

The ICAM-1 (469 T/C) gene polymorphism but not (241 G/A) is associated with Behçet's disease in the Lebanese population

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ABSTRACT

Objective: To investigate the association of the 2 intracellular adhesion molecules-1 (ICAM-1) gene polymorphisms [thymidine/cytidine (T/C) 469 and guanosine/adenosine (G/A) 241] in Behçet's disease in Lebanon.

Methods: We initiated the study in July 2003, and carried out the work in the research laboratory of Beirut Arab University, Beirut, Lebanon. We extracted the DNA by glass fiber matrix mini column. We amplified the ICAM gene by polymerase chain reaction (PCR) and tested the PCR products for the presence of the polymorphisms using a restriction enzyme specific for each polymorphism. We

analyzed the results by agarose electrophoresis.

Results: We demonstrated the association of only one single nucleotide polymorphism (SNP) (K469) with Behçet's disease, while we could not detect the other SNP (G241A) in either controls or patients in the Lebanese population.

Conclusion: The ICAM-1 gene polymorphism 469 T/C, but not 241 G/A, may encode risk for Behçet's disease in the Lebanese population.

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Behçet's disease (BD) is a systemic inflammatory disorder characterized by oral and genital ulcerations, eye lesions, and with neurological and gastrointestinal impairments. It is a rare, chronic disease that proceeds over a long time in a series of remissions and exacerbations, yet still with obscure etiology and pathogenesis.^{1,2} It also involves inflammation of blood vessels through out the body, and is known for its tendency for thromboembolism, which is thought to be due to vascular injury.³ Currently it is assumed to be immune mediated due to the common denominator of non-specific vasculitis, which affects blood vessels of all sizes, in most patients.^{3,4} Diverse adhesion molecules are known

to be involved in various immune responses and, therefore, have been implicated in the pathogenesis of autoimmune diseases. The intercellular adhesion molecule-1 (ICAM-1), a well-characterized surface glycoprotein, is known to be expressed on vascular endothelial cells, thymic epithelial cells, and activated lymphocytes. The ICAM-1 is also known to be involved in various leukocyte functions including antigen presentation and extravasations into lymphoid tissues and inflamed non-lymphoid tissues,^{5,6} and leukocyte tight adherence to activated endothelial cells.⁷ Two single-base ICAM-1 polymorphisms have been described in exon 4 and 6, changing codons 241 and 469 respectively. Both polymorphisms result

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in amino acid changes and can potentially lead to different interactions of ICAM-1 with its ligand. A previous study conducted on Italian patients with BD,⁸ showed that there was no association between ICAM-1 polymorphism at codon 469 [exon 6; thymidine/cytidine (T/C)] and BD, whereas, a strong association exists between ICAM-1 gene polymorphism at codon 241 [exon 4; guanosine/adenosine (G/A)] and BD. Contradictory results were found in a study conducted on Koreans,⁹ where a strong association was observed between ICAM-1 gene polymorphism at codon 469 (T/C) and BD. Moreover, in a study conducted on Palestinian and Jordanian patients with BD,¹⁰ a strong association was found between ICAM-1 gene polymorphism at codon 469 (T/C) and BD, while no association was found between ICAM-1 gene polymorphism at codon 241 (G/A) and BD. In this study, we investigate the association between ICAM-1 469 (T469C) and 241 (G241A) polymorphisms in BD in the Lebanese population.

Methods. Study started July 2003 and ended September 2004. Thirty-nine Lebanese patients diagnosed with BD based on the International Study Group Criteria for BD, attending the American Hospital of Beirut outpatient clinic, and 32 controls without BD, matched for ethnic background were included in the study. Most of the patients were young adults, their ages ranged between 18 and 29 years. They presented soon after the start of disease (1-4 months). Although, they complained of multisystem involvement, none had any history suggestive of thrombosis or occlusion of vessels on the venous or the arterial sides. They had anterior or posterior segment uveitis with frank microvascular involvement secondary to endothelial injury, although, the data at presentation showed no large vessel thrombotic events. It may have been too early in the course of illness for such complications to develop. Peripheral venous blood (5 ml) was drawn from each individual and genomic DNA was extracted from ethylenediaminetetraacetic acid anticoagulated sample using glass fiber matrix kit from Amersham, Biosciences, (Piscataway, USA). Analysis for both ICAM-241 and ICAM-469 gene polymorphisms was performed using the polymerase chain restriction fragment length polymorphism (PCR-RFLP) method. We designed a novel PCR assay to detect the single nucleotide polymorphism. The single base change G to A at codon 241 does not introduce or abolish a restriction site. To overcome this we mutated the forward primer by one base pair (G to T) to introduce the restriction site for the enzyme BsrGI.

