Polymorphisms in the tumor necrosis factor gene and susceptibility to Behcet’s disease: An updated meta-analysis

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Propose: Studies investigating the association between the tumor necrosis factor (TNF) gene polymorphisms and Behcet’s disease (BD) report conflicting results. The aim of this meta-analysis was to assess the association between TNF gene polymorphisms and BD.

Methods: A systematic literature search was conducted to identify all relevant studies. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to estimate the strength of the association.

Results: A total of 16 articles, involving 1,708 patients with BD and 1,910 healthy controls, were included in the meta-analysis. Overall, a significant association was found between BD and the TNF −308A/G polymorphism (OR=0.730, 95% CI=0.608–0.877, p=0.001). Meta-analysis of TNF −238A/G showed significant association with BD (OR=1.512, 95% CI=1.155–1.979, p=0.003). The TNF −1031C allele showed significant association with BD (OR=1.549, 95% CI=1.190–2.015, p=0.001). Similarly, the meta-analysis showed a significant association of the TNF −857T/C polymorphism with BD (OR=0.758, 95% CI=0.593–0.968, p=0.027). Stratification by ethnicity revealed that the −308A/G and −857T/C polymorphisms were associated with BD in the Asian group, while the −238A/G and −1031C/T polymorphisms were associated with BD in the Caucasian population.

Conclusions: The results of our meta-analysis suggest that TNF (−308A/G, −238A/G, −1031C/T, and −857T/C) polymorphisms are associated with susceptibility to BD.

Behcet’s disease (BD) is a chronic relapsing inflammatory disease characterized by recurrent oral and genital mucous ulcers and ocular and skin lesions [1]. BD also involves vessels of all sizes, central nervous system disease, and gastrointestinal tract and thrombotic events, which are less frequent but can be life-threatening [1]. Ocular inflammation is often present at the disease onset of BD and is the initial manifestation in approximately 20% of patients. If not present at disease onset, ocular involvement occurs most commonly within 2–4 years, eventually affecting more than 50% of patients [2]. The typical form of ocular involvement is relapsing remitting uveitis that may cause significant damage to the intraocular structures. Much less frequently, ocular involvement may present in the form of conjunctival ulcers, episcleritis, scleritis, or extraocular muscle paralysis due to neurologic involvement [3-5]. Intraocular inflammation may involve the anterior or posterior segment or, more commonly, both. Since lesions affecting the posterior segment are persistent in nature and correlated with significant vision loss, anterior or posterior classification of uveitis is therapeutically and prognostically important [6]. The pathogenesis of BD remains unknown, but evidence has indicated that genetic and immunological mechanisms are related to BD. During the past two decades, the genetic participation in the pathogenesis of BD has been widely investigated. The HLA-B51 locus is recognized as a genetic marker of susceptibility to BD [7,8]. Two recent genome-wide association studies (GWASs) [9,10] indicated associations between single nucleotide polymorphisms (SNPs) of the major histocompatibility complex (MHC) class I region, some cytokines, and BD susceptibility. Studies have also implicated the abnormality of lymphocyte function in patients with BD, especially for T cell subsets. Saadoun et al. demonstrated the promotion of Th17 responses and the suppression of regulatory T cells (Tregs) that were driven by interleukin (IL)-21 production and that correlate with BD activity [11]. In a study of Japanese patients, Th22 cells played an important role in enhancing the inflammatory response in patients with BD who have uveitis through producing large amounts of IL-22 and tumor necrosis factor-α (TNF-α) [12]. In addition, epidemiological studies found that people genetically originating from an endemic region who emigrated to different nations appear to have a significantly
lower risk of BD, such as Japanese living in Hawaii [13] and
the mainland United States and Turks living in Germany
[14], suggesting that environmental factors may play a role
in BD susceptibility. Bacterial and viral infections, as well
as abnormal antigen presentation, have been implicated
in initiating immunopathological pathways leading to the
disease onset of BD, such as Streptococcus sanguis, Herpes
simplex virus 1, and heat shock proteins 60/65 [15-18]. To
date, the most comprehensive immunopathogenesis hypo-
thesis speculates that the etiology of BD can be triggered by
environmental factors in genetically susceptible individuals,
especially microbiological factors [19].

TNF-α, an important proinflammatory cytokine, is
secreted primarily by mononuclear phagocytic cells [20].
It is implicated in the pathogenesis of several inflamma-
dory disorders. TNF-α is involved in various physiologic
and pathologic processes, such as inflammation initiation,
immunoregulation, proliferation, and apoptosis [21]. Overex-
pression of proinflammatory cytokines from various cellular
sources seems to be related to the severity of inflammatory
responses in BD. Serum levels of TNF-α are increased in
patients with active BD as well as secretion of TNF-α from
stimulated peripheral blood mononuclear cells [22,23]. Indi-
vidual differences in TNF-α production are related to sever-
al single nucleotide polymorphisms (SNPs) in the TNF gene
region [24-26]. Furthermore, monocytes from patients with
BD can spontaneously generate large amounts of TNF-α [27].
Yamashita et al. showed that the levels of TNF-β produced by
the γδT cells in patients with BD were higher than those of
healthy controls [28]. However, treatment with TNF-α inhibi-
tors indicated a dramatic anti-inflammatory effect against
major BD lesions, particularly for uveitis [29-31]. These
findings indicated that TNF-α might play a pivotal role in
the pathogenesis of BD.

