original article

Insertion/deletion polymorphism of the *ACE* gene increased risk of Behcet disease: evidence from a meta-analysis

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BACKGROUND AND OBJECTIVES: Endothelial dysfunction has a role in the development of the Behcet disease (BD). Local renin–angiotensin system (RAS) plays a crucial role in the endothelial control, and angiotensin-converting enzyme (ACE) is the monitoring component of the RAS. We investigated the relationship between the ACE Ins/Del (I/D) variants and the risk of BD.

DESIGN AND SETTINGS: A meta-analysis was conducted from all published studies on the associations between the ACE I/D polymorphism and BD.

METHODS: We systemically searched all published studies from PubMed and EMBASE, and data were quantitatively synthesized. Pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for allele, homozygous, heterozygous, and combined genetic models.

RESULTS: Out of 5 eligible studies, 676 healthy controls and 534 BD cases were included in the present meta-analysis. D allele carrier was significantly associated with increased BD risk (D vs I: *P*=.002; OR=1.321, 95% CI=1.111–1.570). Homozygous mutant DD genotype also revealed 1.5-fold increased risk (DD vs II; *P*=.004; OR=1.573, 95% CI=1.156–2.141). In addition, the dominant genetic model demonstrated an increased risk of developing BD (DD vs II+ID: *P*=.001; OR=1.610, 95% CI=1.242–2.087)

CONCLUSION: The current study suggests that *ACE* gene polymorphism (Ins/Del) contributes an increased susceptibility to BD. However, larger studies with stratified case control population and biological characterization are needed to validate this finding.

Behcet disease (BD) is a chronic inflammatory disorder,[1] characterized by a wide range of clinical manifestations, including recurrent oral and genital ulcers, skin lesions, and uveitis.[2] BD also affects all types and sizes of blood vessels, various joints, the central nervous system, lungs, and gastrointestinal system. Despite of these clinical manifestations, the etiologies of BD remain elusive, and the host genetic factors and environmental features have been attributed to the development of BD.[3]

Angiotensin-converting enzyme (ACE), also known as peptidyl dipeptidase A or kininase II, encoded by the *ACE* gene (GenBank NM_000789.2), is located on the long arm of chromosome 17.4 The local renin–angiotensin system (RAS) in the vessel walls plays a crucial role in the endothelial control of vascular tonus and contributes to the inflammatory process via stimulation of cytokine production.[5] ACE plays a key role in RAS as well as in kallikrein–kininogen systems by hydrolyzing inactive angiotensin I to active angiotensin II and inactivate the bradykinin (naturally occurring inflammatory peptides).[6] Angiotensin II also acts as a potent pro-inflammatory modulator.[7]

A common 287 base pair Ins/Del (I/D) polymorphism (ALU repeat sequence) has been reported in intron 16 of *ACE* gene and known to be associated with serum levels of circulating ACE. Subjects having the extra fragment (Ins allele) is associated with lower circulating ACE and tissue activity, and the absence of this fragment (Del allele) is associated with a comparatively higher ACE activity. However, heterozygous (Ins/Del) subjects display an intermediate level of ACE activity.[8]

Given the functional significance of this genetic variant, it is expected that ACE I/D polymorphism is possibly associated with predisposition to BD. Thus several case–control studies have been conducted to investigate the association between ACE I/D polymorphism and BD in different populations.[9-13] But the existing studies have yielded inconsistent or conflicting results. These controversies may be partly ascribed to small sample sizes, various types of genotyping quality, false-positive results, and publication biases. Meta-analysis is a powerful tool for analyzing cumulative data from studies where individual sample sizes are small and have lower statistical power.[14] Hence, the quantitative synthesis may provide clearer evidence on the association of such genetic polymorphisms with BD. We therefore performed a meta-analysis of the published studies to clarify this inconsistency and to establish a comprehensive picture of the relationship between ACE I/D polymorphisms and BD.

METHODS

Published reports search strategy and data extraction

We carried out a PubMed (Medline) and EMBASE searches and covered all research papers published with a combination of the following key words: "*ACE* gene or ACE polymorphisms and Behcet disease" (last updated on Februrary 2013). We evaluated potentially relevant genetic association studies by examining their titles and abstracts, and all published studies matching the eligible criteria were retrieved.

