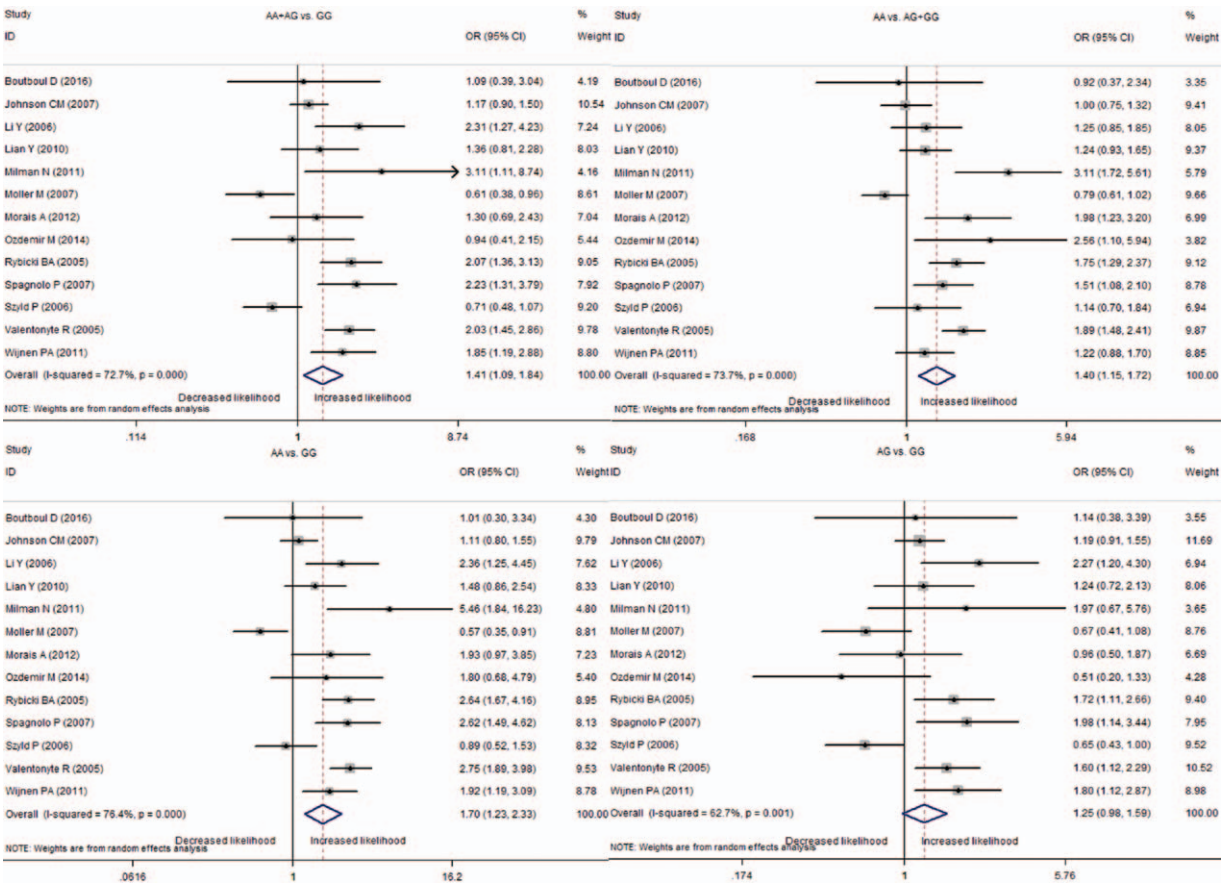

Figure 2. The results of association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility. CI = confidence interval, OR = odds ratio.

