The \textbf{BTNL2 G16071A} gene polymorphism increases granulomatous disease susceptibility

A meta-analysis including FPRP test of 8710 participants

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Abstract

\textbf{Objective:} The butyrophilin-like 2 (\textit{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 \textit{BTNL2} G16071A gene polymorphism and granulomatous disease susceptibility by the meta-analysis including false-positive report probability (FPRP) test.

\textbf{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.

\textbf{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 \textit{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 \textit{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 \textit{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 \textit{BTNL2} G16071A gene polymorphism was truly associated with sarcoidosis susceptibility (A vs G, FPRP < 0.001). Additionally, the FPRP test confirmed that the \textit{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.

\textbf{Conclusion:} The meta-analysis indicated that \textit{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.

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

\textbf{Keywords:} \textit{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).\textsuperscript{1,2} In addition, the fibroblasts and granulocytes also can be counted. The aggregated abnormal granuloma always results in pseudotumorous lesions.\textsuperscript{1,2} 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.\textsuperscript{1,3–6} 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.\textsuperscript{7–9} Saboor et al found that there was a significant proportion of patients with sarcoidosis having mycobacteria in their lungs.\textsuperscript{10} Additionally, the immunohistochemical staining of sarcoid granulomas shows majority of lymphocytes as CD4+ T cells, whereas the periphery of the
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.\[28\]–\[30\] 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 Mihman 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: “granulomatus” 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 information.

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 $\chi^2$ 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 $\chi^2$ 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 FPPR value at a probability level of 0.001 and an OR of 1.5. In the FPPR test, we set a FPPR cutoff value of 0.2 as suggested by a previous study, and only the results with FPPR <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. Further, after full-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.), rather than BTNL2 G16071A polymorphism. Two review articles were eliminated, 1 article was not included because it was not designed as a case–control study, 2 review articles did not extract the available data to further count the pooled OR and 95% CI; 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. Thus, all 15 articles were identified. According to the results of HWE test, 1 of them 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. 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.
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 BTLNL2 G16071A gene polymorphism and granulomatous disease susceptibility. We used the random-effect model to estimate the pooled ORs because the results of \( \chi^2 \) and \( P \) tests suggested a notable heterogeneity (\( P<0.001, \Gamma=72.7\% \)). By total analysis, the results suggested that there is a significant association between the BTLNL2 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 BTLNL2 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 1

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

| First authors | Years | Countries | Ethnicity | Ages, yr (case/control) | Cases | Control | Diseases |
|---------------|-------|-----------|-----------|------------------------|-------|---------|----------|
| 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/38.3±15.53          | 210   | 202     | Sarcoidosis |
| Lian          | 2010  | China     | Asian     | 42/39                  | 286   | 608     | Tuberculosis |
| Milman        | 2011  | Denmark   | Caucasian | 23.6±11.2/27.8±10.9    | 87    | 113     | Sarcoidosis |
| Mochida       | 2007  | Japan     | Caucasian | 23/43                  | 87    | 113     | Sarcoidosis |
| 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 |
| Styld         | 2006  | Germany   | Caucasian | NR                     | 180   | 261     | Wegener granulomatosis |
| Valentonyte   | 2005  | Germany   | Caucasian | 36.4±11/11/NR           | 904   | 427     | Sarcoidosis |
| Wijnen        | 2011  | Netherlands | Caucasian | 40.2±11.7/48.8±10.3   | 632   | 200     | Sarcoidosis |

HWE = Hardy–Weinberg equilibrium.

