Lack of association between CTLA-4 +49A/G and -318C/T polymorphisms and Behçet's disease risk: a meta-analysis

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ABSTRACT

Objective. *To more precisely determine whether there is a significant association of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) gene polymorphisms with the susceptibility for Behçet's disease.*

Methods. *Eight studies that included data from 7 articles were identified using PubMed, Embase, Chinese Biomedical Literature Database (CBM), and Chinese National Knowledge Infrastructure (CNKI) published before March 2012. Meta-analysis was performed for two CTLA-4 gene polymorphisms, +49A/G (rs231775) and -318C/T (rs5742909). Statistical analyses were performed using software Review Manager (version 5.1) and Stata (version 11.0). The pooled odds ratio (OR) with 95% confidence interval (95%CI) were presented.*

Results. *Overall, no significant association was detected in all genetic models when all studies were pooled into the meta-analysis (for +49A/G polymorphism: A vs. G, OR=1.173, 95% CI=0.790–1.743; A/A vs. A/G+G/G, OR=1.422, 95% CI=0.718–2.814; A/A+A/G vs. G/G, OR=1.421, 95% CI=0.729–2.767; and for -318C/T polymorphism: C vs. T, OR=1.051, 95% CI=0.844–1.307; C/C vs. T/T+C/T, OR=1.154 95% CI=0.891–1.495, C/C+C/T vs. T/T, OR=1.044, 95% CI=0.301–3.617). Furthermore, in the subgroup analysis by ethnicity, there was also lack of evidence for the association in Turkish patients.*

Conclusion. *Our study failed to provide evidence for the genetic association between CTLA-4 +49A/G and -318C/T polymorphisms with Behçet's disease based on currently available evidence from literature. Further confirmations in large and well-designed studies including other CTLA-4 gene polymorphisms are needed.*

Introduction

Behçet's disease (BD) is a multisystemic inflammatory disease characterised by recurrent oral aphthae, genital ulcers and uveitis, other manifestations including skin lesions, arthritis, a positive pathergy test, thrombophlebitis, gastrointestinal ulcerations and central nervous system disease (1-3). Until now, the precise pathogenesis of BD is yet unknown and well-established genetic risk factor is associated with the HLA-B51 (4). However, the contribution of HLA-B51 to the overall genetic susceptibility to BD is estimated to be less than 20% (5). This suggests that other genetic elements in addition to HLA-B51 carry a risk for developing BD.

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4, CD152) is a co-stimulatory molecule expressed by activated T cells and interacts with the B7 molecule to transmit an inhibitory signal to T cells (6). There is increasing number of articles examined the relationship between CTLA-4 gene polymorphisms and BD risk (7-15). However, several studies in the literature have inconsistent results. Conflicting results may arise from small sample size, ethnic background, uncorrected publication bias, and multiple hypothesis testing. In 2009, Du *et al.* (8) performed a meta-analysis in order to clarify the effect of CTLA-4 +49A/G polymorphism on the risk of BD which only contained 4 studies. Therefore, we performed an updated meta-analysis of all published studies which allowed for a greater number of subjects. In conclusion, the aim of the current meta-analysis was to generate large-scale evidence on whether CTLA-4 gene polymorphisms are associated with BD susceptibility.

Materials and methods
Literature search strategy
Data were collected from the PubMed,

Embase, Chinese Biomedical Literature Database (CBM), and Chinese National Knowledge Infrastructure (CNKI) with the last search was updated on Mar 9, 2012, using the combinations search terms, "Cytotoxic T-lymphocyte antigen 4 or CTLA-4 or CTLA4", "Behçet Syndrome or Behçet's disease". All searched studies were retrieved, and their bibliographies were checked for other relevant publications. Only published studies with full-text articles were included.