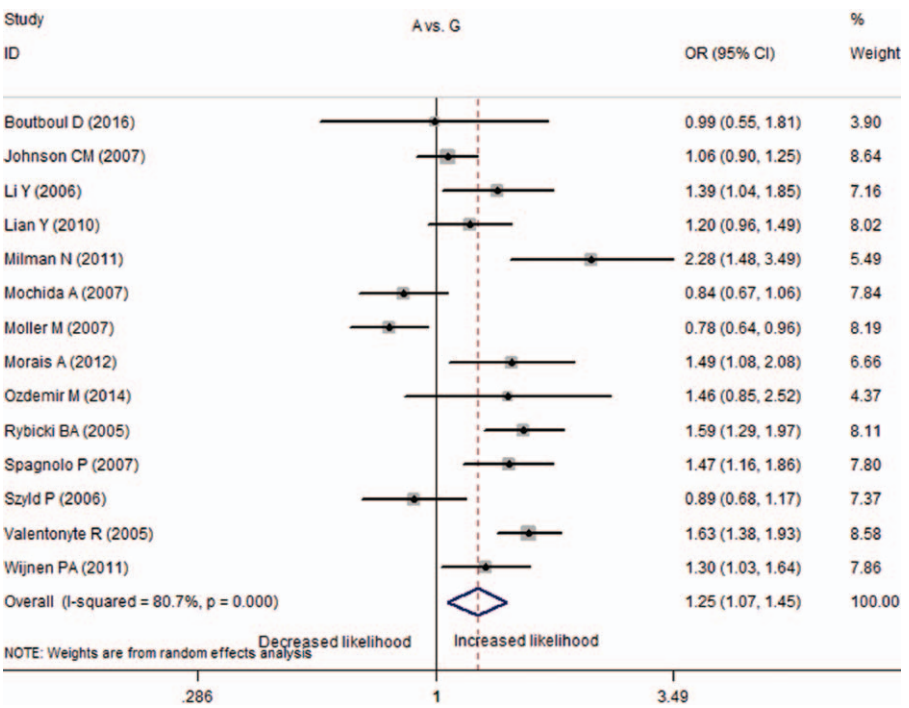

Figure 3. The result of association between granulomatous disease susceptibility and *BTNL2 G16071A* gene polymorphism (A vs G). CI = confidence interval, OR = odds ratio.

Table 3

Summary of the results of total and subgroup analyses in different genetic models.

Genetic models	Groups	OR* (95% CI†)	*P*	I^2 (%)
AA + AG vs GG	Overall	1.41 (1.09–1.84)	0.010	72.7
	Caucasians	1.55 (1.19–2.01)	0.001	67.6
	Asians	1.36 (0.81–2.28)	0.252	0
	Africans	0.61 (0.38–0.96)	0.033	0
AA vs AG + GG	Overall	1.40 (1.15–1.72)	0.001	73.7
	Caucasians	1.52 (1.25–1.84)	<0.001	61.3
	Asians	1.24 (0.93–1.65)	0.142	0
	Africans	0.79 (0.61–1.02)	0.075	0
AA vs GG	Overall	1.70 (1.23–2.33)	0.001	76.4
	Caucasians	1.92 (1.43–2.59)	<0.001	66.2
	Asians	1.48 (0.86–2.54)	0.154	0
	Africans	0.57 (0.35–0.91)	0.019	0
AG vs GG	Overall	1.25 (0.98–1.59)	0.068	62.7
	Caucasians	1.34 (1.04–1.73)	0.025	60.2
	Asians	1.24 (0.72–2.13)	0.441	0
	Africans	0.67 (0.41–1.08)	0.101	0
A vs G	Overall	1.25 (1.07–1.45)	0.005	80.7
	Caucasians	1.37 (1.18–1.58)	<0.001	68.9
	Asians	1.01 (0.71–1.42)	0.969	78.4
	Africans	0.78 (0.64–0.96)	0.018	0

* Odds ratio.
† Confidence interval.

As listed in Table 3, in the subgroup analysis by ethnicity, we found a statistically significant relationship between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility in Caucasians (11 case–control studies) (AA + AG vs GG: OR = 1.55, 95% CI = 1.19–2.01, $P = 0.001$; AA vs AG + GG: OR = 1.52, 95% CI = 1.25–1.84, $P < 0.001$; AA vs GG: OR = 1.92, 95% CI = 1.43–2.59, $P < 0.001$; AG vs GG: OR = 1.34, 95% CI = 1.04–1.73, $P = 0.025$; A vs G: OR = 1.37, 95% CI = 1.18–1.58, $P < 0.001$) and in Africans (only 1 study) (AA + AG vs GG: OR = 0.61, 95% CI = 0.38–0.96, $P = 0.033$; AA vs GG: OR = 0.57, 95% CI = 0.35–0.91, $P = 0.019$; A vs G: OR = 0.78, 95% CI = 0.64–0.96, $P = 0.018$) (Fig. 4), but not among Asians (2 case–control studies).