### Table 2

Distributions of BTLNL2 G16071A allelic and genotype in cases and controls.

| First authors | Years | Cases | Controls | HWEx | Gene methods |
|---------------|-------|-------|----------|------|--------------|
| Boutboul      | 2016  | 19    | 18       | 41   | 35           | 15   | 23 | 11 | 53 | 45 | TaqMan SNP Genotyping Assay |
| Johnson       | 2007  | 194   | 188      | 448  | 504          | 160  | 372 | 228 | 692 | 828 | TaqMan SNP Genotyping Assay |
| Li            | 2006  | 32   | 28      | 123  | 84           | 22   | 36 | 250 | 154 | PCR-RFLP |
| Lian          | 2010  | 127  | 122      | 236  | 164          | 260  | 277 | 59  | 797 | 395 | TaqMan SNP Genotyping Assay |
| Milman        | 2011  | 47   | 35      | 120  | 45           | 31   | 64 | 18  | 126 | 100 | SNaPshot kit |
| Mochida       | 2007  | /    | /        | 248  | 332          | /    | /  | 265 | 299 | /   | PCR-RFLP |
| Moller        | 2007  | 212  | 172      | 596  | 268          | 265  | 183 | 34  | 713 | 251 | TaqMan SNP Genotyping Assay |
| Morais        | 2012  | 67   | 63      | 207  | 105          | 43   | 81 | 26  | 167 | 133 | TaqMan SNP Genotyping Assay |
| Ozdemir       | 2014  | 23   | 13      | 59   | 47           | 12   | 24 | 16  | 48  | 56  | Sequencing |
| Rybicki       | 2005  | 157  | 169      | 488  | 294          | 110  | 182 | 74  | 402 | 330 | PCR-automated fluorescent DNA sequencers |
| Spagnolo      | 2007  | 93   | 113      | 299  | 151          | 142  | 228 | 76  | 512 | 380 | TaqMan SNP Genotyping Assay |
| Styld         | 2006  | 36   | 76      | 148  | 212          | 47   | 135 | 79  | 229 | 293 | TaqMan SNP Genotyping Assay |
| Valentonyte   | 2005  | 419  | 403      | 82   | 1241         | 567  | 134 | 221 | 72  | 489 | 365 | TaqMan SNP Genotyping Assay |
| Wijnen        | 2011  | 271  | 294      | 67   | 836          | 428  | 76  | 88  | 36  | 240 | TaqMan SNP Genotyping Assay |

HWE = Hardy–Weinberg equilibrium.

1 Not reported.
2 Polymerase chain reaction-restriction fragment length polymorphism.
3 Polymerase chain reaction-sequence-specific primer.
4 Polymerase chain reaction-fluorescent 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.
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 = 1.32, 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 (*I*² = 0.743) and Egger (*I*² = 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 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 (0.96–2.54) | 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.
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         |

$^\dagger$ Odds ratio.

$^\ddagger$ Confidence interval.
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.
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). 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. There have been a number of studies indicating association between T cell, HLA class II antigens, and sarcoidosis. However, the role of

![Figure 7. The result of sensitivity analysis on association between BTNL2 G18071A gene polymorphism and granulomatous disease susceptibility. CI = confidence interval.](image)

