The influence of genotype on disease severity and concomitant diseases in familial Mediterranean fever patients

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ABSTRACT

Objective. *To evaluate differences between the patients with familial Mediterranean fever (FMF) with homozygous (Hom), heterozygous (Het) and compound heterozygous (cHet) MEFV mutations in terms of clinical features and severity of the disease, as well as frequency of concomitant disorders, without focusing on Exon 10 mutations.*

Methods. *The patients with FMF were diagnosed using the Tel-Hashomer diagnostic criteria. The presence of MEFV mutations was investigated in exons 2,3,5 and 10 by multiplex-PCR reverse hybridisation method. All the patients were questioned for the presence of concurrent disorders, and the medical records of these patients were revised retrospectively.*

Results. *259 unrelated patients (female: 143, male: 116; mean age: 33.5±12 years) were included in this study. Hom and Het mutations were found in 79 (31.9%) and 88 (35.6%) patients with FMF, respectively. cHet mutations were found in 68 (27.5%) FMF patients. Early onset and early diagnosis of FMF were found in Hom group compared to Het and compound Het groups. The number of the patients with a higher severity score was significantly higher in Hom group (n=40, 50.6%) than Het (n=12, 13.6%) and cHet groups (n=10, 14.7%), (p<0.0001). No significant differences were found between the groups in terms of clinical features, except for erysipelas like erythema (ELE) (Hom group: 69.6% vs. Het group 37.5%, p<0.0001). Amyloidosis and concomitant disorders were found in 22 FMF patients with Hom MEFV mutations, 16 FMF patients with heterozygous mutations, 7 FMF patients with cHet mutations.*

Conclusion. *While the presence of homozygous mutations creates tendency for a severe disease phenotype, the development of concomitant disorders seems to be independent of homozygous mutations.*

Introduction

Familial Mediterranean fever (FMF) is an auto-inflammatory disease characterised by recurrent serositis and fever attacks. The disease is caused by mutations in a gene named Mediterranean fever (*MEFV*), which encodes a protein called pyrin. This protein is likely to exert a down-regulating influence upon the response of neutrophils to inflammatory stimuli (1).

Although some patients with FMF have heterozygous mutations for *MEFV* gene, there is no consensus as to whether heterozygosity for *MEFV* mutations are responsible for disease symptoms. Jeru *et al*. suggested that heterozygosity for *MEFV* mutation should be considered as a susceptibility factor and is not responsible for classical Mendelian FMF (2). Soriona *et al*. showed that carriers for heterozygous mutation may have a mild or incomplete disease (3). In many studies, no second mutation in the FMF gene has been found in FMF patients with heterozygous mutation (4, 5). Authors have suggested that modifying other genes or environmental factors may account for the clinical manifestations of FMF patients. On the other hand, full-blown FMF manifestations may also be reported in patients with heterozygous mutations (6, 7). Another interesting feature of FMF is that it evokes tendency towards other inflammatory diseases and accompanies them (8, 9). As far as development of secondary amyloidosis is concerned, we know that the presence of M694V homozygous mutation poses a risk (10). However, we do not know exactly how homozygous and heterozygous genotypes generate impact on concomitant diseases that occur during the course of FMF. Although frequencies and clinical manifestations of homozygous and heterozygous genotypes have been reported in some other ethnic groups, the phenotype of the disease may present a different course in different ethnic com-

munities and different environmental conditions.

In this study, we aimed to evaluate differences between the patients suffering from FMF with homozygous (Hom), heterozygous (Het) and compound heterozygous (cHet) *MEFV* mutations in terms of clinical features and severity of the disease, and also to investigate whether there is a difference between these three patient groups as regards concomitant disorders and amyloidosis observed in the course of FMF. Our study is the first to seek an answer to this question as far as FMF patients are concerned.

Methods

The patients with FMF were diagnosed based on Tel-Hashomer diagnostic criteria (11). Any patients with phenotype 2 FMF were included in the analyses. Severity of FMF was determined according to Tel-Hashomer severity score (12). Severity scores were calculated depending on the clinical manifestations prior to the colchicine therapy. All clinical manifestations and their features were reviewed. All the patients were questioned for the presence of concurrent disorders, and their medical records were revised retrospectively. A previous diagnosis of a concomitant disease was taken into consideration if it met the relevant criteria. This study was approved by the local ethics committee. Informed consent was obtained from all the individual participants of our study.