As summarized in Table 5, by the subgroup analysis by types of diseases, we observed a significant association between *BTNL2 G16071A* gene polymorphism and sarcoidosis susceptibility (9 case–control studies) (AA + AG vs GG: OR = 1.90, 95% CI = 1.59–2.27, $P < 0.001$; AA vs AG + GG: OR = 1.65, 95% CI = 1.46–1.87, $P < 0.001$; AA vs GG: OR = 2.40, 95% CI = 1.98–2.91, $P < 0.001$; AG vs GG: OR = 1.60, 95% CI = 1.33–1.93, $P < 0.001$; A vs G: OR = 1.52, 95% CI = 1.39–1.66, $P < 0.001$) (Fig. 5). However, we did not find any significant association between *BTNL2 G16071A* gene polymorphism and CD, WG, and TB susceptibility (2, 1, 2 case–control studies, respectively).

3.4. Publication bias and sensitivity analysis

The funnel plot is a symmetrical inverted funnel (Fig. 6). No publication bias was found in the Begg ($P = 0.743$) and Egger ($P = 0.695$) tests. Additionally, we executed a sensitivity analysis by sequentially excluding studies from the meta-analysis to investigate the influence of each study on the pooled results. The result of sensitivity analysis revealed that the pooled ORs were not materially altered, suggesting the stability of our meta-analysis (Fig. 7).

Table 4

Univariate meta-regression analyses of potential source of heterogeneity.

	t	*P*	95% CI*	τ^2	Adjusted R^2 (%)
AA + AG vs GG					
Ethnicity	−2.07	0.063	0.427–1.026	0.110	30.85
Type	−4.92	<0.001	0.600–0.823	0.002	98.88
Study quality	−0.30	0.770	0.486–1.732	0.177	−11.10
Test methods	<0.001	1	0.856–1.169	0.197	−13.92
AA vs AG + GG					
Ethnicity	−2.29	0.043	0.550–0.988	0.048	46.47
Type	−2.54	0.027	0.674–0.972	0.040	54.07
Study quality	0.43	0.673	0.674–1.800	0.104	−18.40
Test methods	0.08	0.936	0.891–1.133	0.117	−15.98
AA vs GG					
Ethnicity	−2.64	0.023	0.363–0.912	0.123	46.67
Type	−4.10	0.002	0.532–0.826	0.049	78.92
Study quality	−0.05	0.959	0.462–2.088	0.259	−12.35
Test methods	0.15	0.887	0.840–1.219	0.294	−13.03
AG vs GG					
Ethnicity	−1.55	0.149	0.478–1.136	0.096	21.05
Type	−4.01	0.002	0.630–0.874	0	100
Study quality	−0.56	0.590	0.475–1.560	0.138	−13.10
Test methods	−0.08	0.935	0.860–1.150	0.150	−18.07
A vs G					
Ethnicity	−2.92	0.013	0.607–0.930	0.032	50.34
Type	−3.84	0.002	0.720–0.913	0.020	68.72
Study quality	<0.001	0.998	0.687–1.454	0.072	−11.15
Test methods	−0.05	0.959	0.909–1.095	0.081	−11.81

* Confidence interval.