Inclusion and exclusion criteria

To minimize heterogeneity and facilitate the interpretation of our results, studies included in the current meta-analysis had to meet all the following criteria: (a) evaluation of the ACE I/D and BD risk, (b) use of a case–control design, (c) recruitment of confirmed BD patients and BD free controls, (d) have available genotype frequency in case and control, and (e) have publication in English language. Additionally, when the case–control study was included by more than 1 article using the same case series, we selected the study that included the largest number of individuals. The major reasons for excluding studies were as follows: (a) overlapping data, (b) case-only studies, (c) family-based studies, and (d) review articles.

Data extraction and quality assessment

For each publication, the methodological quality assessment and data extraction were independently abstracted in duplicate by 2 independent investigators using a standard protocol and data-collection form according to the inclusion criteria listed above to ensure the accuracy of the data. In the case of disagreement on any item of the data, the problem was fully discussed to reach a consensus. Characteristics abstracted from the studies included the name of the first author, the year of publication, the country of origin, the sources of cases and controls, the number of cases and controls, the types of studies, genotype frequencies, and minor allele frequencies in the controls with Hardy–Weinberg (HWE) *P* value.

Statistical analysis

We calculated the combined ORs and corresponding 95% CIs to evaluate the association between the ACE I/D polymorphism and BD risk. Heterogeneity in meta-analysis refers to the variation in study outcomes between different studies. Heterogeneity assumption was checked by the chi-square–based Q-test,[15] and a *P* value >.10 indicates a lack of heterogeneity among the studies. Besides this, the pooled OR was calculated by the fixed effects model;[16] otherwise, the random-effects model was used.[17] In addition, I^2 statistics was used to quantify inter-study variability.[18] HWE in the control group was assessed via chi-square test and a *P* value <.05 was considered significant. Publication bias was assessed by the visual inspection of funnel plots in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was also assessed by Egger linear regression test, a linear regression approach to measure funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined by the *t*-test (*P*<.05 was considered representative of statistically significant publication bias).[19] All statistical analysis for meta-analysis was performed by comprehensive meta-analysis (CMA) V2 software (Biostat, USA). CMA V2 has several advantages over other software available for computing meta-analyses (*http://www.meta-analysis.com/pages/comparisons.html*).

RESULTS

Characteristics of published studies

A total 13 articles were achieved by published report searches from PubMed (Medline) and EMBASE. All retrieved articles were examined by reading the titles and abstracts, and the full texts for the potentially relevant publications were further checked for their suitability for this meta-analysis (**Figure 1**). Besides the database search, the reference lists of the retrieved articles were

screened for other potential articles. Studies either using ACE polymorphism to predict survival or considering ACE variants as indicators for response to therapy were excluded. Studies investigating the levels of ACE mRNA or protein expression or review article were also excluded. We included only case–control or cohort design studies having frequency of all 3 genotypes. After careful screening and following inclusion and exclusion criteria, 5 eligible original published studies were included in this study (**Table 1**). The distribution of genotypes in the controls did not deviate from HWE (**Table 2**).

Publication bias

Begg funnel plot and Egger test were performed to assess the publication bias in the studies included for meta-analysis. The shape of funnel plots (figures not shown) and Egger test did not show any evidence of publication bias (**Table 3**).

Test of heterogeneity

Heterogeneity among studies was assessed by Q test and I2 statistics. Results are shown in **Table 3**. Heterogeneity was not observed in all the models, thus the fixed effects model was used for calculating OR and 95% CI.

Meta-analysis results

We pooled all the 5 studies that comprised 676 controls and 534 BD cases and used fixed effects model (based on heterogeneity test) to assess the overall association between the ACE I/D polymorphism and the risk of BD. The variant D allele was significantly associated with the risk of developing BD in terms of frequency with when compared with wild allele (D vs I: *P*=.002; OR=1.321, 95% CI=1.111-–1.570). Similarly homozygous mutant genotype DD significantly altered the risk for the occurrence of BD as compared with the wild-type homozygous II genotype (DD vs II; *P*=.004; OR=1.573, 95% CI=1.156–2.141). In addition, the analysis of the dominant genetic model indicated 1.6-fold increased risk of developing BD (DD vs II+ID: *P*=.001; OR=1.610, 95%CI=1.242–2.087) (**Figure 2**). However, Heterozygous genotype ID (ID vs II: *P*=.322; OR=1.047, 95% CI=0.772–1.420) and recessive model (TT+CT vs CC: *P*=.194; OR=0.853, 95%CI=0.623–1.169) did not demonstrate an increased risk of developing BD compared with the II genotype (**Figure 3**).