Inclusion and exclusion criteria
Studies that were included in the meta-analysis had to meet all of the following criteria, 1) using a case-control design; 2) evaluation of the CTLA-4 +49A/G or -318C/T polymorphism with BD; 3) the frequencies of alleles or genotypes in case and control groups could be collected; 4) the publication was in English or Chinese; 5) the diagnosis criteria was based on the International Study Group criteria for BD or the revised criteria of BD (16). The exclusion criteria were as follows, 1) abstracts and reviews; 2) if the studies reported overlapped or duplicated sample, only the study with the largest sample numbers was included.

Data extraction
All data were extracted independently by two authors, complied with the selection criteria. In case of disagreement, the result was reviewed by a third investigator, and then reached a consensus on all items. The following characters were extracted, first author's name, country, year of publication, number of cases and healthy controls and polymorphisms be analysed in this article. Disagreement was resolved by discussion.

Statistical analysis
We examined the relationship between the allele and susceptibility to BD. Genotype contrasts, both the dominant and recessive effects models, were also used to assess the association with BD risk. The odds ratio (OR) and 95% confidence interval (95% CI) were estimated for each study. The between-study heterogeneity was assessed by the Chi-square test based Q-statistic

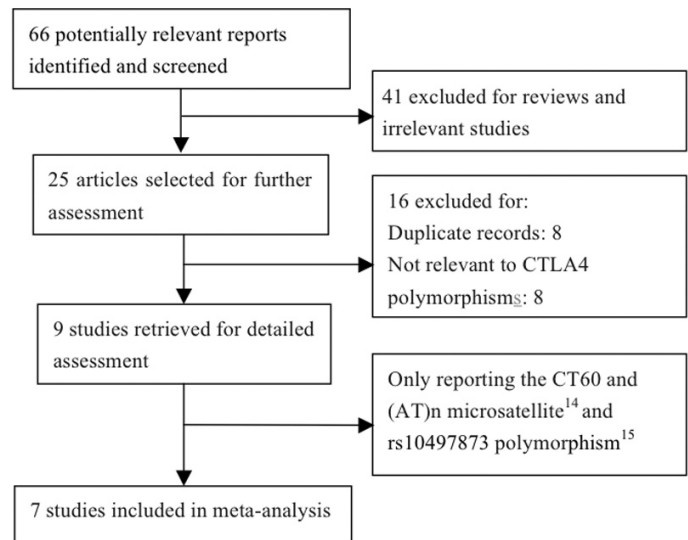**Fig. 1.** Flow diagram for study selection process.

($p \leq 0.10$ was considered to be representative of statistically significant heterogeneity). A fixed-effects model was used when there was no heterogeneity of the results; otherwise, the random-effects model was used. The significance of the pooled OR was determined by using a Z-test, and $p < 0.05$ was considered statistically significant. The forest plots were performed using Review Manager Software 5.1 (Cochrane Collaboration, Oxford, United Kingdom) and other statistical analyses were conducted by Stata 11.0 (StataCorp, College Station, Texas, USA). We also computed the power for a given number of sampling unites to detect the association between the CTLA-4 gene polymorphisms and BD with QUANTO version 1.2.4.

Results
Characteristics of studies
Figure 1 presents the flow of the literature search. There were 65 results identified relevant to the searching words and 1 result identified by manual searches. After reviewing of the titles and abstracts, 25 articles were included for detailed selection; however, 8 results were excluded for duplicate records and 8 not relevant to CTLA-4 gene polymorphisms. Thus, 9 articles were remained for detailed assessment, 2 were excluded for not relevant to -318C/T and +49A/G polymorphisms (14, 15). Then 7 articles were remained for data extraction. One of these studies contained data on two different subpopulations, and we treated them independently (11). The genotype fre-

Table I. Characteristics of studies included in the meta-analysis.