Sense: 5'- CCGTGGTCTGTTCCCTGTAC 3'

Antisense: 5'- GAAGGAGTCGTTGCCATAGG-3'

A total of 100 ng genomic DNA was amplified in a 25ul PCR reaction containing 1 x ammonium (NH₄) buffer (bioline), 2.5 mM, 0.2 mM dNTP's (New England, Biolabs, Hitchin, Herts, UK), 5 pmol of each primer, and 0.5 U Taq polymerase (New England, Biolabs, Hitchin, Herts, UK). The PCR cycles were as follows, 95°C for 2 minutes followed by 35 cycles each for 45 seconds, 50°C for 45 seconds, 72°C for 45 seconds. The final extension was carried out at 72°C for 2 minutes. The presence of product (110 bp) was verified on a 2% agarose gel stained with ethidium bromide. The BsrGI will digest the PCR product only when the mutant A allele is present, yielding DNA fragments of 90 and 20 base pairs. To amplify the target DNA in the 469 polymorphic region of ICAM-1 we used the following primers:

Sense: 5'-TGGACGAGAGGGATTGTC3'

Antisense: 5'-AGAGCACATTCACGGTCAC-3'

The PCR was carried out in a volume of 25ul, contained 1 x PCR buffer (including magnesium chloride (MgCl₂) 1.5 mmol/L), Taq polymerase 0.025 U/ul, dNTP 200 umol/L, primer 0.4 mmol/L and 50 ng of DNA. After denaturation for 10 minutes at 94°C, 14 cycles of denaturation at 95°C for 30 seconds, annealing at 61°C for 45 seconds, and extension at 72°C for 45 seconds (with a touchdown program decreasing annealing temperature by 0.5°C every cycle), 30 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 45 seconds, extension at 72°C for 7 minutes. The PCR product (223 bp) was digested with restriction enzyme BstUI (6 ul PCR product and 3 U enzyme, digestion overnight at 37°C), separated on a 2.5% agarose gel, and photographed under ultraviolet light.

Allele and genotype frequencies were analyzed by generating two-by-two contingency tables and statistical analysis was performed using the chi-squared test. Allele and genotype frequencies in all subjects were calculated by direct counting. The level of significance was determined by X² and Fisher's exact tests. The strength of the gene association was indicated by the odds ratio (OR). The core of the analysis consisted of comparing heterozygous and homozygous carriers of the C allele with homozygous carrier of the T allele. A *p*-value <0.05 was considered statistically significant. The SPSS was used in the statistical analysis.

Results. ICAM-1 K469E. All patients were of the same ethnic background representing the 5 provinces in Lebanon. A single base T to C transition in exon 6 of the ICAM-1 gene leads to an amino acid substitution at codon 469 from lysine (K) to glutamine (E). After digestion, the fragment length of the wild type (TT)

Table 1 - Allele and genotype frequencies for intracellular adhesion molecules-1 469 T/C polymorphism in patients with Behçet's disease and matched controls.

Genotypes	Patients (N=39)	Controls (N=32)
TT	16 (41%)	26 (81.25%)
TC	17 (43.6%)	5 (15.6%)
CC	6 (15.4%)	1 (3.1%)
<i>Alleles</i>		
Thymidine	10.9%	37.1%
Cytidine	89%	62.8%
TT - wild type, TC - heterozygote, CC - homozygote,		

genotype was 223 bp, the fragment lengths of the heterozygote (TC) genotype are 223, 136 and 87 bp. The ICAM-1 469 T/C genotypes are presented in **Table 1**. The distribution of the ICAM-1 genotypes was significantly different between patients with BD and controls ($p < 0.01$), Chi-square = 11.9. Genotypes carrying the CC and TC are increased in frequency in the patients versus controls, this is reflected by the increased allele C frequency in patients compared to control OR=4.792, CI=1.579-14.59.

ICAM-1 arginine/glycine. We did not find the ICAM-1 241 gene polymorphism in either the patient group (n=39) or the controls (n=32).

Discussion. In this study, we identified an increased frequency of the ICAM-1 exon 6 E469 allele in patients compared to controls. The reported disease associations with ICAM-1 E/K 469 polymorphism showed that the ICAM-1 is strongly expressed in vascular endothelial cells and perivascular inflammatory infiltrates in BD.⁸ The ICAM-1 gene polymorphisms have been implicated in the susceptibility to inflammatory diseases, such as multiple sclerosis, inflammatory bowel diseases,^{11,12} and BD.¹⁰ Mycko et al,¹³ showed the existence of a strong association between ICAM gene polymorphism at codon 469 (exon 6) and multiple sclerosis. Similar results were obtained by Nejentsev et al,¹⁴ where an increased risk of multiple sclerosis was observed with (K469E) polymorphism of the ICAM-1 gene. A recent study showed that the K469E polymorphism of the ICAM-1 gene is associated with plasma fibrinogen level in type 2 diabetes, suggesting that fibrinogen is a candidate, which links the ICAM-1 gene polymorphism to atherosclerosis.¹⁵ Conversely, Lee et al,¹⁶ showed lower a frequency of the ICAM-1 K469E allele in Korean patients with rheumatoid

arthritis than that in healthy controls. The frequency of the R241G allele was demonstrated to be low in patients with BD and controls in a previously reported study of European extraction.¹⁰ We could not find this polymorphism in both patients with BD and controls, and similar results concerning the absence of the R241G polymorphism were found in Korean subjects.¹⁶ Although the number of controls in our study is small, we can confirm the absence of association between 241 ICAM-1 polymorphism and BD in Lebanon.

In conclusion, the ICAM-1 469T/C polymorphism may carry a risk for the development of BD in the Lebanese population. However, this needs to be confirmed in a larger population.

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