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Relationship between HLA-DR, HLA-DQ alleles and MEFV gene mutations in familial mediterranean fever (FMF) patients

Ailevi Akdeniz Ateşi'nde (FMF) MEFV gen mutasyonları ve HLA-DR, HLA-DQ allelleri arasındaki ilişki

Gülay KINIKLI[1], Mehmet BEKTAŞ[2], Müge MISIRLIOĞLU[3], Aşkın ATEŞ[1], Murat TURGAY[1], Serdar TUNCER[3], Sami KINIKLI[4], Güner TOKGÖZ[1]

Departments of [1]Clinical Immunology, Rheumatology and [2]Gastroenterology, Ankara University School of Medicine, Ankara

[3]MSc, Metis Biotechnology Ltd., Ankara

[4]Ministry of Health Ankara Hospital, Ankara

Background/aims: *Three missense mutations clustered on the carboxyl-terminal portion of the MEFV gene (M680I, M694V, and V726A) have been observed in over 80% of affected alleles in several ethnic groups of familial Mediterranean fever patients. Several immunologic abnormalities were found both in cellular and humoral components in Mediterranean fever patients. Those observations have pointed the way for analysis of the HLA region in Mediterranean fever. We intended to compare HLA DR/DQ alleles with those major mutations in the MEFV gene in Mediterranean fever patients.* ***Methods:*** *The distribution of MEFV gene mutations and HLA-DR, HLA-DQ alleles were analyzed in 40 index Turkish Mediterranean fever patients, 28 family members and 42 healthy controls. M680I, M694V, and V726A mutations were studied by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) analysis. HLA-DR and DQ allele subgroups were studied using SSP-PCR technique.* ***Results:*** *A total of 37 (92.5%) patients in 40 Mediterranean fever index patients were found to carry one of the three missense mutations. The HLA-DR4 allele frequency was significantly higher in the Mediterranean fever patient group. When comparisons were made between Mediterranean fever mutations and HLA allele frequencies, M694V mutation with HLA DR3, DR11/5 and DR 13/6 and M680I mutation with DR7 allele subgroups were statistically significant. DQ6/1, DQ7/3, and DQ8/3 allele with M694V, DQ2 allele with M680I, and DQ6/1 with V726A mutations were also statistically significant.* ***Conclusions:*** *Our results indicate a relationship between some HLA-DR/DQ alleles and MEFV mutations in Mediterranean fever patients. We suggest HLA-DR/DQ alleles and their role in the pathogenesis of Mediterranean fever need further analysis and comparative studies.*

Key words: Familial Mediterranean fever, MEFV gene mutations, HLA alleles

Amaç: *Farklı etnik gruptan birçok Ailevi Akdeniz Ateşi hastasında MEFV geninin karboksiterminal parçasında üç missense mutasyonu allellerin %80'inde gözlenmiştir. Ailevi Akdeniz Ateşi hastalarında hem hücresel hem de humoral immun sistemde anormallikler bulunmuştur. Bu gözlemler Ailevi Akdeniz Ateşi'nde HLA bölgesinin analiz yolunu işaret etmiştir. Biz Ailevi Akdeniz Ateşi hastalarında HLA-DR/DQ allelleri ile MEFV genindeki üç major mutasyonun karşılaştırmasını planladık.* ***Yöntem:*** *MEFV gen mutasyonları ve HLA DR/DQ allellerinin dağılımına 40 indeks Türk Ailevi Akdeniz Ateşi hastasında, 28 Ailevi Akdeniz Ateşli aile bireylerinde ve 42 sağlıklı kontrol grubunda bakıldı. M680I, N694V ve V726 A mutasyonları ARMS-PCR yöntemiyle; HLA DR ve DQ allel subgrupları ise SSP-PCR tekniği kullanılarak çalışıldı.* ***Bulgular:*** *42 Ailevi Akdeniz Ateşi hastasının 37'sinde (%92.5) üç mutasyondan biri mevcuttu. HLA-DR4 allel frekansı Ailevi Akdeniz Ateşi hasta grubunda belirgin olarak yüksekti. Ailevi Akdeniz Ateşi mutasyonları ve HLA allel frekansları karşılaştırıldığında M694V mutasyonu ile HLA-DR3, DR11/5, DR13/6; M680I mutasyonu ile DR7 allel subgrubu ilişkisi istatistiksel olarak anlamlıydı. DQ6/1, DQ7/3 ve DQ8/3 alleli ile M694V; DQ2 alleli ile M680I ve DQ6/1 ile V726A mutasyonu birlikteliği istatistiksel olarak anlamlıydı.* ***Sonuç:*** *Bizim sonuçlarımızda Ailevi Akdeniz Ateşi hastalarında HLA-DR/DQ allelleri ve MEFV mutasyonları arasındaki ilişki gösterilmiştir. HLA-DR/DQ allelleri ve onların Ailevi Akdeniz Ateşi patogenezindeki rolleriyle ilgili ileri analizlere ve karşılaştırılmalı çalışmalara ihtiyaç vardır.*