The TNF gene is encoded in the class III region of the
MHC on chromosome 6p21.3 [32]. Over the last decade,
numerous studies have investigated the relationship between
TNF gene polymorphisms and BD risk [23,33-47]. However,
the results of previous studies are not consistent. The discord
may be attributable to small sample size, various racial and
ethnic backgrounds, uncorrected multiple hypothesis testing,
and publication bias.

Meta-analysis is a statistical method for combining the
results of several studies to produce a single estimate of the
major effect with enhanced precision. Meta-analysis is consid-
ered a powerful tool for pooling inconsistent results from
different studies [48]. Touma et al. performed a meta-analysis
to assess the association between TNF gene polymorphisms
and BD risk, but this meta-analysis included only ten studies
[49]. More studies concerning the association between SNPs
and BD risk have been reported in recent years [43-47]. Thus,
it seems necessary to perform a meta-analysis that includes
the most updated data to investigate the relationships between
TNF gene polymorphisms and the risk of BD.

METHODS

Publication search: A systematic literature search in PubMed,
Elsevier Science Direct, the China National Knowledge
Infrastructure database (CNKI), and the Chinese Biomedical
database (CBM) was performed to identify articles. References
in the studies were reviewed to find additional studies
regarding the association between TNF gene polymorphisms
and BD risk. The text words were as follows: “Behcet’s
disease or Behcet syndrome” and “tumor necrosis factor or
tumor necrosis factor gene” combined with “single nucleo-
tide polymorphism or polymorphism or polymorphisms.”
The languages were limited to English and Chinese. The last
search was updated on August 1, 2012.

Inclusion and exclusion criteria: The inclusion criteria
were defined as follows: a) The design was a case-control or
cohort study; b) the studies evaluated the association between
TNF gene polymorphisms (−308A/G, −238A/G, −1031C/T,
−857T/C, −863A/C, −376A/G) and BD risk; c) the studies
provided sufficient data to calculate the odds ratio (OR); and
d) genotype distribution of the control population is in Hardy–
Weinberg equilibrium (HWE). Studies were excluded if one
of the following existed: a) The studies contained overlapping
data, or b) studies included family members who had been
studied because of analysis based on linkage considerations.

Data extraction: Data were collected by two independent
investigators (Xu and Wen). The characteristics of the
selected articles are shown in Table 1, including first author,
year of publication, study population, ethnicity, number of
cases and controls, findings about the polymorphisms inves-
tigated in these studies, and HWE (p value). The study popu-
lations comprised Koreans, Lebanese, Iranians,Moroccans,
Tunisians, Turks, and Germans. The Asian subgroup included
Korean, Lebanese, and Iranian populations. Moroccan and
Tunisian populations were classified in the African subgroup
and others in the Caucasian subgroup.

Statistical analysis: Allele frequencies at the TNF gene poly-
morphisms from the individual study were determined by the
counting method. HWE was tested using the χ2 test (signifi-
cant at the 0.05 level). The strength of association between
the gene polymorphisms and BD susceptibility was assessed
with ORs and 95% confidence intervals (CIs).
| First author | Year | Population | Ethnicity | Case | Control | Genotyping methods | Association | HWE (p value) |
|--------------|------|------------|-----------|------|---------|--------------------|-------------|--------------|
| Lee          | 2003 | Korean     | Asian     | 94   | 94      | PCR-SSP           | TNF −308A/G | NS           | 0.667       |
| Duymaz-Tozkir| 2003 | Turkish    | Caucasian | 99   | 96      | PCR-RFLP          | TNF −308A/G | NS           | 0.806       |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.793       |
|              |      |            |           |      |         |                    | TNF −308A/G | NS           | 0.254       |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.88        |
| Ates         | 2006 | Turkish    | Caucasian | 107  | 102     | PCR               | TNF −308A/G | NS           | 0.254       |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.88        |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.88        |
| Akman        | 2006 | Turkish    | Caucasian | 99   | 103     | PCR-RFLP          | TNF −1031C/T | p=0.018     | 0.084       |
| Park         | 2006 | Korean     | Asian     | 254  | 344     | PCR-RFLP          | TNF −308A/G | p=0.010     | 0.988       |
|              |      |            |           |      |         |                    | TNF −1031C/T | p=0.030     | 0.354       |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.88        |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.88        |
| Chang        | 2007 | Korean     | Asian     | 115  | 114     | PCR               | TNF −308A/G | NS           | 0.322       |
|              |      |            |           |      |         |                    | TNF −1031C/T | p=0.015     | 0.99        |
|              |      |            |           |      |         |                    | TNF −376A/G | NS           | 0.735       |
|              |      |            |           |      |         |                    | TNF −1031C/T | p=0.023     | 0.595       |
|              |      |            |           |      |         |                    | TNF −1031C/T | p=0.008     | 0.382       |
|              |      |            |           |      |         |                    | TNF −857T/C | NS           | 0.456       |
|              |      |            |           |      |         |                    | TNF −863A/C | NS           | 0.873       |
| Alayli       | 2007 | Turkish    | Caucasian | 80   | 105     | PCR-SSP           | TNF −308A/G | p=0.001     | 0.264       |
| Kamoun       | 2007 | Tunisian   | African   | 89   | 157     | PCR-RFLP          | TNF −1031C/T | p=0.015     | 0.99        |
| Storz(1)     | 2008 | German     | Caucasian | 92   | 51      | PCR               | TNF −308A/G | NS           | 0.701       |
| Storz(2)     | 2008 | Turkish    | Caucasian | 30   | 20      | PCR               | TNF −1031C/T | p=0.023     | 0.595       |
| Akman        | 2008 | Turkish    | Caucasian | 82   | 77      | PCR               | TNF −308A/G | NS           | 0.707       |
| Araysssi     | 2008 | Lebanese   | Asian     | 48   | 90      | NA                | TNF −308A/G | NS           | 0.707       |
|              |      |            |           |      |         |                    | TNF −1031C/T | NS           | 0.666       |
|              |      |            |           |      |         |                    | TNF −857T/C | NS           | 0.284       |
|              |      |            |           |      |         |                    | TNF −863A/C | NS           | 0.873       |
| Dilek        | 2009 | Turkish    | Caucasian | 97   | 127     | PCR-SSP           | TNF −308A/G | NS           | 0.1         |
| Bonyadi      | 2009 | Turkish    | Caucasian | 53   | 79      | PCR-RFLP          | TNF −308A/G | NS           | 0.277       |
|              |      |            |           |      |         |                    | TNF −1031C/T | p<0.001     | 0.909       |
| Ates         | 2010 | Turkish    | Caucasian | 102  | 102     | ARMS-PCR          | TNF −308A/G | NS           | 0.359       |
| Amirzargar   | 2010 | Iranian    | Asian     | 147  | 137     | PCR-SSP           | TNF −308A/G | NS           | 0.052       |
| First author | Year | Population | Ethnicity | Case | Control | Genotyping methods | Association | HWE (p value) |
|--------------|------|------------|-----------|------|---------|-------------------|-------------|--------------|
| Radouane     | 2012 | Moroccan   | African   | 120  | 112     | PCR               | TNF −308A/G NS | 0.521        |
|              |      |            |           |      |         |                   | TNF −238A/G NS | 0.448        |
|              |      |            |           |      |         |                   | TNF −857T/C NS | 0.355        |
|              |      |            |           |      |         |                   | TNF −863A/C NS | 0.147        |
|              |      |            |           |      |         |                   | TNF −376A/G NS | 0.658        |