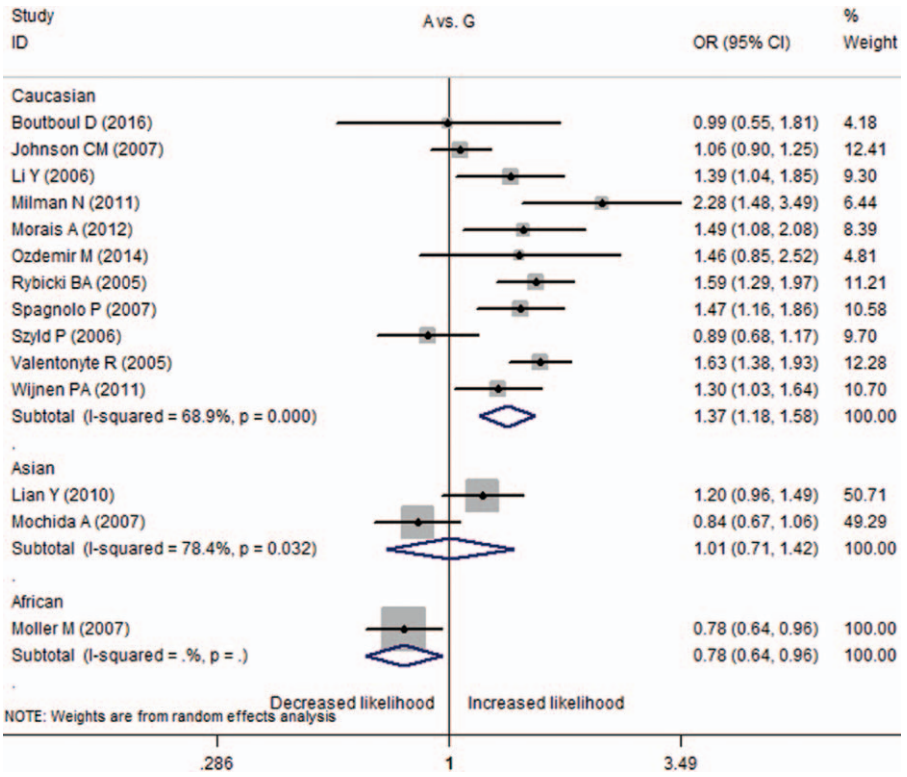

Figure 4. The result of the association between granulomatous disease susceptibility and *BTNL2 G16071A* gene polymorphism: subgroup analysis by ethnicity. CI = confidence interval, OR = odds ratio.

3.5. FPRP test results

Moreover, we further investigated the significant associations ($P < 0.05$) found in the present meta-analysis by the FPRP test sheet. As listed in Table 6, according to the results of FPRP test, we confirmed that the *BTNL2 G16071A* gene polymorphism was associated with sarcoidosis susceptibility in all gene models. On the other hand, the FPRP test suggested a truly significant association of *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility only in Caucasians.

Table 5

Summary results of subgroup analysis by disease types in different genetic models.

Genetic models	Subgroups	OR* (95% CI†)	*P*	I^2 (%)
AA+AG vs GG	Sarcoidosis	1.90 (1.59–2.27)	<0.001	0
	Tuberculosis	0.90 (0.41–1.97)	0.790	80.6
	Crohn disease	1.17 (0.90–1.50)	0.240	0
	Wegener granulomatosis	0.71 (0.48–1.07)	0.101	0
AA vs AG+GG	Sarcoidosis	1.65 (1.46–1.87)	<0.001	43.9
	Tuberculosis	0.98 (0.63–1.53)	0.945	80.9
	Crohn disease	1.00 (0.75–1.32)	0.985	0
	Wegener granulomatosis	1.14 (0.70–1.84)	0.599	0
AA vs GG	Sarcoidosis	2.40 (1.98–2.91)	<0.001	0
	Tuberculosis	0.91 (0.35–2.33)	0.840	85.4
	Crohn disease	1.11 (0.80–1.55)	0.525	0
	Wegener granulomatosis	0.89 (0.52–1.53)	0.673	0
AG vs GG	Sarcoidosis	1.60 (1.33–1.93)	<0.001	22.2
	Tuberculosis	0.90 (0.49–1.65)	0.725	64.1
	Crohn disease	1.19 (0.91–1.55)	0.212	0
	Wegener granulomatosis	0.65 (0.43–1.00)	0.052	0
A vs G	Sarcoidosis	1.52 (1.39–1.66)	<0.001	4.7
	Tuberculosis	0.97 (0.64–1.46)	0.871	87.1
	Crohn disease	0.96 (0.77–1.21)	0.735	61.1
	Wegener granulomatosis	0.89 (0.68–1.17)	0.416	0

* Odds ratio.
† Confidence interval.