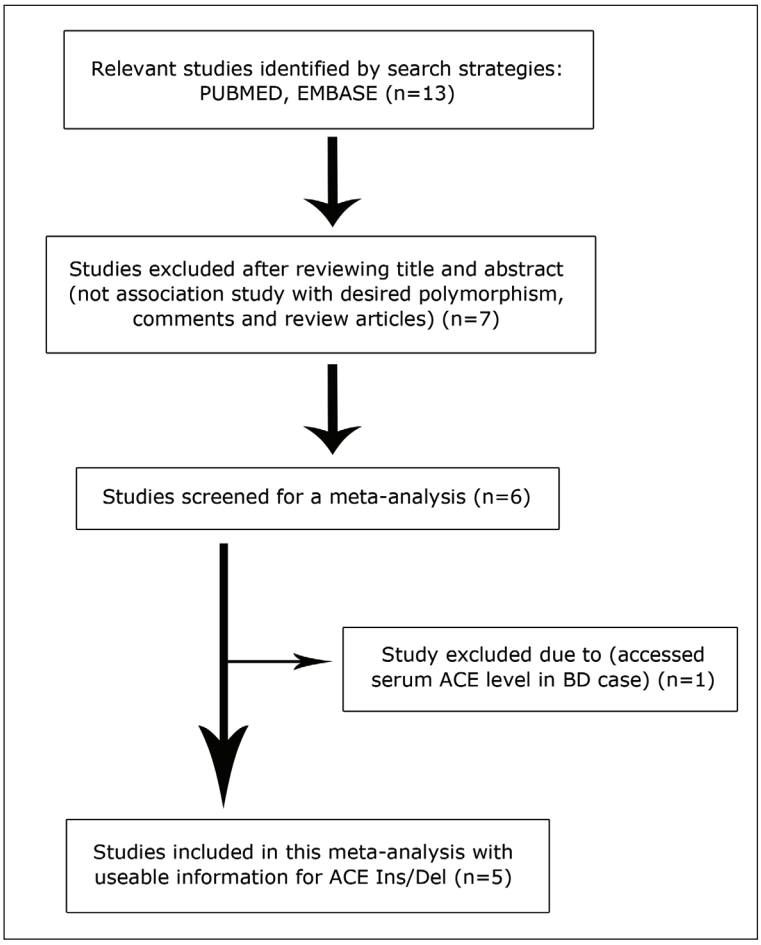


Figure 1. Flow diagram identifying potential studies for meta-analysis.

DISCUSSION

It is well known that BD is a multifactorial disease in which multiple genetic factors in combination with environmental factors and infectious agents are probably of importance in determining susceptibility. As a result, the number of candidate genes was investigated to assess the probable association between modulations of BD risk across different populations. The prevalence and incidence of the condition and its constituent manifestations show marked variability among different populations.[20] Unbiased epidemiological investigations with large the sample sizes of gene polymorphisms can provide insights into the relationship between candidate genes and diseases. In the present study, we performed a meta-analysis to examine the relationship between the ACE I/D variant in the intron 16 of the *ACE* gene and the risk of BD. The purpose of this study was to summarize the collected data of 5 studies and explore whether an association exists between the ACE I/D and the occurrence of BD risk.

ACE is a membrane-bound enzyme localized in endothelial cells and it is also present in the smooth muscle cells and the adventitial layers of the blood vessels.[21] Endothelial cell injury and/or pathologic activation are characteristic features of BD, and this enzyme plays an

Table 1. Main characteristics of all five studies included in the meta-analysis.