NA: not available; NS: not significant, HWE: Hardy–Weinberg equilibrium.
The \( \chi^2 \) test-based Q statistic was used to examine the heterogeneity of between-studies [50]. The \( I^2 \) statistic measures the degree of inconsistency in the studies by computing what percentage of the total variation across studies was due to heterogeneity rather than by chance. A high \( I^2 \) value indicated a higher probability of the existence of heterogeneity (\( I^2 =0\% \) to \( 25\% \), no heterogeneity; \( I^2 =25\% \) to \( 50\% \), moderate heterogeneity; \( I^2 =50\% \) to \( 75\% \), large heterogeneity; and \( I^2 =75\% \) to \( 100\% \), extreme heterogeneity). If the \( p \) value of the heterogeneity Q statistic was less than 0.10, the random effects model was selected. Otherwise, a fixed-effects model was adopted.

Publication bias was estimated using Egger’s linear regression test and a funnel plot. If the \( p \) value was less than 0.05, statistically significant publication bias might exist [51].

All the statistical analyses for the meta-analysis were performed with STATA statistical software (version 11.0 STATA Corp, College Station, TX).

**RESULTS**

**Literature search and study characteristics:** The process for selecting the studies is shown in Figure 1. Fifty potentially relevant studies were reviewed, and 16 articles met the inclusion criteria and were finally included in our meta-analysis. Of the 16 articles, one study [40] included two cohorts; therefore, each cohort was considered a separate study. Finally, a total of 17 case-control studies in 16 articles were identified [23,33-47], including 1,708 patients with BD and 1,910 healthy controls. There were 11 studies on −308A/G, eight studies on −238A/G, seven studies on −1031C/T, four studies on −857T/C, three studies on −863A/C, and three studies on −376A/G. Nine studies involved Caucasian populations [23,34,35,38,40,41,43-45], five studies involved Asian populations [33,36,37,42,46], and two studies involved African populations [39,47]. The main characteristics of each study included in this meta-analysis are shown in Table 1.

**Meta-analysis of tumor necrosis factor gene polymorphisms in Behcet’s disease:** A summary of the meta-analysis of the relationship between TNF gene polymorphisms and BD is listed in Table 2.

**Tumor necrosis factor −308A/G polymorphism and Behcet’s disease:** Eleven studies determined the relationship between the −308A/G polymorphism and BD risk [33,37,42-47]. The total sample size for patients with BD and healthy controls was 1,232 and 1,397, respectively. Meta-analysis revealed an association between −308A and BD risk (OR=0.730, 95% CI=0.608–0.877, \( p=0.001 \); Figure 2). Stratification by ethnicity indicated that the −308A allele was significantly associated with BD risk in the Asian population (OR=0.676, 95% CI=0.511–0.894, \( p=0.006 \); Figure 2).