Author	Year	Country	Polymorphisms	Sample size*		*p* (HWE for controls)	
				Case	Control	+49A/G	-318C/T
Düzgün N	2010	Turkey	+49A/G	217	154	0.191	–
Du L	2009	China	+49A/G, -318C/T, C/T60,-1661A/G	228	207	0.776	0.443
Park KS	2009	Korea	+49A/G, -318C/T, -1722T/C, -1661A/G	285	287	0.985	0.021
Ben Dhifallah I	2009	Tunisia	+49A/G	135	151	0.198	–
Bye L (UK)**	2008	UK	+49A/G, -318C/T	61	129	–	–
Bye L (ME)**	2008	Middle East	+49A/G, -318C/T	175	51	–	–
Gunesacar R	2007	Turkey	+49A/G, -318C/T	123	179	0.396	0.453
Sallakci N	2005	Turkey	+49A/G	59	99	0.161	-

*The number of cases and controls may be slightly different in specific SNP. **Only the information of allele frequency available.

Table II. Results of meta-analysis for *CTLA4* polymorphisms and BD.

Polymorphism	Populat.	No. of studies	Model	Test of association				Heterogeneity			Publication bias			
				OR	95%CI	Z	*p* value	Q	*p* value	I²(%)	Begg's test	*p* value	Egger's test	*p* value
+49A/G														
A vs. G	Overall	8	Random	1.173	0.790, 1.743	0.79	0.428	70.50	0.000	90.0	1.11	0.266	0.29	0.779
	Turks	3	Fixed	1.046	0.843, 1.297	0.40	0.686	0.95	0.622	0.0	1.04	0.296	1.30	0.416
A/A vs. A/G+G/G	Overall	6	Random	1.422	0.718, 2.814	1.01	0.312	47.68	0.000	89.5	0.75	0.452	0.83	0.453
	Turks	3	Fixed	1.064	0.806, 1.403	0.44	0.663	0.83	0.660	0.0	0.00	1.000	1.04	0.486
A/A+A/G vs. G/G	Overall	6	Random	1.421	0.729, 2.767	1.03	0.302	36.09	0.000	86.1	0.38	0.707	0.79	0.474
	Turks	3	Random	1.120	0.389, 3.221	0.21	0.834	6.50	0.039	69.3	0.00	1.000	0.64	0.639
-318C/T														
C vs. T	Overall	5	Fixed	1.051	0.844, 1.307	0.44	0.658	3.68	0.450	0.0	1.71	0.086	2.84	0.066
C/C vs. T/T+C/T	Overall	3	Fixed	1.154	0.891, 1.495	1.09	0.276	1.40	0.496	0.0	1.04	0.296	1.84	0.317
C/C+C/T vs. T/T	Overall	2	Fixed	1.044	0.301, 3.617	0.07	0.946	0.50	0.478	0.0	0.00	1.000	-	-
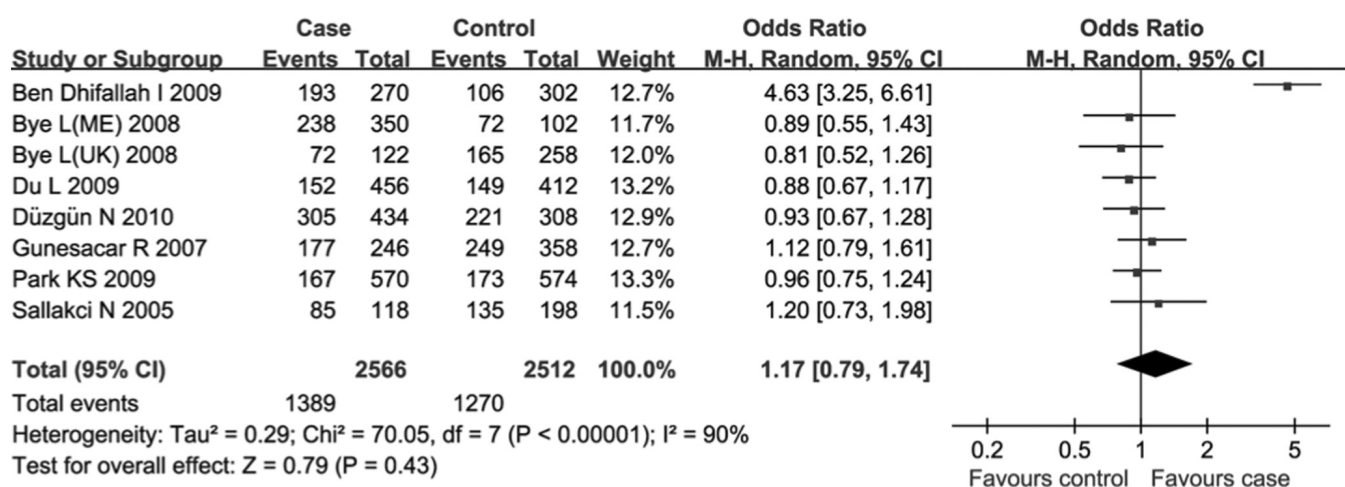
Fig. 2. Forest plot of overall BD risk associated with +49A/G polymorphism (A vs. G).
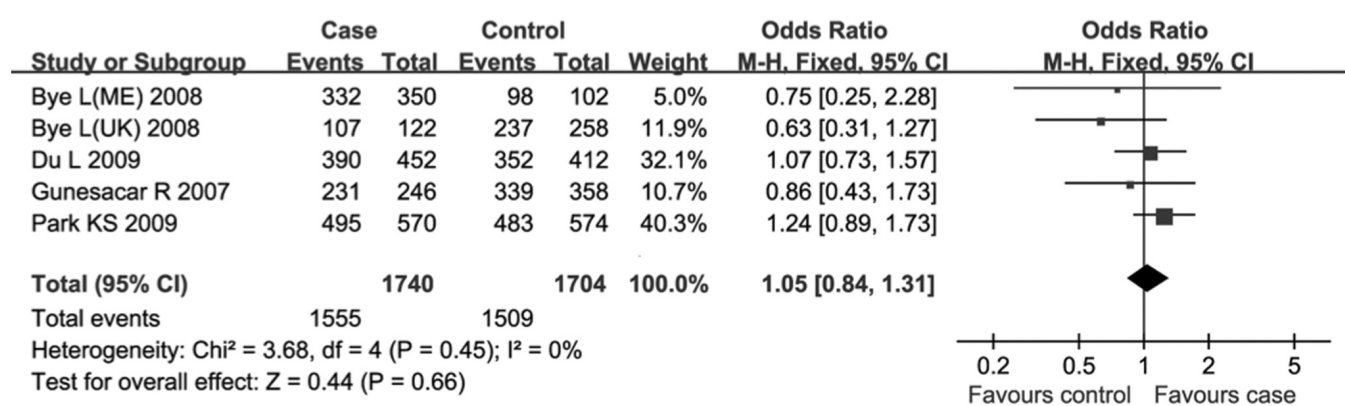
Fig. 3. Forest plot of overall BD risk associated with the -318C/T polymorphism (C vs. T).