First authors	Year	Country	Study design	Genotyping method	Controls	Cases	Source of genotyping
Yigit et al	2013	Turkey	HB	PCR	300	266	Blood
Dursun et al	2009	Turkey	HB	PCR	90	73	Blood
Ozturk et al	2004	Turkey	HB	PCR	30	90	Blood
Chang et al	2004	Korea	HB	PCR	106	70	Blood
Turgut et al	2005	Turkey	HB	PCR	150	35	Blood

HB-Hospital-based

Table 2. Distribution of gene polymorphism of studies included in the meta-analysis.

Authors and year	Control				Case				HWE
	Genotype			Minor allele	Genotype			Minor allele	
	II	ID	DD	MAF	II	ID	DD	MAF	*P* value
Yigit et al 2013[8]	128	95	77	0.41	112	50	104	0.48	<.001
Dursun et al 2009[9]	23	35	32	0.55	12	29	32	0.63	.26
Ozturk et al 2004[10]	5	16	9	0.56	12	56	22	0.55	.63
Chang et al 2004[11]	42	44	20	0.39	25	28	17	0.44	.17
Turgut et al 2005[12]	19	44	87	0.72	2	7	26	0.84	.008

MAF-Minor allele frequency, HWE-Hardy Weinberg equilibrium

Table 3. Statistics to test publication bias and heterogeneity in meta-analysis.

Comparisons	Egger's regression analysis				Heterogeneity analysis		Model used for meta-analysis
	Intercept	95% Confidence Interval	*P* value	Q value	Phetero-geneity	I^2 (%)	
D vs I	0.19	-3.44 to 3.83	.87	2.88	0.57	<0.0001	Fixed
DD vs II	0.21	-1.72 to 2.14	.75	1.27	0.86	<0.0001	Fixed
ID vs II	2.23	-0.30 to 4.78	.06	6.28	0.17	36.30	Fixed
DD+ID vs II	1.26	-0.09 to 2.63	.05	2.52	0.64	<0.0001	Fixed
DD vs II+ID	-1.59	-5.10 to 1.91	.24	3.96	0.41	<0.0001	Fixed

integral role in the regulatory system responsible for endothelial control.[22,23] Increased *ACE* gene expression and its activity in the vessel wall led to an increased conversion of angiotensin I to angiotensin II. Angiotensin II is a growth factor that plays an active role in vascular inflammation.[24,25]

Additionally, early response to inflammation is associated with an upregulation of angiotensinogen levels. A high activity of ACE has been observed in inflammatory sites of monocytes/macrophages.[26,27] Studies have indicated that ACE inhibition improves endothelial function.[28] The levels of tissue and circulating ACE activities are regulated under tight genetic control.[6] Genetic variation in *ACE* gene plays a major role in determining ACE levels in human T lymphocytes.[29] Hence, ACE I/D polymorphism could be a genetic factor for inter-individual differences in susceptibility to BD.

In the present study, we combined published results from 5 case–control studies and found an overall increased BD risk for carriers of 1 and 2 variants allele compared with the wild allele (I) and homozygous (II) genotype. When we stratified by dominant and reces-

sive genetic model, the dominant model (DD vs II+ID) had an increased risk of BD (1.6-fold). It has been speculated that ACE I/D polymorphism accounts for approximately one-half of the variance in ACE plasma levels.[30] In earlier studies, Lee et al also reported the overall risk of ACE I/D polymorphism with BD risk.[31]

Chang et al was the first to investigate the association between the incidence of BD and ACE I/D polymorphism.[12] Thereafter, more and more studies were conducted to further assess the association of this polymorphism with BD; however, the results are inconsistent. Our meta-analysis result suggested significant association of ACE I/D polymorphism with the risk of developing BD. However, the etiology of BD is not fully understood and the single genetic variant is usually insufficient to predict the risk of this disease. One important property of this gene polymorphism is that their incidence can vary substantially between different racial or ethnic populations.[32]

Meta-analysis is a highly cost-effective method that combines the findings of independent similar studies and derives a definitive conclusion.[33] In this study we investigated the well-known I/D polymorphism of *ACE* gene and BD risk and found that this polymorphism is associated with an increased risk of BD. Our study has some advantages; first, it provides an update for this polymorphism and BD risk. Second, our results indicate that this polymorphism is associated with an increased risk of BD. Third, the methodological issues for meta-analysis, such as heterogeneity and publication bias, are well investigated.