**Tumor necrosis factor −238A/G polymorphism and Behcet’s disease:** Eight case-control studies including 842 cases and
## Table 2. Meta-analysis of the TNF gene polymorphisms in BD

| Polymorphisms | Population | Number of studies | Sample size | Test of association | Test of heterogeneity | Egger's test (P) |
|---------------|------------|-------------------|-------------|---------------------|-----------------------|------------------|
|               |            |                   | Case control | OR (95%CI) | Z | P | Model | $\chi^2$ | P | $I^2$ (%) |
| TNF −308A/G   | Overall    | 11                | 1232/1397   | 0.730(0.608–0.877) | 3.37 | 0.001 | F | 13.28 | 0.208 | 24.7 | 0.317 |
|               | Asian      | 5                 | 654/779     | 0.676(0.511–0.894) | 2.75 | 0.006 | F | 4.24  | 0.375 | 5.7  | 0.066 |
|               | Caucasian  | 5                 | 458/506     | 0.833(0.627–1.108) | 1.25 | 0.21  | F | 7.85  | 0.11  | 47   | 0.565 |
|               | African    | 1                 | 120/112     | 0.638(0.400–1.017) | 1.89 | 0.059 | NA | NA    | NA    | NA   | NA |
| TNF −238A/G   | Overall    | 8                 | 842/938     | 1.512(1.155–1.979) | 3.01 | 0.003 | F | 5.96  | 0.544 | 0    | 0.002 |
|               | Asian      | 5                 | 654/779     | 0.676(0.511–0.894) | 2.75 | 0.006 | F | 4.24  | 0.375 | 5.7  | 0.066 |
|               | Caucasian  | 4                 | 458/506     | 0.833(0.627–1.108) | 1.25 | 0.21  | F | 7.85  | 0.11  | 47   | 0.565 |
|               | African    | 1                 | 120/112     | 0.638(0.400–1.017) | 1.89 | 0.059 | NA | NA    | NA    | NA   | NA |
| TNF −1031C/T  | Overall    | 7                 | 738/964     | 1.549(1.190–2.015) | 3.26 | 0.001 | R | 13.54 | 0.035 | 55.7 | 0.89 |
|               | Asian      | 3                 | 413/548     | 1.421(0.876–2.303) | 1.42 | 0.154 | F | 0.66  | 0.72  | 0    | 0.627 |
|               | Caucasian  | 4                 | 309/278     | 1.556(1.074–2.253) | 2.34 | 0.019 | F | 5.2   | 0.158 | 42.3 | 0.02 |
|               | African    | 1                 | 120/112     | 1.548(0.790–3.033) | 1.27 | 0.203 | NA | NA    | NA    | NA   | NA |
| TNF −857T/C   | Overall    | 4                 | 533/660     | 0.758(0.593–0.968) | 2.22 | 0.027 | F | 0.45  | 0.93  | 0    | 0.949 |
|               | Asian      | 3                 | 326/310     | 0.757(0.583–0.983) | 2.09 | 0.037 | F | 0.45  | 0.799 | 0    | 0.974 |
|               | African    | 1                 | 120/112     | 0.763(0.375–1.553) | 0.75 | 0.456 | NA | NA    | NA    | NA   | NA |
| TNF −863A/C   | Overall    | 3                 | 489/570     | 1.101(0.707–1.713) | 0.43 | 0.671 | R | 6.31  | 0.043 | 68.3 | 0.45 |
|               | Asian      | 2                 | 369/458     | 1.091(0.551–2.158) | 0.25 | 0.803 | R | 6.15  | 0.013 | 83.7 | NA |
|               | African    | 1                 | 120/112     | 1.082(0.623–1.878) | 0.28 | 0.779 | NA | NA    | NA    | NA   | NA |
| TNF −376A/G   | Overall    | 3                 | 326/310     | 0.438(0.188–1.024) | 1.9  | 0.057 | F | 0.24  | 0.889 | 0    | 0.756 |
|               | Caucasian  | 2                 | 206/198     | 0.476(0.142–1.597) | 1.2  | 0.23  | F | 0.19  | 0.66  | 0    | NA |
|               | African    | 1                 | 120/112     | 0.405(0.123–1.334) | 1.49 | 0.137 | NA | NA    | NA    | NA   | NA |

BD: Behcet’s disease, OR: odds ratio, CI: confidence interval, F: fixed effects model, R: random effects model, NA: not available *Adjusted using the “trim and fill” method.
938 controls identified an association between the TNF −238A/G polymorphism and BD risk [35-38,40,42,47]. The pooled OR (95% CI, p value) in the A versus G allele was 1.512 (1.155–1.979, p=0.003). In the subgroup analysis by ethnicity, we found that the BD cases had a significant higher frequency of A versus G (OR=1.556, 95% CI=1.074–2.253, p=0.019) than that in the controls in the Caucasian populations. The forest plot is shown in Figure 3.

Tumor necrosis factor −1031C/T polymorphism and Behcet’s disease: Seven studies containing 738 cases and 964 controls examined the association of TNF −1031C/T and BD [23,36,37,39,41,42,44]. Results indicated a significant association between the TNF −1031C/T polymorphism and BD (OR=1.549, 95% CI=1.190–2.015, p=0.001). Stratifying by ethnicity, we found a significant association in the Caucasian population (OR=2.171, 95% CI=1.581–2.981, p=0.001).

Tumor necrosis factor −857T/C, −863A/C, and −376A/G polymorphisms and Behcet’s disease: Four studies focused on the association between the TNF −857T/C polymorphism and BD risk [36,37,42,47]. The total sample size for patients with BD and healthy controls was 533 and 660, respectively. A significant association was observed in the T versus C allele (OR=0.758, 95% CI=0.593–0.968, p=0.027). Ethnicity-specific analysis showed the −857T allele was significantly associated with BD in the Asian subjects (OR=0.757, 95% CI=0.583–0.983, p=0.037). For two other SNPs, results from the meta-analysis showed that the TNF −863A/C and −376A/G polymorphisms were not susceptible to BD. Detailed results are presented in Table 2.