Anahtar kelimeler: Ailevi Akdeniz Ateşi, MEFV gen mutasyonları, HLA allel

Address for correspondence: Gülay KINIKLI
Fakülteler Mah. Yazgan Sokak. No: 34/15 Cebeci, Ankara, Turkey
Phone: +90 312 310 33 33/2192 • Fax: +90 312 310 63 71
E-mail: gkinikli@hotmail.com

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INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive disease which occurs most commonly in Sephardic Jews, Armenians, Arabs and Turks. FMF is characterized by recurrent and self-limited attacks of fever accompanied by peritonitis, pleuritis, synovitis, or erysipelas-like erythema (1).

Several immunologic abnormalities have been found both in cellular and humoral components in FMF patients (2). Those observations pointed the way for analysis of the HLA region in FMF. The previous linkage analysis studies did not demonstrate a relationship between HLA markers in patients with FMF (3-7). The localization of the disease-associated mutations on exon 10 was described in the MEFV gene in 1997 (8, 9). Three missense mutations clustered in the carboxyl-terminal portion of the MEFV gene (M680I, M694V, and V726A) were observed in the previously indicated ethnic panel for FMF patients accounting for 80% of affected alleles (8-10). In this study, we intended to compare HLA DR/DQ alleles with those major mutations in the MEFV gene in FMF patients.

MATERIALS AND METHODS

Forty adult Turkish FMF patients, 28 family members, and 42 healthy controls followed up at the Department of Immunology and Rheumatology of Ankara University were included in this study. Genomic DNA was extracted from peripheral blood lymphocytes according to standard procedures. HLA-DR and DQ allele subgroups were studied using low resolution SSP-polymerase chain reaction (PCR) technique (Dynal A.S., Oslo, Norway). M680I, M694V, and V726A mutations were analyzed by amplification refractory mutation system (ARMS)-PCR technique. The MEFV region covering three mutations was amplified using previously reported primers and PCR conditions (11). The amplified products were analyzed by electrophoresis on 2% agarose gel. Statistical significance of frequencies was determined by chi-square tests.

RESULTS

The distribution of FMF mutations in the study groups is given in Table 1. M694V was the most frequently observed mutation, as in the previous reports (8-10). In 40 index patients, 37 (92.5%) were found to carry one of the three missense mutations. Eleven of the 40 patients were homozygotes and 14 were compound heterozygotes for one mutation. In three patients no mutation was identified, and in 12 patients only a single mutation was observed. A statistically significant difference in mutation frequency was observed in the FMF

Table 1. The distribution of FMF mutations

	FMF		Family		Healthy controls	
Mutations	**(n=40)**	**(%)**	**(n=26)**	**(%)**	**(n=42)**	**(%)**
694M-V/694M-V	10	25	0		0	
680M-I/680M-I	1	2.5	0		0	
694M-I/726V-A	7	17.5	6	23.1	0	
694M-V/680M-I	6	15	1	3.8	2	4.8
726V-A/680M-I	1	2.5	0		0	
694M-V/?	11	27.5	7	26.8	5	11.9
726V-A/?	1	2.5	1	3.8	1	2.3
680M-I/?	0	0	1	3.8	0	
Total	37	92.5	16	61.5	8	19.0
Unknown	3	7.5	10	38.5	34	81.0

Table 2. HLA subgroup distribution

	FMF (n=40)	**Family (n=26)**	**Healthy controls (n=42)**
HLA-DR			
3	9	1	4
4	17*	5	11
5/1	8	5	13
7	5	9	9
9	5	2	0
10	5	2	1
11/5	20	11	14
13.6	8	6	12
15/2	3	3	9
HLA-DQ			
2	11	9	12
5/1	17	13	13
6/1	7	7	8
7/3	15	13	14
8/3	14	2	6