Mutation analysis

DNA was extracted from peripheral blood leukocytes using standard protocols (Invisorb® Spin Blood Kit, STRATEC Molecular GmbH, D-13125; Berlin, Germany). Molecular analyses were performed within the framework of routine genetic testing. The presence of *MEFV* mutation was investigated in exons 2, 3, 5, and 10 by the multiplex-PCR reverse hybridisation method.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) software, version 15.0 statistical package program (IBM Inc.; Chicago, IL, USA). Demographic and clinical variables were summarised as proportions. Chi-square testing was performed for the comparison of categorical variables. A p-value <0.05 was considered statistically significant.

Results

259 unrelated patients (female: 143, male: 116; mean age: 33.5±12 years) who met the Tel-Hashomer diagnostic criteria for FMF and phenotype 2 FMF patients were included in this study. In 12 of 259 patients, *MEFV* mutation analysis was not performed. No mutation was determined in 8 of the FMF patients (3.2%). Hom and Het mutations were found in 79 (31.9%) and 88 (35.6%) patients with FMF, respectively. cHet mutations were found in 68 (27.5%) FMF patients (Table I). Early onset and early diagnosis of FMF were found in Hom group compared to Het and cHet group (Table II). The number of patients with a higher severity score was significantly higher in hom group (n=40, 50.6%) than Het (n=12, 13.6%) and cHet group (n=10, 14.7%), (p<0.0001) (Table III). No significant difference was found between groups in terms of clinical features except for erysipelas like erythema (ELE) (Hom group: 69.6% *vs*. Het group 37.5%, p<0.0001). We also compared disease severity in only 49 heterozygous patients with a copy of M694V and 38 without. No significant difference was found between these two groups in terms of disease severity score (M694V group: 7.84; others: 7.34; p=0.4) and the number of patients with severe disease severity score (12% *vs*. 10.2 %; p=0.8).

Amyloidosis and concomitant disorders were found in 22 (9 amyloidosis, 1 Behçet's disease, 12 ankylosing spondylitis) FMF patients with homozygous MEFV mutations, 16 (1 AA-amyloidosis, 5 Behçet's disease, 10 ankylosing spondylitis) FMF patients with heterozygous mutations, 7 (2 AA-amyloidosis, 2 Behçet's disease, 3 ankylosing spondylitis) FMF patients with cHet mutations (Table IV). Amyloidosis was significantly higher in Hom group than Het group (9 *vs*. 1; p<0.006) and cHet group (9 *vs*. 2; p<0.006). All the concomitant disorders and amyloidosis are presented in Table IV.

Table I. Mutations distribution in patients with familial Mediterranean fever.

Genotype	n (%)
Homozygous	
M694V/ M694V	72 (30.1)
M680I/ M680I	4 (1.7)
E148Q/ E148Q	1 (0.4)
V726A/ V726A	1 (0.4)
R761H/ R761H	1 (0.4)
Heterozygous	
M694V/ wt	49 (20.5)
E148Q/ wt	14 (5.9)
M680I/ wt	10 (4.2)
V726A/ wt	6 (2.5)
K695R/ wt	3 (1.3)
A744S/ wt	2 (0.8)
R202Q/ wt	2 (0.8)
P369S/ wt	1 (0.4)
Compound heterozygous	
M694V/ V726A	20 (8.4)
M694V/ E148Q	13 (5.4)
M694V/ M680I	11 (4.6)
M680I/ V726A	9 (3.8)
M694V/ R761H	4 (1.7)
M694V/ R202Q	2 (0.8)
M694I/ V726A	2 (0.8)
E148Q/ V726A	2 (0.8)
M694V/ E148Q/ R202Q	2 (0.8)
M680I/ K695M / G678E	1 (0.4)
M694V/ E148Q / V726A	1 (0.4)
M680I/ E148Q	1 (0.4)
V726A/ R761H	1 (0.4)
M680I/ R761H	1 (0.4)
E148Q/ P369S	1 (0.4)
M694V/ K695R	1 (0.4)
M694V/ A744S	1 (0.4)
Total	239

Discussion

In this study, we evaluated frequencies of Hom, Het, and cHet mutations in FMF patients, and we assessed whether there were differences between these groups in terms of clinical findings and disease severity. Additionally, for the first time in the literature, we investigated frequency of concomitant diseases in line with mutation distribution and whether there any differences occurred between the groups as regards concomitant diseases and amyloidosis. We grouped the patients as Hom, Het and cHet without taking into account the types of the variants, which resulted in the combination of exon 10 variants with exon 2 or 3 variants in some patients as cHet. It has been sug-

Table II. Comparison of some demographic features between groups.