| 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 vs AG + 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 | 0.902 |
| 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 | 0.008 |
| Africans | 0.61 | 0.38–0.96 | 0.351 | 0.033 | 0.902 |
| Sarcoidosis | 1.90 | 1.59–2.27 | 0.005 | <0.001 | 0.902 |
| 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 | 0.391 | 0.019 | 0.653 |
| 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.20-23 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.20,39 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.124 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.49 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.19 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 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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References
[1] Soler P, Bassot F. Morphology and distribution of the cells of a sarcoid granuloma: ultrastructural study of serial sections. Ann NY Acad Sci 1976;278:147–60.
[2] Vanhagen P, Kreining E, Reubi J, et al. Somatostatin analogue scintigraphy in granulomatous diseases. Eur J Nucl Med 1994;21:497–502.
[3] Ulrichs T, Kosmiadi GA, Trusov V, et al. Human tubercul granulomas induce peripheral lymph follicle-like structures to orchestral host defense in the lung. J Pathol 2004;204:217–28.
[4] Mahr A, Guillevin L, Poirsonnet M, et al. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegner’s granulomatosus, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. Arthritis Care Res (Hoboken) 2004;51:92–9.
[5] Matsumoto T, Nakamura S, Jin-No Y, et al. Role of granuloma in the immunopathogenesis of Crohn’s disease. Digestion 2001;63(suppl 1):43–7.
[6] Society AT. Statement on sarcoidosis. Am J Respir Crit Care Med 1999;160:736–55.
[7] Sartor RB. Mechanisms of disease: pathogenesis of Crohn’s disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006;3:390–407.
[8] Hunninghake G, Keogh B, Line B, et al. Pulmonary sarcoidosis: pathogenesis and therapy. Basic and Clinical Aspects of Granulomatous Diseases 1980;NewYork:Elsevier/North-Holland, 275–290.
[9] Comstock G. Tuberculosis in twins: a re-analysis of the Prophit survey. Am Rev Respir Dis 1978;117:621–4.
[10] Sobo S, McFadden J, Johnson NM. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet 1992;339:1012–5.
[11] Agostini C, Adam F, Semeruzato G. New pathogenetic insights into the sarcoid granuloma. Curr Opin Rheumatol 2000;12:71–6.
[12] Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association studies and Danish ancestry informative markers identify new susceptibility loci for Crohn disease. Nat Genet 2008;40:955–62.
[13] Li H, Zhang T, Zhou Y, et al. SLC11A1 (formerly NRAMP1) gene polymorphisms and tuberculous susceptibility: a meta-analysis. J Tuberc Lung Dis 2006;10:3–12. [review article].
[14] Seitzer U, Swider C, Stüber F, et al. Tumour necrosis factor alpha promoter gene polymorphism in sarcoidosis. Cytokine 1997;9:787–90.
[15] Kishore A, Petrak M. Immunogenetics of sarcoidosis. Int Immunol 2013;1:43–53.
[16] Rossman MD, Thompson B, Frederick M, et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. Am J Hum Genet 2003;73:720–35.

[17] Iannuzzi MC, Malaiark M, Possin LM, et al. Sarcoidosis susceptibility and resistance HLA-DQB1 alleles in African Americans. Am J Respir Crit Care Med 2003;167:1225–31.

[18] Tong X, Chen L, Liu S, et al. Polymorphisms in HLA-DRB1 gene and the risk of tuberculosis: a meta-analysis of 31 studies. Lung 2015;193:309–18.

[19] Stammers M, Rowen L, Rhodes D, et al. BTL1-II: a polymorphic locus with homology to the butyrophilin gene family, located at the border of the major histocompatibility complex class II and class III regions in human and mouse. Immunogenetics 2000;51:373–82.

[20] Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol 2002;2:116–26.

[21] Riley J, June CH. The CD28 family: a T-cell rheostat for therapeutic control of T-cell activation. Blood 2005;105:13–21.

[22] Rhodes D, Stammers M, Malcherek G, et al. The cluster of BTN genes in the major histocompatibility complex class II and class III regions in human. Hum Genet 2012;131:703.

[23] Wacholder S, Chanock S, Garcia-Closas M, et al. Assessing the significance of linkage disequilibrium with HLA-DRB1 associated with ulcerative colitis in the Japanese under strong linkage disequilibrium. J Natl Cancer Inst 2004;96:434–42.

[24] Milman N, Svendsen CB, Nielsen FC, et al. The BTNL2 A allele variant is frequent in Danish patients with sarcoidosis. Clin Respir J 2011;5:324–7.

[25] Snyder P, Jajiello P, Cvaenok E, et al. On the Wegener granulomatosis associated region on chromosome 6p21. J. BMC Med Genet 2006;7:21.

[26] Boulboul D, Vince N, Mahevas M, et al. TNFA, ANXA11 and BTNL2 polymorphisms in CVID patients with granulomatous disease. J Clin Immunol 2016;36:110–2.

[27] Ozturk M, Saydam F, Kurt E, et al. Is there a genetic predisposition for Turkish patients with sarcoidosis in the 329-bp region containing the BTNL2 rs2076530 polymorphism? Turk J Med Sci 2014;44:590–4.