*$p<0.05$

Table 3. Allele frequencies of mutations with HLA subgroups in FMF patients

		M694V (44/80)*	M680I (9/80)*	V726A (9/80)*
HLA-DR	**n (chromosome)**			
3	12	7**	3	1
4	20	13	1	2
7	10	4	4**	1
9	10	7	0	0
10	8	3	0	0
11/5	32	18**	0	0
13/6	10	9**	1	2
16/2	16	3	0	1
HLA-DQ				
2	22	10	6**	2
5/1	30	17	1	2
6/1	14	7**	1	4**
7/3	26	15**	3	4
8/3	16	9**	1	1

*Allele/Chromosome number, **p<0.005

patient group when compared with the other study groups. HLA subgroup distributions in FMF patients, healthy FMF family members, and healthy controls are shown in Table 2. HLA-DR4 positivity was significant in FMF patients ($p<0.05$ respectively) when compared with healthy FMF family members and the healthy control group. The comparison of mutations with HLA-DR and HLA-DQ subgroups in 40 FMF patients is shown in Table 3. M694V mutated allele frequency was significantly higher in HLA DR3, DR11/5 and DR13/6 subgroups. M680I mutation was observed in four of 10 in the DR7 subgroup, which is statistically significant. In the analysis of DQ subgroups, the difference in frequency was significant in DQ6/1, DQ7/3, and DQ8/3 with M694V, in DQ2 with M680I, and in DQ6/1 with V726A.

DISCUSSION

In 1997, the International Consortium and French Consortium independently cloned the gene for the disease on the short arm of chromosome 16 and identified four missense mutations on FMF carrier chromosomes (8, 9). Four FMF-associated mutations (M694V, M680I, V726A and M694I) were reported, which accounted for 85% of carrier chromosomes (9).

We have evaluated the three most prevalent FMF mutations (M694V, M680I, V726A) in three different study groups, including healthy controls. These mutations were observed in 92.5%, 61.5% and 11.9% of FMF patients, healthy FMF family members and the control group, respectively. The M694V allele accounted for the majority of the three mutations as observed in other studies (8-10, 12). Akar et al. (12) investigated seven missense mutations and genotype-phenotype correlation in 230 Turkish FMF patients, and the M694V, M680I, and V726A mutations were found in 43.5%, 12%, 11.1% of the patients, respectively. We found these mutations at rates of 67.5%, 11.3%, and 11.3% in FMF patients and of 25%, 3.9%, and 13.5% in the healthy family group, respectively. Among the healthy controls, 8.3% (M694V), 2.4% (M680I), and 1.2% (V726A) were found to carry one of the three missense mutations. These results confirm the genetic heterogenecity of FMF among the Turkish population.

Many immunologic abnormalites have been described during FMF attacks, including decrease in suppressor T-cell activity, increase in total complement activity, elevation of IgG, IgA and IgM, and increases in serum haptoglobin, C-reactive protein and fibrinogen (2, 13, 14). Based on these findings, the HLA region on chromosome 6 has become an important area of investigation for possible association with FMF, although no linkage could be demonstrated in previous studies, where HLA typing was not conducted in the majority (3, 4, 15, 16). Shohat et al. (17) reported the relationship between FMF genetic markers and HLA-DR4 alleles. During FMF attacks, HLA-DR expression elevates. Musabak et al. (18) reported that 40 patients with FMF in an attack period had higher levels of HLA-DR than control groups consisting of 20 healthy blood donors and 15 patients with inactive Behcet's disease. We have also observed a statistically significant higher frequency of DR4 allele in FMF patients with the additional MEFV mutational analysis data. The relationships between M694V with HLA DR3, DR11/5, DR13/6, DQ6/1, DQ7/3, DQ8/3, of M680I with DR7 and DQ2, and of V726A with DQ6/1 were also significant in our study. Blood samples were collected during the attack-free period.

These laboratory findings may confirm the relationship of HLA DR4 subgroups and positive patients with FMF. However, this conclusion requires the study of a larger series. Interesting findings have been observed with additional possible linkages when the comparison was made between HLA subgroups and FMF mutations. This significance might have a role in the explanation of the FMF disease pathogenesis. However, this requires carefully designed investigative studies.

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