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Intercellular adhesion molecule-1 gene polymorphisms in patients with familial mediterranean fever

Research Article

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Abstract: Familial Mediterranean fever (FMF) is a disease of unknown etiology characterized by recurrent attacks of polyserositis and fever. Intercellular adhesion molecule-1 (ICAM-1) is known to contribute inflammatory conditions by regulating leukocyte localization at inflammatory sites. The aim of this study was to evaluate the probable association of ICAM-1 G/R 241 and ICAM-1 E/K 469 polymorphisms according to susceptibility with FMF. Sixty-seven FMF patients and 83 healthy volunteers were included in the study. Genomic DNA was extracted from EDTA-preserved whole blood of whole series of patients and controls, and genotyped by polymerase chain reaction (PCR) and allele-specific oligonucleotide techniques for ICAM-1 polymorphisms G/R at codon 241 and E/K at codon 469. The ICAM-1 241 genotype and allele frequencies of FMF patients and healthy volunteers were similar. The frequency of ICAM-1 K469 homozygosity was significantly lower in FMF patients than in the controls (32.8% vs 50.7% subsequently, p=0.03). Moreover, ICAM-1 E469 allele was more frequent in FMF patients than in controls (44.8% vs 32.3%, p:0.03). Our results showed that ICAM-1 469 gene polymorphism could contribute to the pathogenesis of FMF.

Keywords: *Familial Mediterranean fever • Intercellular adhesion molecule • Genetic polymorphism*

1. Introduction

Familial Mediterranean fever (FMF) is an inherited disease, and its symptoms include silent periods and acute attacks characterized with fever, peritonitis, pleuritis and erizipel-like skin lesions. Despite cloning of MEditerranean FeVer(MEFV), identification of the gene responsible for FMF and several FMF-related mutations in this gene, the pathogenesis of the disease is stil unclear.

Intercellular adhesion molecule-1 (ICAM-1) is expressed on a wide range of cells including leukocytes, epithelium and endothelial cells. ICAM-1 is upregulated in inflammatory conditions by cytokines such as tumor necrosis factor-beta(TNF-β), interleukin-1(IL-1) and interferon-gamma(IF-γ), and plays an important role in transendothelial migration of neutrophils and T-cell activation. Direskeneli et al. [1] found an increased soluble form of ICAM-1 (sICAM-1) levels in acute attacks and in the remission phase of FMF. They suggest that this observation mıght indicate a regulatory role of sICAM-1 during the inflammatory course of the disease.

Genetic polymorphisms in adhesion molecules have been implicated in susceptibility to diseases that have an inflammatory component. ICAM-1 gene is known to contain two polymorphic sites, situated in codons 241 (G/R 241) and 469 (E/K 469). ICAM-1 polymorphisms were found to be associated with inflammatory bowel

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disease, multiple sclerosis, chronic allograft failure, rheumatoid arthritis (RA), and Behçet's disease in different studies [2-7]. Though the functional role of these polymorphisms remain unclear, these studies suggest that alterations in the amino acid sequences of ICAM-1 molecule may influence ICAM-1 ligand interactions and hence the intensity and the duration of the inflammatory response.

The aim of our study was to evaluate the probable association of ICAM-1 G/R 241 and ICAM-1 E/K 469 polymorphisms with susceptibility to FMF.

2. Material and Methods

Sixty-seven FMF patients, according to Tel-Hashomer criteria [8], whom were admitted to our hospital Rheumatology and Gastroenterology departments, were included in the study. Eighty-three healthy volunteers were consisted in the study as the control group. Written informed consent was obtained from all subjects. Blood samples were taken from FMF patients and healthy volunteers for genotyping by polymerase chain reaction (PCR) for ICAM-1 polymorphisms G/R at codon 241 and E/K at codon 469.

2.1. Molecular analysis of ICAM-1 polymorphisms [9]

Genomic DNA was isolated from EDTA-preserved whole blood samples of all patients and controls. The first amino acid polymorphism consisted of a substitution of arginine (R) for glycine (G) at codon 241. This bi-allelic polymorphism was examined as follows: a novel PCR assay was used to detect this single nucleotide polymorphism. Because the single base change G to A at codon 241 does not introduce or abolish a restriction site, the forward primer was mutated by one base pair (G or T) to introduce the restriction site for the enzyme *Bsr*GI.