quency data were not available in the study by Bye *et al.* (11). Characteristics of studies included in the current meta-analysis are presented in Table I.

Quantitative data synthesis
There were 8 case-control studies in 7 articles between +49A/G and BD. Heterogeneity was observed, and the original data were combined by the random-effects model (Table II). Overall, no significant association were observed between +49A/G polymorphism and BD risk (OR=1.173, 95%CI=0.790-1.743 for A *vs*. G; OR=1.422, 95%CI=0.718-2.814 for A/A *vs*. A/G+G/G; OR=1.421, 95%CI=0.729-2.767 for A/A+A/G *vs*. G/G). Ben's study (10) probably is the major reason for the existence of heterogeneity, after removing it, the results were not materially altered (data not shown). In order to look for an ethnic effect, we performed the subgroup meta-analysis but this did not support

associations between this polymorphism and BD in Turks (Table II). There was no evidence of heterogeneity for -318C/T polymorphism (see Table II). The combined results based on 5 case-control studies in 4 articles showed that -318C/T polymorphism was not associated with the risk of BD under the allele, dominant, and additive models (Table II). After removed the study which was not in Hardy–Weinberg equilibrium for -318C/T polymorphism, the results were not materially altered (data not shown). The forest plots of allele comparison (A *vs*. G, C *vs*. T) were shown in Figures 2 and 3. However, we could not to perform further subgroup stratifications analysis for the limited number of published studies.