Some limitations of our meta-analysis should be acknowledged when interpreting the results. First, since our assessment included published studies in English that were indexed by the selected electronic databases for data analysis, it is possible that some relevant studies in other databases or some unpublished studies were not included, and this may have biased our conclusions. Second, we did not study the possible association with BD severity due to unavailability of data in the included studies.

In conclusion, we found that I/D polymorphism of *ACE* gene could be a risk factor for BD. However, the sample size was small in our study, future well-designed studies including larger sample sizes with environmental factors are necessary to validate the role of this association in different populations. Such studies might eventually lead to a better and more comprehensive understanding of the association between the *ACE* gene polymorphism and BD risk.

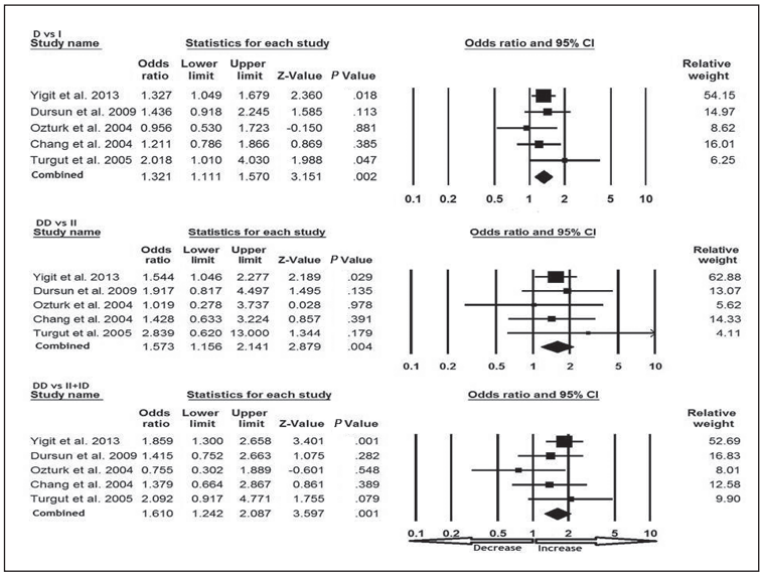
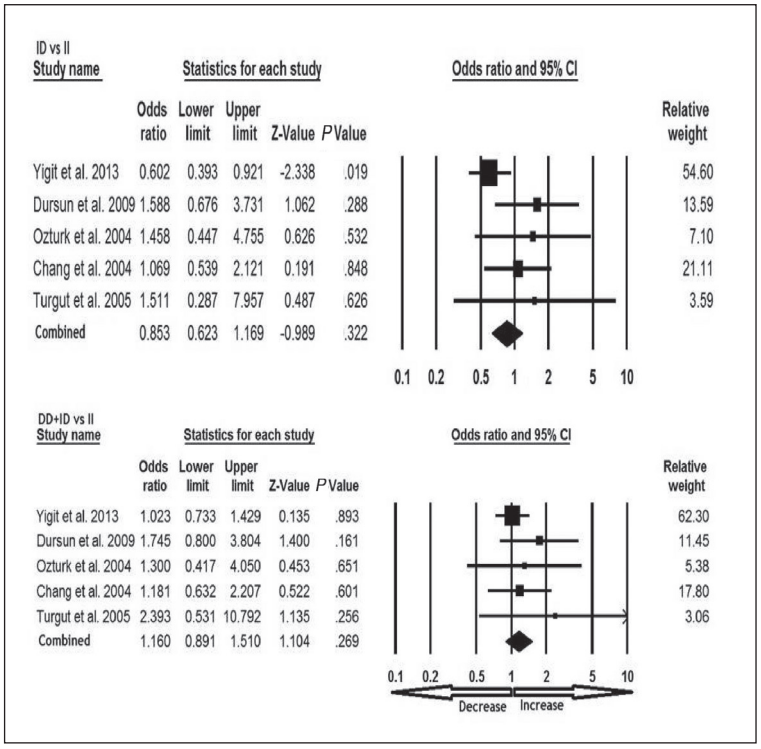


Figure 2. Forest plot of overall BD risk associated with ACE Ins/Del polymorphism. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight of the respective study.



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