Heterogeneity and publication bias: Heterogeneity of the included studies regarding each polymorphism is presented in Table 2. Heterogeneity was found between the TNF −1031C/T and −863A/C polymorphisms and overall BD susceptibility ($\chi^2=13.54$, I²=55.7%, p=0.035; $\chi^2=6.31$, I²=68.3%, p=0.043, respectively). For the TNF −863A/C polymorphism, after stratifying the analyses by ethnicity, we detected significant heterogeneity in the Asian populations ($\chi^2=6.15$, I²=83.7%, p=0.013). Evidence of publication bias was observed for the meta-analysis of the TNF −238A/G in all study subjects and the Caucasian group with a p value for Egger’s linear regression test: 0.002 and 0.020. Thus, the “trim and fill” method was used to adjust for publication bias. The adjusted OR calculation using the “trim and fill” technique remained significant (OR=1.521, 95% CI=1.159–1.995; OR=1.574, 95% CI=1.083–2.288, respectively), suggesting that these results might not be affected by publication bias.
DISCUSSION

Since the clear pathogenesis of BD remains to be elucidated, it is highly suggestive that multiple host genetic factors are involved in the development of BD [18]. TNF-α is a multifunctional cytokine secreted by monocytes that plays a central role in initiating and regulating the immune response [52]. Recently, genetic variants of the TNF gene have drawn increasing interest in the etiology of several autoimmune diseases [53,54]. Several studies have shown an association of TNF gene polymorphisms in patients with BD, but the results of individual studies were inconsistent. Radouane et al. observed that TNF −1031C constitutes a susceptibility allele for BD and genital ulcers, and reported a strong association between the −238A allele and the absence of uveitis, indicating that the −238A allele could be a good prognostic factor for anterior uveitis [47]. In contrast, Chang et al. discovered no significant difference in the allele frequency of TNF −1031C/T between patients with BD and controls in a Korean population, and the analysis of the influences of the TNF gene on various clinical manifestations of BD showed that TNF −1031C was not related to the presence of clinical features, such as oral and genital ulceration and uveitis [37]. To comprehensively analyze these associations between TNF gene polymorphisms and BD susceptibility, a meta-analysis was performed.

Overall, to our knowledge, this is the first study to confirm the association between the TNF −308A/G polymorphism and BD susceptibility. Significant associations were also identified between the TNF −238A/G, −1031C/T, and −857T/C polymorphisms and BD risk, whereas the TNF −863A/C and −376A/G polymorphisms did not appear to have a significant association with overall BD risk. These results were similar to those observed by Touma et al. in the previous meta-analysis [49].

The findings of the present study seem to contradict individual studies included in the meta-analysis, which are non-significant studies. In this meta-analysis, we found significant differences after pooling all individual studies. The reasons for this disagreement may arise from two aspects. On the one hand, although some studies are non-significant, the ORs (95% CIs) of the individual studies [34,36,37,44,46,47] draw near critical values as shown in Figure 2 and Figure 3. If these individual studies increased the sample size, they might yield significant association. On the other hand, meta-analysis is a means of increasing the effective sample size under investigation through pooling data from individual association
studies, and can overcome the limitations of individual studies, resolve inconsistencies, and reduce the likelihood that random errors are responsible for false-positive or false-negative associations; therefore, meta-analysis can enhance the statistical power of the analysis for estimating genetic effects.

In the present study, we also performed subgroup analyses by ethnicity for these polymorphisms. Our results revealed that the −308A/G and −857T/C polymorphisms were associated with BD only in Asians, while the −238A/G and −1031C/T polymorphisms were associated with BD in Caucasians. The meta-analysis of the −1031C/T polymorphism showed a significant association with Africans, but it might not be reliable because only two published articles in African population were included in the present study. Therefore, additional large sample size case-control studies should be performed in this group.

The diverse roles of the same gene polymorphism in subgroup analysis by ethnicity could be ascribed to the following major aspects. First, BD is a complex autoimmune disease, and genetic heterogeneity exists in different populations. GWASs on BD have confirmed this genetic heterogeneity [9, 10]. Similarly, rheumatoid arthritis is also a complex autoimmune disease, and genetic heterogeneity exists in different populations. GWASs have determined genetic heterogeneity for TRAF1/C5 [55, 56]. Second, autoimmune diseases are multifactorial and caused by an interaction of genetic and environmental factors. Gene-environment interactions of different populations are not all the same, and are partly affected by the various environment backgrounds, which may often play a different role in autoimmune diseases susceptibility [13, 14]. Genetic and environment factors play a key role in disease initiation of systemic lupus erythematosus as well as its evolution. A previous study demonstrated that TNF −238A/G was associated with systemic lupus erythematosus in Caucasian populations, not in African and Mexican populations, suggesting the interactions between different environments and gene might be different [57]. Third, different linkage disequilibrium (LD) patterns may contribute to the discrepancy. The TNF gene is located at the class III region of the HLA complex, adjacent to HLA-B [32], and the MHC/HLA complex is the most polymorphic genetic region [58, 59]. A polymorphism may be in LD with a nearby causal variant in one ethnic group, but not in another.

Compared with the previous meta-analysis [49], the current study involved a total of 16 articles, which is larger than the data from the previous meta-analysis. Moreover, we performed subgroup analyses by ethnicity to look at the ethnic effect on the risk of BD. In addition, several studies have reported significant associations between genetic polymorphisms and diseases when the genotype distribution of the control population deviated from HWE, but deviation from HWE in the control population might imply potential selection biases of controls or genotype errors. Therefore, we excluded studies in which HWE was absent in the controls. Thus, our meta-analysis might draw a more reliable conclusion.