Evaluation of publication bias
Begg's funnel plot and Egger's test were performed to access the publication bias of literatures and no bias was found (Table II).

Power analysis
The target sample size of 1283 cases and 1256 controls referred to the +49A/G polymorphism and the minor allele frequency (MAF) in controls was 49.44%. The power is approximately 98.80% under the dominant model (OR=1.5, α=0.05). 870 cases and 852 controls with MAF of -318C/T polymorphism in controls being 11.44% provides approximately 95.40% statistical power to detect the association between the -318C/T polymorphism and BD under the dominant model (OR=1.5, α=0.05).

Discussion
In the present work, we could not discover a significant association between CTLA-4 gene polymorphisms and BD. BD is prevalent in Middle Eastern countries with the highest rate (between 20 and 421 per 100,000) found in Turkey (17), similarly, ethnic-specific subgroup analyses also indicated no evidence of association between the two polymorphisms and BD in Turks. BD is at the crossroad between autoimmune and autoinflammatory syndromes (18). Th1-dominant immune responses and cytokines may be associated with the pathogenesis of certain active BD

patients (19, 20). CTLA-4 can inhibit T cell activation and proliferation by outcompeting CD28 for B7 ligands, and the B7-CD28/CTLA-4 co-stimulatory pathway can provide a signal pivotal for T cell activation (21). Disruption of the normal physiologic control provided by the CTLA-4 or change of CTLA-4 expression on the cell surface due to change of CTLA-4 protein caused by genetic polymorphisms can contribute to the pathogenesis of autoimmune diseases, such as ulcerative colitis (22) and Graves' disease (23).

Our results did not support a genetic association between the polymorphisms and BD risk in the general and Turkish populations. Interestingly, Remmers EF *et al*. reported that rs10497873 polymorphism in the 3' of the CTLA-4 gene associated with BD (p=0.013) (15). The frequency of +49A allele and the AA genotype were significantly higher in patients with ocular involvement (OR=9.67, p=0.011; and OR=9.56, p=0.015, respectively) and also BD patients with erythema nodosum-like lesions had a higher A-allele frequency (OR=6.24, p=0.04) (13). Whereas we could not to perform further subgroup analysis by clinical manifestations due to limited data. In addition, serum sCTLA4 levels in BD patients were significantly lower, especially in BD patients with the CTLA-4 +49G allele, than those in healthy controls (9). The active BD patients have significantly more expression of CTLA-4 in CD4$^+$ T cells after stimulation compared with the healthy group (24), likewise, the mRNA for CTLA-4 were highly expressed compared to remission BD and healthy controls (25). These conflicting results could have resulted from a combination of one or more of the following possibilities: first, the sample size and numbers of studies were still relatively small for the meta-analysis, which may lower the power in validity. Second, heterogeneity was detected in some comparisons, and may distort the meta-analysis. Third, publication bias may present in results due to the negative result missed or small sample size studies or language bias. Last, BD disease as a complex disease, both genetic and environmental factors

contribute to the development of it. The heterogeneity of disease phenotype and susceptibility factors may exist in different regions. Thus, all of these results should be interpreted with caution. So additional primary studies may be necessary to provide evidence of more specific association between the CTLA-4 gene polymorphisms and BD.

Our analysis differs from a previous meta-analysis on the CTLA-4 gene +49A/G polymorphism and BD have been published already (8), though we get similar results. We included three more studies (7, 9-10) and analysed the -318C/T polymorphism. In addition, we performed ethnicity-specific meta-analyses of Turkish patients.

Taken together, the present meta-analysis suggests that CTLA-4 gene polymorphisms (+49A/G, -318C/T) were unlikely to be associated with risk of BD on the basis of published literature.

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