Forward: 5' CCG TGG TCT GTT CCC TG<u>T</u> AC 3'
Reverse: 5' GAA GGA GTC GTT GCC ATA GG 3'

*Bsr*GI digested the PCR product only when the mutant A allele was present yielding DNA products of 90 and 20 base pairs.

100 ng genomic DNA was amplified in a 25 µl PCR reaction containing 1XNH_4 buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 pmol of each primer, 0.5 U Taq polymerase and 1 mM Betaine. The DNA was denatured at 95ºC for 2 minutes (min.), followed by 35 cycles at: 95ºC for 45 seconds (s), 58ºC for 5 s, 50ºC for 45 s, 72ºC for 45 s, and 72ºC for a final 2 min. The presence of product was verified in a 2% agarose gel stained with ethidium bromide. PCR products were digested with *Bsr*GI in a 15µl final volume. This contained 7µl PCR product, 1XNE buffer, 1X bovine serum albumin, and 5 U *Bsr*GI. The digest was incubated overnight at 37ºC and the products of the digest were then visualized on a 3% agarose gel stained with ethidium bromide.

The second amino acid polymorphism consisted of a substitution of lysine (K) for glutamic acid (E) at codon 469. The following primers were used for amplification of this region:

Forward: 5' AGG ATG GCA CTT TCC CAC T 3'
Reverse: 5' GGC TCA CTC ACA GAG CAC AT 3'

100 ng genomic DNA was amplified in a 25 µl PCR reaction containing 10XKCl buffer, 3.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 pmol of each primer, 1U of Taq polymerase, and 4 mM Betaine. The DNA was denatured at 95ºC for 5 min with the temperature cycling set at 95ºC for 45 s, 56ºC for 45 s, 72ºC for 45 s, followed by a final extension at 72ºC for 2 min. Analysis of the PCR product was performed by enzyme digestion using *Bsr*UI, which cuts the product from the E469 allele, but does not cut the K469, and yields one fragment of 140 bp for the homozygous KK allele. The digestion was incubated overnight at 37ºC and the products were then visualized on a 3% agarose gel stained with ethidium bromide.

2.2. Statistical Analysis

The distribution of genotypes and allele frequencies in FMF patients were compared with healthy subjects and evaluated using a χ^2 test.

3. Results

FMF patients and healthy controls included in our study were matched for age, sex, and ethnic background (FMF patients: 43 female, 24 male, mean age; 34.8±9.6 years, range 19-57, healthy controls: 42 female, 41 male, mean age; 33.6± 8.4, range 18-55). The mean disease duration of FMF patients was 8 ± 4,3 years. All of the patients were on treatment with colchicine.The mean number of acute attacks in the last year was 3±3.4 while 17 patients did not experience any attacks. The clinical presentation of the disease during acute attack was fever in 75%, peritonitis in 97.5%, pleuritis in 35%, and arthritis in 32.5% of the patients. Only seven patients were diagnosed as having secondary amyloidosis.

3.1. ICAM-1 G/R 241 polymorphism

The ICAM-1 G/R 241 genotype distribution and allele frequencies of FMF patients and healthy controls were similar (Table 1). G241 homozygote genotype and G241

Table 1. ICAM-1 G/R 241 genotype distribution and allele frequencies of FMF patients and healthy controls.

Groups	Genotypes(NS*)			Alleles (NS*)	
	R/R	G/R	G/G	R	G
FMF	0 (0%)	2 (3%)	65 (97%)	2 (1.5%)	132 (98.5%)
Controls	2 (2.4%)	1 (1.2%)	80 (96.3%)	5 (3.1%)	161 (96.9%)

** Not significant: ICAM-1 G/R genotype distribution and allele frequencies of FMF patients and controls were not significantly different (p>0.05).*

Table 2. ICAM-1 E/K 469 genotype distribution and allele frequencies of FMF patients and healthy controls.