Some limitations of the present study should be considered. First, this study could not analyze the potential gene-environment interactions and gene susceptibility haplotypes owing to lack of data, such as data on environmental risk factors and genotypes. Second, ocular involvement is frequent and severe, but this study could not assess the association between TNF gene polymorphisms and ocular inflammation because of the insufficient data. Third, our literature search was dependent on English and Chinese; language bias might be considered. Fourth, although adjustment using the "trim and fill" method did not affect the results of the meta-analysis, publication bias still existed, and it might have influenced the current meta-analysis. Finally, different genotyping methods and disease status might affect the data interpretation of the included studies.

In summary, this updated meta-analysis suggests that TNF −308G, −238A, −1031C, and −857C alleles might be risk alleles for BD susceptibility. However, a large sample size including more ethnic groups with careful matching between cases and controls should be considered in future association studies to confirm the results of our meta-analysis.

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REFERENCES

1. Sakane T, Takeno M, Suzuki N, Inaba G. Behcet’s disease. N Engl J Med 1999; 341:1284-91. [PMID: 10528040].
2. Evrekleoglu C. Current concepts in the etiology and treatment of Behcet disease. Surv Ophthalmol 2005; 50:297-350. [PMID: 15967189].
3. Matsuo T, Itami M, Nakagawa H. The incidence and pathology of conjunctival ulceration in Behcet’s syndrome. Br J Ophthalmol 2002; 86:140-3. [PMID: 11815335].
4. Colvard DM, Robertson DM, O’Duffy JD. The ocular manifestations of Behcet’s disease. Arch Ophthalmol 1977; 95:1813-7. [PMID: 911254].
5. Zamir E, Bodaghi B, Tugal-Tutkun I, See RF, Charlotte F, Wang RC, Wechsler B, LeHoang P, Anteby I, Rao NA. Conjunctival ulcers in Behcet’s disease. Ophthalmology 2003; 110:137-41. [PMID: 12799237].

6. Atmaca LS. Fundus changes associated with Behcet’s disease. Graefes Arch Clin Exp Ophthalmol 1989; 227:340-4. [PMID: 2777102].

7. de Menthon M, Lavalle MP, Maldini C, Guillevin L, Mahr A. HLA-B51/B5 and the risk of Behcet’s disease: a systematic review and meta-analysis of case-control genetic association studies. Arthritis Rheum 2009; 61:1287-96. [PMID: 19790126].

8. Busch R, De Riva A, Hadjinicolou AV, Jiang W, Hou T, Mellins ED. On the perils of poor editing: regulation of peptide loading by HLA-DQ and H2-A molecules associated with celiac disease and type 1 diabetes. Expert Rev Mol Med 2012; 14:e15. [PMID: 22805744].

9. Remmers EF, Cosan F, Kirino Y, Ochsendorf FR, Keitel W, Stadler R, Wollina U, Proksch E, Mikulicz N, Namba K, Horie Y, Takeno M, Sugita S, Mochizuki M, Ito N, Kera J, Okada E, Yatsu K, Song YW, Lee EB, Kitaichi YH, Bang D, O'Shea J, Wallace GR, Gadina M, Kastner DL, Gil A. Genome-wide association study identifies IL23R–IL12RB2 and IL10 as Behcet’s disease susceptibility loci. Nat Genet 2010; 42:698-702. [PMID: 20622878].

10. Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, Ito N, Kera J, Okada E, Yatsu K, Song YW, Lee EB, Kitachi N, Namba K, Horie Y, Takeno M, Sugita S, Mochizuki M, Bahram S, Ishigatsubo Y, Inoko H. Genome-wide association studies identify IL23R–IL12RB2 and IL10 as Behcet’s disease susceptibility loci. Nat Genet 2010; 42:703-6. [PMID: 20622879].

11. Geri G, Terrier B, Rosenzwaig M, Wechsler B, Touzot M, Seilhean D, Tran TA, Bodaghi B, Musset L, Soumelis V, Klatzmann D, Cacoub P, Saadoun D. Critical role of IL-21 in modulating T(H)17 and regulatory T cells in Behcet disease. J Allergy Clin Immunol 2011; 128:655-64. [PMID: 21724243].

12. Sugita S, Kawazoe Y, Imai A, Kawaguchi T, Horie S, Keino H, Takahashi M, Mochizuki M. Role of IL-12- and TNF-α-Producing Th22 Cells in Uveitis Patients with Behcet’s Disease. J Immunol 2013; 190:5799-808. [PMID: 23630362].

13. Hirohata T, Kuratsune M, Nomura A, Jimi S. Prevalence of Behcet’s syndrome in Hawaii: with particular reference to the comparison of the Japanese in Hawaii and Japan. Hawaii Med J 1975; 34:244-6. [PMID: 1165185].

14. Zouboutis CC, Kötter I, Djawari D, Kirch W, Kohl PK, Ochsendorf FR, Keitel W, Stadler R, Wollina U, Proksch E, Söhnchen R, Weber H, Gollnick HP, Hölzle E, Fritz K, Licht T, Orfános CE. Epidemiological features of Adamantianos-Behcet’s disease in Germany and Europe. Yonsei Med J 1997; 38:411-22. [PMID: 9509911].