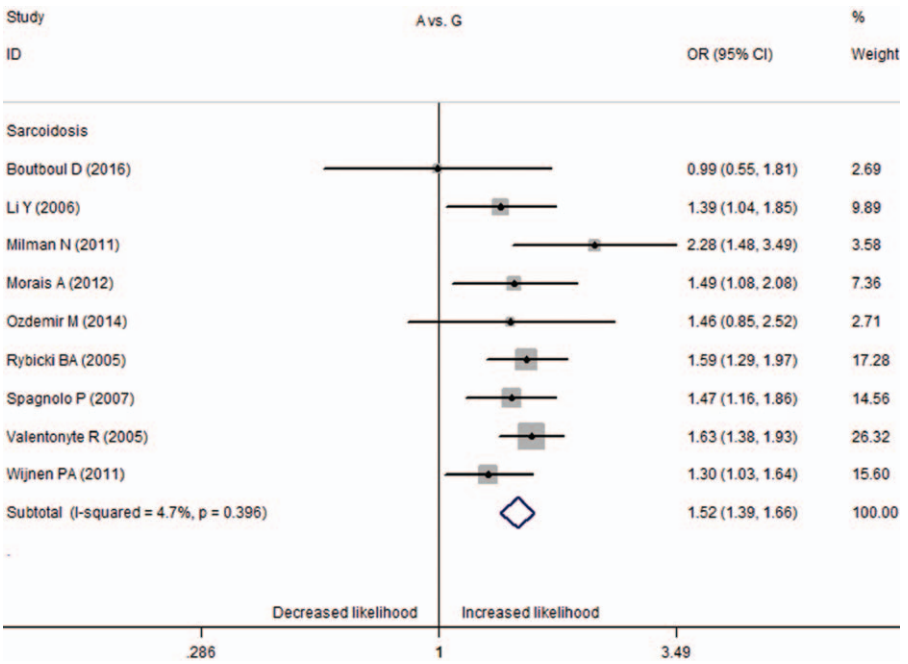

Figure 5. The result of the association between sarcoidosis susceptibility and *BTNL2 G16071A* gene polymorphism. CI = confidence interval, OR = odds ratio.

4. Discussion

In the current meta-analysis, by the total analysis, we found that *BTNL2 G16071A* gene polymorphism significantly increased the likelihood of granulomatous diseases. However, a notable heterogeneity was observed. Although the heterogeneity can be taken into account by using the random-effect model, it would increase the probability of type I error. Combining the results of the previous studies[51–53] and those of the present study, we speculated that the following factors may have contributed to the significant heterogeneity: different types of granulomatous diseases may be caused by some different mechanisms; there are different genetic and demographic characteristics of Caucasian, Asian, and African populations; each study had different genotyping methods; included studies had different quality; the included studies were of differing sample size; included studies involved different sex and age. Hence, we conducted univariate meta-regression analyses to identify the source of heterogeneity. The results suggested that the ethnicity and different types of granulomatous diseases could explain a large proportion of the heterogeneity (A vs G: adjusted $R^2 = 50.34\%$, 68.72%, $P = 0.013$, 0.002, respectively), but the quality of included studies and genotyping methods did not explain much of it.

Therefore, we further conducted subgroup analyses by ethnicity and types of diseases. By ethnicity, the meta-analysis indicated that *BTNL2 G16071A* gene polymorphism may increase granulomatous disease susceptibility among Caucasians and Africans, but not in Asians. Interestingly, the results by types of diseases suggested that the likelihood of sarcoidosis would be observably increased in people who carry A allele of *BTNL2 G16071A* polymorphism, whereas the *BTNL2 G16071A* gene polymorphism did not increase the CD, TB, and WG susceptibility.

Several previous studies indicated that the published "statistically significant" results for genetic variants have been proven to be a false-positive finding, even for large and well-designed studies.[54,55] Fortunately, as we know, we can use the FPRP test to estimate the true significant associations. The FPRP is calculated based on the statistical power of the test, the observed *P* value, and a given prior probability for the association. So, for the positive consequences of the present meta-analysis, we further investigated whether an association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility is "noteworthy." The results of FPRP test detected that the *BTNL2 G16071A* gene polymorphism actually increases sarcoidosis susceptibility in all gene models. Additionally, the FPRP test also confirmed that the *BTNL2 G16071A* gene polymorphism could increase granulomatous disease likelihood only among Caucasians. Surprisingly, the significant association of African group in the present meta-analysis was proved to be false-positive at the level of a prior probability, which was 0.001.

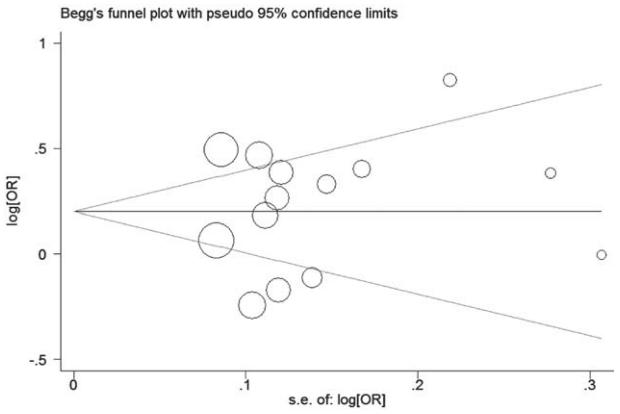

Figure 6. Funnel plot for evaluation of publication bias on association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility. OR = odds ratio, s.e. = standard error.