		n	mean ± SD	Median(Q1-Q3)	*p*
Age (yrs)	Homozygous	79	32.3 ± 10.7	31 (24-38)	ns
	Heterozygous	88	33.7 ± 12.8	31.5 (24-41,8)	
	Compound heterozygous	68	34.3 ± 13.1	33.5 (23.3-42)	
Onset age (yrs)	Homozygous	79	8.4 ± 8	7 (2-12)	*p*1=0.001
	Heterozygous	88	14.2 ± 11.5	12 (4.3-20.8)	*p*2=0.004
	Compound Heterozygous	68	12.7 ± 9.1	11.5 (6-18.8)	
Diagnostic age (yrs)	Homozygous	79	23.3 ± 11.5	20 (15-30)	*p*3=0.046
	Heterozygous	88	27.9 ± 11.8	25 (19.3-33)	*p*4=0.004
	Compound Heterozygous	68	29 ± 11.3	29 (20.3-35)	
Delay in diagnosis	Homozygous	79	14.9 ± 11.3	13 (6-23)	ns
	Heterozygous	88	13.6 ± 11.6	11 (3-21)	
	Compound Heterozygous	68	16.3 ± 12.1	14 (7-22)	

*p*1: Hom *vs*. Het. *p*2: Hom *vs*. cHet. *p*3: Hom *vs*. Het. *p*4: Hom *vs*. cHet.

Table III. Comparison of groups according to severity score, concomitant disorders and amyloidosis in FMF patients.

	Hom n=79	Het n=88	Com Het n=68
Low disease severity (Score:2-5)	8 (10.1%)	17 (19.3%)	13 (19.1%)
Moderate disease severity (Score: 6-10)	31 (39.2%)	59 (67%)	45 (66.2%)
Severe disease severity (Score>10)	40 (50.6%)*	12 (13.6%)	10 (14.7%)
Amyloidosis	9 (11.4%)**	1 (1.1%)	2 (2.9%)
Behçet's disease	1 (1.3%)	5 (5.7%)	2 (2.9%)
Spondyloartropathy	12 (15.2%)	10 (11.4%)	3 (4.4%)

*p <0.0001; Hom group *vs*. Het group and cHet. **p<0.006; Hom *vs*. Het and cHet.

Table IV. All concomitant disorders and AA-amyloidosis in FMF patients.

Diseases	n (%)
FMF+ spondyloartropathy (SpA)	24 (9.27)
FMF+ amyloidosis	6 (2.31)
FMF+ Behçet's disease (BD)	6 (2.31)
FMF+ fibromyalgia	5 (1.93)
FMF+ psoriasis	4 (1.54)
FMF (Phenotype 2)	4 (1.54)
FMF+ Henoch-Schonlein purpura	3 (1.16)
FMF+ MPGN+ psoriasis	1 (0.38)
FMF+ ichthiosis	1 (0.38)
FMF+ MPGN	1 (0.38)
FMF+ BD+ SpA	1 (0.38)
FMF+ Crohn's disease	1 (0.38)
FMF+ SpA + chronic renal failure (CRF)	1 (0.38)
FMF+ CRF + haemodialysis (phenotype 2)	1 (0.38)
FMF+ IgA nephropathy	1 (0.38)
FMF+ ulcerative colitis	1 (0.38)
FMF+ SLE	1 (0.38)
FMF+ BD + fibromyalgia	1 (0.38)
FMF+ amyloidosis + SpA	1 (0.38)
FMF+ amyloidosis + CRF+ peritoneal dialysis + SLE	1 (0.38)
FMF	194 (75)
Total	259 (100)

MPGN: membranoprolipherative glomerulonephritis; SLE: systemic lupus erythematosus.

gested that exon 2 or exon 3 variants are uncertain of significance or variants that are not the genetic cause of FMF (13). Grouping the patients according to variants could be more valuable to draw meaningful conclusions. However, because of limited number of patients with having exon 2 or 3 variants and considering the fact that exon 2 or exon 3 variants can lead to full-blown manifestations of FMF (14, 15), we did not exclude those patients from analysis. Moreover, all our patients met Tel-Hashomer diagnostic criteria for FMF and were put on colchicine. Different ethnicity and different environmental factors, in addition to gene variants, may be a determinant factor for a disease phenotype.