15. Mizushima Y, Matsuda T, Hoshi K, Ohno S. Induction of Behcet’s disease symptoms after dental treatment and streptococcal antigen skin test. J Rheumatol 1988; 15:1029-30. [PMID: 3418627].

16. Tojo M, Zheng X, Yanagihori H, Oyama N, Takahashi K, Nakamura K, Kaneko F. Detection of herpes virus genomes in skin lesions from patients with Behcet’s disease and other related inflammatory diseases. Acta Derm Venereol 2003; 83:124-7. [PMID: 12735641].

17. Lehner T. The role of heat shock protein, microbial and autoimmune agents in the aetiology of Behcet’s disease. Int Rev Immunol 1997; 14:21-32. [PMID: 9203024].

18. Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D. New insights into the pathogenesis of Behcet’s disease. Autoimmun Rev 2012; 11:687-98. [PMID: 22197900].

19. Direskeneli H. Behcet’s disease: infectious aetiology, new autoantigens, and HLA-B51. Ann Rheum Dis 2001; 60:996-1002. [PMID: 11602462].

20. Beutler B, Cerami A. The biology of cachectin/TNF-A primary mediator of the host response. Ann Rev Immunol 1989; 7:625-55. [PMID: 2540776].

21. Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol 1992; 10:411-52. [PMID: 1590993].

22. Sayinlap N, Ozcebe OI, Ozdemir O, Haznedaroğlu IC, Dündar S, Kirazli S. Cytokines in Behcet’s disease. J Rheumatol 1996; 23:321-2. [PMID: 8882039].

23. Akman A, Sallakci N, Coskun M, Bacanli A, Yavuzer U, Alpsoy E, Yegin O. TNF-alpha gene 1031 T/C polymorphism in Turkish patients with Behcet’s disease. Br J Dermatol 2006; 155:350-6. [PMID: 16882174].

24. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997; 94:3195-9. [PMID: 9096369].

25. Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H, Itoh K. Polymorphism of the 5′-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 1998; 51:605-12. PMID: 9694352 [PMID: 9694352].

26. Kroeger KM, Carville KS, Abraham LJ. The −308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997; 34:391-9. [PMID: 9293772].

27. Mege JL, Dilsen N, Sanguedolce V, Gul A, Bongrand P, Roux H, Ocal L, Inanç M, Capo C. Overproduction of monocyte derived tumor necrosis factor alpha, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behcet’s disease. A comparative study with familial Mediterranean fever and healthy subjects. J Rheumatol 1993; 20:1544-9. [PMID: 8164212].

28. Yamashita N, Kaneoka H, Kaneko S, Takeno M, Oneda K, Koizumi H, Kogure M, Inaba G, Sakane T. Role of gammahdelta T lymphocytes in the development of Behcet’s disease. Clin Exp Immunol 1997; 107:241-7. [PMID: 9030859].
29. Robertson LP, Hickling P. Treatment of recalcitrant orogenital ulceration of Behcet’s syndrome with infliximab. Rheumatology (Oxford) 2001; 40:473-4. [PMID: 11312390].

30. Sfikakis PP, Theodossiadias PG, Katsiari CG, Kaklamanis P, Markomichelakis NN. Effect of infliximab on sight-threatening panuveitis in Behcet’s disease. Lancet 2001; 358:295-6. [PMID: 11498218].

31. Iwata S, Saito K, Yamaoka K, Tsujimura S, Nawata M, Suzuki K, Tanaka Y. Effects of anti-TNF-alpha antibody infliximab in refractory entero-Behcet’s disease. Rheumatology (Oxford) 2009; 48:1012-3. [PMID: 19465589].

32. Complete sequence and gene map of a human major histo-compatibility complex, The MHC sequencing consortium. Nature 1999; 401:921-3. [PMID: 10553908].

33. Lee EB, Kim JY, Lee YJ, Park MH, Song YW. TNF and TNF receptor polymorphisms in Korean Behcet’s disease patients. Hum Immunol 2006; 64:614-20. [PMID: 12770792].

34. Duyumaz-Tozikir J, Gül A, Uyar FA, Ozbek U, Saruhan-Direskeneli G. Tumour necrosis factor-alpha gene promoter region –308 and –376 G → A polymorphisms in Behcet’s disease. Clin Exp Rheumatol 2003; 21:S15-8. [PMID: 14727453].

35. Ateş A, Kinikli G, Düzgün N, Duman M. Lack of association of tumor necrosis factor-alpha gene polymorphisms with disease susceptibility and severity in Behcet’s disease. Rheumatol Int 2006; 26:348-53. [PMID: 15875188].

36. Park K, Kim N, Nam J, Bang D, Lee ES. Association of TNFA promoter region haplotype in Behcet’s Disease. J Korean Med Sci 2006; 21:596-601. [PMID: 16891799].

37. Chang HK, Jang WC, Park SB, Nam YH, Lee SS, Park YW, Kim SK. The novel -G646A polymorphism of the TNF alpha promoter is associated with the HLA-B51 allele in Korean patients with Behcet’s disease. Scand J Rheumatol 2007; 36:216-21. [PMID: 17657677].

38. Alayli G, Aydin F, Coban AY, Süllü Y, Cantürk F, Bek Y, Durupinar B, Cantürk T. Helper 1 type cytokines polymorphisms: association with susceptibility to Behcet’s disease. Clin Rheumatol 2007; 26:1299-305. [PMID: 17211678].

39. Kamoun M, Chelbi H, Houman MH, Lachef J, Hamzaoui K. Tumor necrosis factor gene polymorphisms in Tunisian patients with Behcet’s disease. Hum Immunol 2007; 68:201-5. [PMID: 17349875].