[28] Sutton AJ, Abrams KR, Jones DR, et al. Methods for Meta-Analysis in Medical Research. Vol 348. Chichester:Wiley; 2000.

[29] Sogo GS, Batterwith AS, Sanderson S, et al. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects. Genet Med 2009;11:139–46.

[30] Evangelou E, Ioannidis JP, Paterson NA. Uncertainty in heterogeneity estimates in meta-analyses. BMJ 2007;335:914–6.

[31] Sterne JA, Davey Smith G. Sifting the evidence—what’s wrong with significance tests? BMJ 2001;322:226–31.

[32] Ioannidis JP, Ntzani EE, Trikalinos TA, et al. Replication validity of genetic association studies. Nat Genet 2001;29:106–9.

[33] Mortaz E, Masjedi MR, Tabarsi P, et al. Immunopathology of sarcoidosis. Iran J Allergy Immunol 2014;13:300.

[34] Agostini C, Trettin L, Perin A, et al. Regulation of alveolar macrophage-T cell interactions during Th1-type sarcoid inflammatory process. Am J Physiol Lung Cell Mol Physiol 1999;277:L240–50.

[35] Traherne JA, Barcellos LF, Sawyer SJ, et al. Association of the truncating splice site mutation in BTNL2 with multiple sclerosis is secondary to HLA-DRB1*15. Hum Mol Genet 2006;15:155–61.

[36] Orozco G, Erlingham P, Sánchez E, et al. Analysis of a functional BTNL2 polymorphism in type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus. Hum Immunol 2003;66:1235–41.

[37] Cziraky T, Gombos M, Koch S, et al. Identification of a novel nonsense mutation in the BTNL2 gene. Hum Genet 2002;111:585–9.

[38] Cozzi D, Ruiz-Varela E, McKinnon C, et al. Identification of a novel nonsense mutation in the BTNL2 gene. Hum Genet 2002;111:585–9.

[39] Smith G, Brownell I, Sanchez M, et al. Advances in the genetics of sarcoidosis. Clin Genet 2008;73:401–12.

[40] Coudurier M, Freymond N, Assaous S, et al. Homozygous variant rs1076530 of BTNL2 and familial sarcoidosis. Sarcoidosis Vascul Disse Thorax Lung Dis 2009;26:162–6.

[41] Adrianto I, Lin CP, Hale JJ, et al. Genome-wide association study of African and European Americans implicates multiple shared and ethnic specific loci in sarcoidosis susceptibility. PLoS One 2012;7:e43907.

[42] Suzuki H, Ota M, Meguro A, et al. Genetic characterization and susceptibility for sarcoidosis in Japanese patients: risk factors of BTNL2 gene polymorphisms and HLA class II alleles. In: Splettstoss O, Ed. Histocompatibility Testing 2012;51:7109–15.

[43] Moller M, Kwiatkowski R, Nebel A, et al. Allelic variation in BTNL2 gene and susceptibility to tuberculosis in a South African population. Microbes Infect 2007;9:522–8.

[44] Li Y, Wollnik B, Pabst S, et al. BTNL2 gene variant and sarcoidosis. Thorax 2006;61:273–4.

[45] Wijnen PA, Voorter CE, Nelemans P, et al. Butyrophilin-like 2 in pulmonary sarcoidosis: a factor for susceptibility and progression? Hum Immunol 2011;72:342–7.

[46] Leyv S, Jajiello P, Cvaenok E, et al. On the Wegener granulomatosis associated region on chromosome 6p21. J. BMC Med Genet 2006;7:21.

[47] Traherne JA, Barcellos LF, Sawcer SJ, et al. Association of the truncating splice site mutation in tuberculosis, leprosy and Crohn disease appears to be secondary to DRB1 exon 2 position 1101: a study of Cree. Genome Med 2016;8:74.