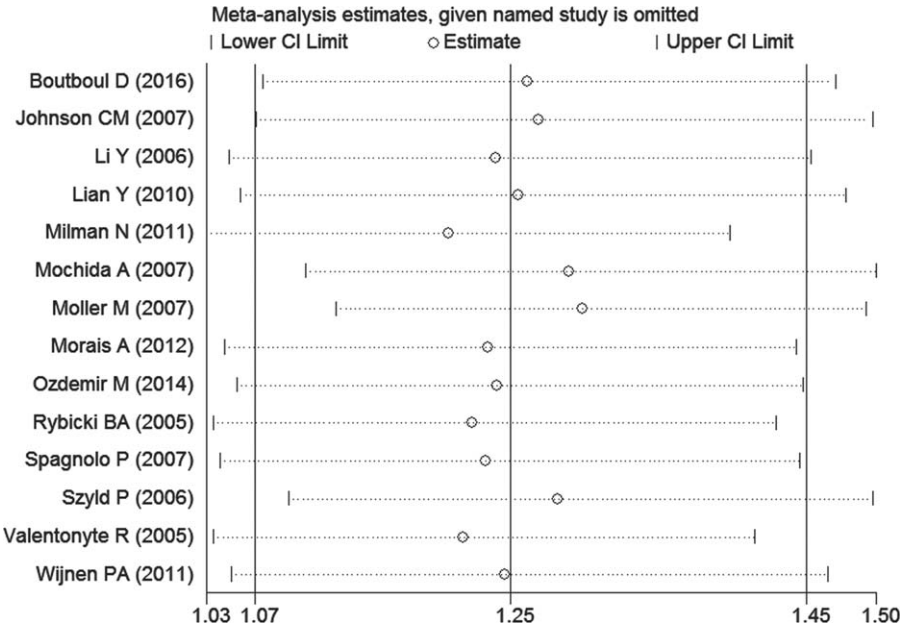

Figure 7. The result of sensitivity analysis on association between *BTNL2 G16071A* gene polymorphism and granulomatous disease susceptibility. CI = confidence interval.

Granulomas is thought to form as a consequence of a crippled immunological response against an unidentified antigen resulting in the progressive accumulation and activation of Th1 clones ($CD4^+$ and $CD8^+$ T cells).[56,57] In addition, the percentage of T cells was increased in bronchoalveolar lavage fluid from patients with sarcoidosis, where they typically made up 20% to 60% of the total cell count. Moreover, a series of studies found that the presence of both α/β T-cell receptors that recognize antigens in a major histocompatibility complex class II–restricted manner is essential for T-cell activation in sarcoidosis.[58] There have been a number of studies indicating association between T cell, HLA class II antigens, and sarcoidosis.[16] However, the role of

Table 6

The results of FPRP test in each gene model.

Gene models	OR*	95% CI†	Power (OR = 1.50)	*P* value	Prior probability = 0.001 (FPRP‡ value)
AA + AG vs GG					
Overall	1.41	1.09–1.84	0.676	0.011	0.944
Caucasians	1.55	1.19–2.01	0.402	<0.001	0.702
Africans	0.61	0.38–0.96	0.351	0.033	0.902
Sarcoidosis	1.90	1.59–2.27	0.005	<0.001	<0.001
AA vs AG + GG					
Overall	1.40	1.15–1.72	0.744	0.001	0.646
Caucasians	1.52	1.25–1.84	0.446	<0.001	0.038
Sarcoidosis	1.65	1.46–1.87	0.068	<0.001	<0.001
AA vs GG					
Overall	1.70	1.23–2.33	0.218	<0.001	0.816
Caucasians	1.92	1.43–2.59	0.053	<0.001	0.268
Africans	0.57	0.35–0.91	0.256	0.019	0.986
Sarcoidosis	2.40	1.98–2.91	<0.001	<0.001	<0.001
AG vs GG					
Caucasians	1.34	1.04–1.73	0.807	0.025	0.968
Sarcoidosis	1.60	1.33–1.93	0.250	<0.001	0.004
Wegener granulomatosis	0.65	0.43–1.00	0.454	0.050	0.991
A vs G					
Overall	1.25	1.07–1.45	0.992	0.003	0.764
Caucasians	1.37	1.18–1.58	0.894	<0.001	0.017
Africans	0.78	0.64–0.96	0.931	0.019	0.953
Sarcoidosis	1.52	1.39–1.66	<0.001	<0.001	<0.001

* Odds ratio.
† Confidence interval.
‡ False-positive report probability.