40. Storz K, Löffler J, Koch S, Vonthein R, Zouboulis CC, Fresko I, Yazici H, Kötter I. IL-6 receptor, IL-8 receptor and TNF-alpha238 (G/A) polymorphisms are not associated with Behcet’s disease in patients of German or Turkish origin. Clin Exp Rheumatol 2008; 26:S103-6. [PMID: 19026125].

41. Akman A, Sallakei N, Kacaroglu H, Tosun O, Yavuzer U, Alpasoy E, Yegin O. Relationship between periodontal findings and the TNF-alpha Gene 1031T/C polymorphism in Turkish patients with Behcet’s disease. J Eur Acad Dermatol Venereol 2008; 22:950-7. [PMID: 18355201].

42. Arayssi TK, Hamdan AR, Touma Z, Shamsheddeen W, Uthman IW, Hourani HB, Farra CG. TNF polymorphisms in Lebanese patients with Behcet’s disease. Clin Exp Rheumatol 2008; 26:S130-1. [PMID: 19026135].

43. Dilek K, Ozçimen AA, Sarıcaoğlu H, Saba D, Yücel A, Yurtkuran M, Yurtkuran M, Oral HB. Cytokine gene polymorphisms in Behcet’s disease and their association with clinical and laboratory findings. Clin Exp Rheumatol 2009; 27:S73-8. [PMID: 19796538].

44. Bonyadi M, Jahanafroz Z, Esmaeili M, Kolahi S, Khabazi A, Ebrahimi AA, Hajialilo M, Dastgiri S. TNF-alpha gene polymorphisms in Iranian Azeri Turkish patients with Behcet’s Disease. Rheumatol Int 2009; 30:285-9. [PMID: 19774383].

45. Ateş O, Dalyan L, Hatemi G, Hamuryudan V, Topal-Sarıkaya A. Topal-Sarıkaya, Analyses of functional IL10 and TNF-α genotypes in Behcet’s syndrome. Mol Biol Rep 2010; 37:3637-41. [PMID: 20191386].

46. Amirzargar A, Shahrar F, Nikooppour E, Rezaee N, Saeedfar K, Ziaei N, Davatchi F. Proinflammatory cytokte gene polymorphisms in Behcet’s disease. Eur Cytokine Netw 2010; 21:292-6. [PMID: 21059493].

47. Radouane A, Oudghiri M, Chabik A, Bennani S, Toutou I, Barat-Houari M. SNPs in the TNF-α gene promoter associated with Behcet’s disease in Moroccan patients. Rheumatology (Oxford) 2012; 51:1595-9. [PMID: 22711844].

48. Xu WD, Peng H, Zhou M, Zhang M, Li BZ, Han HF, Ye DQ. Association of RANTES and MBL gene polymorphisms with systemic lupus erythematosus: a meta-analysis. Mol Biol Rep 2013; 40:941-8. [PMID: 23065234].

49. Touma Z, Farra C, Hamdan A, Shamsheddeen W, Uthman I, Hourani H, Arayssi T. TNF polymorphisms in patients with Behcet disease: a meta-analysis. Arch Med Res 2010; 41:142-6. [PMID: 20470944].

50. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21:1539-58. [PMID: 1211919].

51. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315:629-34. [PMID: 9310563].

52. Brennan FM, Maini RN, Feldmann M. TNF alpha—a pivotal role in rheumatoid arthritis? Br J Rheumatol 1992; 31:293-8. [PMID: 1581770].

53. Negoro K, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Sato J, Shimosegawa T, Toyota T. Crohn’s disease is associated with novel polymorphisms in the 5′-flanking region of the tumor necrosis factor gene. Gastroenterology 1999; 117:1062-8. [PMID: 10535868].

54. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 2011; 7:33-42. [PMID: 21119608].

55. Kurreeman FA, Padyukov L, Marques RB, Schrod SI, Seddighzadeh M, Steoeken-Rijsergen G, van der Helm-van Mil AH, Allaart CF, Verduyn W, Houwing-Duistermaat J, Alfredsson L, Begovich AB, Klareskog L, Huizinga TW, Toes RE. A candidate gene approach identifies the TRAF1/2.
56. Kurreeman FA, Goulielmos GN, Alizadeh BZ, Rueda B, Houwing-Duistermaat J, Sanchez E, Bevova M, Radstake TR, Vonk MC, Galanakis E, Ortego N, Verduyn W, Zervou MI, Roep BO, Dema B, Espino L, Urcelay E, Boumpas DT, van den Berg LH, Wijmenga C, Koeleman BP, Huizinga TW, Toes RE, Martin J. AADEA Group; SLEGEN Consortium. The TRAF1–C5 region on chromosome 9q33 is associated with multiple autoimmune diseases. Ann Rheum Dis 2010; 69:696-9. [PMID: 19433411].

57. Zou YF, Feng XL, Pan FM, Su H, Tao JH, Ye DQ. Meta-analysis of TNF-alpha promoter −238A/G polymorphism and SLE susceptibility. Autoimmunity 2010; 43:264-74. [PMID: 20166876].

58. Campbell RD, Trowsdale J. Map of the human MHC. Immunol Today 1993; 14:349-52. [PMID: 8363724].

59. Charron D. HLA, immunogenetics, pharmacogenetics and personalized medicine. Vox Sang 2011; 100:163-6. [PMID: 21175666].