It still remains to be seen whether heterozygote mutations lead to full-blown disease in FMF, which is known to be autosomal recessive. However, in case series studies and in medium-sized FMF cohorts, heterozygous mutation was found in 16.5-33.8% of the patients who fulfilled the FMF diagnostic criteria (16, 17). In our cohort, 35.6% of the patients had a heterozygous mutation. We do not discount the possibility of referring bias. However, we cannot ignore that phenotypic properties might be affected by ethnicity and environmental factors. There have been variable results on the distribution of *MEFV* mutation in different population, even in the population of different regions of the same country. In Turkey, Ece *et al*. reported that 26.5% and 2.7% of FMF patients had heterozygous and homozygous for E148Q in southeast part of Turkey, respectively (18). Topaloğlu *et al*. evaluated the phenotypic features of the patients with E148Q mutation (14). They suggested that E148Q is a disease-causing *MEFV* mutation.

In another part of Turkey, Yigit *et al*. showed increased frequency of homozygous R202Q mutation in FMF patients compared to healthy controls (15). It has been reported that R202Q mutation is most commonly observed mutation in the Mediterranean region of Turkey (19). 57 patients with FMF-related AA amyloidosis were investigated for mutational spectrum of *MEFV* gene by Nursal *et al*. (20). The most commonly observed mutation was homozygous M694V. The R202Q allele frequencies were significantly different between patients and control group. Authors suggested that other alleles should not be disregarded in FMF-related amyloidosis. These studies indicate that Exon 2 or Exon 3 mu-

tations can lead to FMF phenotype and showed the presence of heterogenous mutation spectrum in Turkish population. However, Exon 2 or 3 variants carrier rate is high in Turkish population and these variants may only contribute as a modifier gene to other inflammatory disorders. Therefore, it is very difficult to suggest Exon 2 and 3 variants responsible for the full blown FMF phenotype unless appropriate control groups are included in the study design.

We did not find any differences between the homozygous FMF patients and the other 2 groups in terms of clinical presentation other than erysipelas like erythema (ELE), but the disease had appeared earlier in homozygous patients and the diagnosis had been established earlier. In addition, the frequency of amyloidosis was higher in homozygous patients than in het. and cHet ones. In a study by Moradion *et al*. the onset of the disease in patients with homozygous mutation was at an earlier age than that of heterozygous ones (4).

The relationship between M694V homozygosity of *MEFV* and disease severity is well known. We found that the same feature was also valid when we considered patients with all homozygous mutations. There was no difference between the heterozygotes and compound heterozygotes in terms of disease severity. These results were in parallel with the results reported by Moradian *et al*. (4). An interesting part of this study was that heterozygous M694V variant was not different from other heterozygous variant in terms of disease severity. This result may imply that heterozygous M694V variant loses its effect on the severity of FMF, unlike the homozygous M694V variant.

MEFV mutations lead to a pro-inflammatory condition even in carriers (21), which creates a subclinical inflammation. *MEFV* mutations have been suggested to be a modifier for other inflammatory diseases, and because of this feature, concomitant inflammatory diseases in FMF compared to the general population (6). However, it is not clearly known whether mutations play a role in the emergence of these diseases and, if so, which mutations play have a part. We previously found that, in comparing of patients with positive M694V homozygous or heterozygous mutation to those with other mutations, patients with M694 mutation had a higher frequency of concomitant diseases and amyloidosis (7). In addition, spondyloarthritis/ankylosing spondylitis was seen as the second most frequent disease. Akar *et al*. demonstrated that patients with FMF having sacroiliitis more commonly had M694V mutation compared to those without sacroiliitis (22). In our study, we examined whether there were any differences in terms of concomitant diseases and amyloidosis in 3 different groups. We found that amyloidosis was more common in homozygous patients. Spondyloarthritis did not achieve a statistical significance although it was more common in homozygous patients. Interestingly, while homozygosity created a risk in terms of amyloidosis, it presented no difference from heterozygotes as regards other concomitant diseases. Spondyloarthritis and Behçet's disease were also observed in heterozygous and compound heterozygous patients.

In our study, we had limitations such as the number of patients being low, with the study having a retrospective design. To evaluate the relationship of concomitant diseases with mutations, there should be a higher number in the groups. Symptomatic heterozygous patients are more likely to come to the hospital and this may lead to Bergson's bias. Another limitation is that patients with concomitant disease are more likely to apply to a hospital, another condition that is leading to Bergson's bias. We were unable to form a disease control group because of financial reasons. Formation of the diseased control group could have minimised the likelihood of bias.

In conclusion, the presence of homozygous *MEFV* mutations in contrast to heterozygous mutations creates a tendency for early onset of the disease, early diagnosis, frequent ELE, amyloidosis and severe disease phenotype. Concomitant disorders seem to be independent from homozygous mutations.

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