BTNL2, which is located in close proximity to the HLA class II and class III regions, was unknown.

In fact, *BTNL2* is a small immunoglobulin-like ectodomain with a C-terminal transmembrane helix and an N-terminal signal peptide anchoring the protein to the membrane of antigen-presenting cells.[20,22] A previous study found that *BTNL2* has a structural homology with B7 family of costimulatory molecule protein, which plays an important role in cross-talk between B and T lymphocytes.[20,59] Furthermore, a recent study determined that *BTNL2* can inhibit T-cell proliferation in an IL-2–independent manner and also has a further undefined interaction with B cells in a mouse model.[24] These studies showed that the BTNL2 molecule functions to inhibit T-cell activation, which has impacts in granulomatous diseases such as sarcoidosis. Interestingly, the *BTNL2 G16071A* polymorphism could cause the BTNL2 protein to turn into a truncated product lacking the immunoglobulin C domain and the transmembrane helix, further disrupting the membrane localization and influencing the immunological function.[45] Despite the fact that the mechanisms by which *BTNL2 G16071A* gene polymorphism is involved in the granulomatous disease susceptibility are still unclear, combined with the results of previous studies and those of the present study, our hypothesis was that individuals with the *BTNL2* truncated product could have a stronger T-cell response or an uncontrolled proliferation of activated T cells, depending on which T-cell subset was affected the most. This could be a disadvantage in granulomatous diseases, especially sarcoidosis, because the pathogenesis of granulomatous diseases presumably involves an instigating antigen that is presented to T cells.

Moreover, despite the fact that a previous study has found the strong linkage of the *BTNL2 G16071A* with *HLA-DRB1* and *-DQB1* genes, Valentonyte et al and Rybicki et al have confirmed that the association between *BTNL2* gene polymorphism and sarcoidosis susceptibility was independent of HLA class II alleles, which represent the strongest genetic likelihood factors to sarcoidosis identified to date.[25,30] Furthermore, the study conducted by Spagnolo et al had suggested that the *BTNL2 G16071A* gene polymorphism played a role independent of HLA-DRB1 alleles in non-Löfgren sarcoidosis.[31] However, a series of studies still reported that there is an association between the *BTNL2 G16071A* and the likelihood of granulomatous diseases (sarcoidosis, CD, and TB), which resulted from linkage disequilibrium with HLA-DRB1.[31,33,34] In addition to this, it has been recently found that strong associations between *BTNL2 G16071A* gene polymorphism and a number of diseases including type 1 diabetes, multiple sclerosis, and Graves disease were actually due to linkage disequilibrium with various HLA-DRB1 alleles, suggesting the difficulty to assign primary associations to particular HLA or non-HLA genes because of the highly variable and long-range linkage disequilibrium (LD) within this genomic area.[60–62] Unfortunately, because sufficient primary data for included studies were lacking, we failed to further perform analyses to evaluate whether the *BTNL2 G16071A* is an independent likelihood factor for granulomatous diseases. Hence, to explore whether the *BTNL2* gene polymorphism is independent of the known HLA-DRB1 alleles, finer mapping and more robust LD analyses across HLA region will be needed in the future.

Even so, several limitations of our meta-analysis were also found. First, only published studies were included in a few databases, and a publication bias may have occurred. Second, because sufficient data for each included study were lacking, we failed to perform further subgroup analysis to investigate the granulomatous disease likelihood factors such as gene–-environment/gene–gene interaction, sex, age, etc., which might affect our results. Third, the data of this meta-analysis were mainly from Caucasians, so the results might apply to only the ethnic group. Finally, the sample size (Africans, Asians, CD, TB, and WG groups) is relatively small, so the meta-analysis results involving those groups had insufficient power to reveal the reliable association. Despite these limitations, we minimized the likelihood of bias through the whole process by taking a detailed protocol, by performing study identification, data selection, and statistical analysis, as well as in the control of publication bias. Anyway, the reliability of the results is guaranteed.

In summary, the present study suggested that *BTNL2 G16071A* gene polymorphism may be a likelihood factor for granulomatous disease susceptibility, especially for sarcoidosis. And it may be strongly associated with granulomatous disease susceptibility among Caucasians. However, further well-designed studies are needed in order to confirm the results in the future.

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