Groups	Genotypes(NS*)			Alleles (S**)	
	K/K	E/K	E/E	K	E
FMF	22 (32.8%)	30 (44.8%)	15 (22.4%)	74 (55.2%)	60 (44.8%)
Controls	42 (50.7%)	28 (34%)	13 (15.3%)	112 (67.7%)	54 (32.3%)

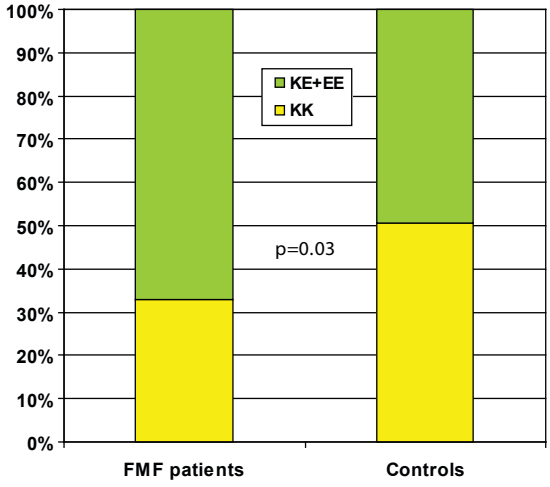
** Not significant: ICAM-1 E/K 469 genotype distribution of FMF patients and controls were not significantly different (p>0.05)*
*** Significant: ICAM-1 469 allele frequencies of FMF patients and controls were significantly different (p=0.03)*

allele were markedly frequent both in FMF patients and controls (97%, 98.5% vs 96.3%, 96.9% subsequently). None of the FMF patients had R241 homozygote genotype.

3.2. ICAM-1 E/K 469 polymorphism

The ICAM-1 E/K 469 genotype distribution of FMF patients were not significantly different from controls (Table 2). However, the frequency of K469 homozygote was significantly lower in FMF patients than in controls (32.8% vs 50.7% subsequently, p=0.03, Figure 1). ICAM-1 469 allele frequencies of FMF patients and controls were significantly different (p=0.03, Table 2). ICAM-1 E469 allele was more frequent in FMF patients than controls (44.8% vs 32.3%).

Figure 1. Significantly less frequent ICAM-1 K469 homozygote genotype in FMF patients compared with controls.



4. Discussion

In this study, we demonstrated an association of FMF with a genetic region external to the MEFV gene, the ICAM-1 gene at codon 469. The frequency of E469 allele was significantly higher in patients with FMF compared to healthy controls and there was a corresponding decrease in the frequency of K469 homozygosity among patients.

The functional consequences of polymorphisms in the ICAM-1 gene are not clear at present; however, they occur in regions that could potentially cause alterations in leukocyte binding and/or costimulatory activity of the ICAM-1 molecule [10,11]. Changes in the function of the ICAM-1 molecule due to polymorphisms could influence the strength of leukocyte binding to endothelial and/or other cells at sites of inflammation. Other than the well known MEFV gene mutations in the pathogenesis of FMF, these potential modifier genes like ICAM-1 polymorphisms may contribute to disease susceptibility and progression by modulating the immune response.

There are several reported disease associations with ICAM-1 E/K 469 polymorphisms: K469 allele with multiple sclerosis and RA, E469 allele with renal allograft failure, and Behçet's disease [3-6]. Gaetani et al. showed that the K469E polymorphism of the ICAM-1 gene is a risk factor for peripheral arterial occlusive disease [12]. Verity et al. identified an increased frequency of ICAM-1 E469 allele in patients with Behçet's disease compared to controls (47.6% vs 38.3%) and a decreased frequency of K469 homozygosity among patients [5]. Lee et al. [6] found significantly lower ICAM-1 K469 allele frequencies in Korean patients with RA than those in healthy controls (59.8% vs 71.7%). In our study, the frequencies of E469 allele in patients with FMF and controls were 44.8% and 32.3% subsequently. In all these studies, the associated alleles were not rare in the control populations.

This is the first study about ICAM-1 polymorphisms and FMF in literature. Our results indicate that ICAM-1 E469 allele may have a role in FMF, however the association is weak since the associated allele was also frequent in the control population. Only seven of the patients with FMF in our study had amyloidosis and we could not investigate the probable role of ICAM-1 polymorphisms on amyloidosis development, the main field for searching modifier genes. Further studies on larger numbers of FMF patients with and without amyloidosis and in different ethnic groups would contribute to our understanding about the influence of ICAM-1 polymorphisms on disease susceptibility and progression.

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