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SHORT REPORT

The differential contribution of MEFV mutant alleles to the clinical profile of familial Mediterranean fever

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterised by recurring attacks of fever and serositis. Five sequence alterations (M694V, V726A, M680I, M694I and E148Q), in the MEFV gene, account for the majority of FMF chromosomes. The wide clinical variability of the disease has been related to MEFV allelic heterogeneity. M694V homozygotes have a severe form of the disease. Mutations E148Q and V726A have reduced penetrance. The clinical features, associated with the M680I and the complex V726A–E148Q allele, are not well defined. This study aims to further characterise the phenotypic profile associated with the major MEFV mutations. We investigated 220 FMF patients, in whom both FMF alleles have been identified, and found that different genotypes are characterised by a specific allelic related clinical profile and penetrance. Homozygotes for the M694V mutation and the complex V726A–E148Q allele are the most severely affected and often endure renal amyloidosis. Homozygotes for the M680I and V726A alleles and compound heterozygotes for either the M694V or the V726A–E148Q alleles in combination with either the E148Q, the V726A or the M680I alleles are significantly less severely affected. The morbididity associated with the complex V726A–E148Q allele by far outweighs that associated with the V726A allele, bearing evidence to the fact that the E148Q mutation is not a benign polymorphism. These findings increase our understanding of the role of allelic variability in disease expression.
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Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disorder (MIM# 249100), particularly common in populations of Mediterranean extraction.[1] It is characterised by recurrent acute self-limited episodes of fever and peritonitis and attacks of pleuritis, arthritis and erysipelas-like skin disease.[1] The clinical variability is wide. In some patients systemic amyloidosis, expressed most notably in the kidneys, develops.[1]

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Mutations in the pyrin/marenostrin (MEFV) gene have been identified in the majority of FMF patients.[2,3] These include four conservative missense mutations (M680I, M694V, M694I, V726A), clustered in exon 10, which, together with mutation E148Q, in exon 2, account for the vast majority of FMF chromosomes identified in our patients.[4–6] It has been established, that the phenotypic variablility of the disease is, at least, partly due to allelic heterogeneity. Mutation M694V is associated with a severe phenotype and amyloidosis, and mutation V726A with a milder form of the disease.[7,8] Few studies, however, profess that the different mutations are not strictly correlated with phenotypic variations and that V726A can be associated with a severe phenotype and amyloidosis.[9] Recent population based studies have shown that the frequency of FMF mutant alleles, in our general population, by far exceed those

deduced from the prevalence of the disease and assert that the majority of individuals, who comply with the genetic definition of FMF, remain unaffected.[4–6] This is mainly attributed to the high frequency of mutations of low penetrance (E148Q, V726A) and partly to the role of unknown, genetic and/or environmental, modifiers.

To further characterise the phenotypic profile associated with the predominant MEFV mutations, particularly M680I and the complex V726A–E148Q allele, we performed a genotype-phenotype correlation analysis on a large cohort of Jewish and Arab FMF patients in whom both FMF alleles have been charaterised.

Patients and methods

The study group comprises 220 FMF patients, who were offered a genetic test as part of their diagnostic work-up and in whom both mutant alleles have been characterised. It includes 94 pediatric cases, referred to the pediatric rheumatology clinic, 84 adults referred to the genetic counseling clinic at Rambam Medical Center, and 42 patients, with amyloidosis, recruited at the FMF clinic of the Sheba Medical Center. Clinical features – prior to onset of colchicine therapy – were recorded through a standardised form featuring an established set of clinical criteria,[10] such as fever, abdominal, thoracic, articular, skin and renal manifestations, duration and frequency of attacks and colchicine dosage required. Disease severity was calculated using the Tel-Hashomer key.[11] The study had the approval of the hospitals' IRB. Patients were stratified according to ethnic descent, namely: Jews of North African descent, Ashkenazi and non-Ashkenazi Jews and Arabs of Muslim, Druze and Christian origin.

Mutations M694V, M680I, M694I, V726A and E148Q were investigated by PCR amplification and digestion with appropriate enzymes made to distinguish the wild type from the mutant allele, as previously described.[3,12] Both M680I mutations (either a or b) abolish a constitutive *Hinf*I restriction site and cannot be distinguished by our assay.

The significance of differences between groups was calculated by either the χ^2 test or the *t*-test. All statistical tests were two-sided.

Results

Genetic characterisation

The cohort includes 220 individuals (129 Jewish and 91 Arabs; 124 males and 96 females), with FMF, originating from 178 unrelated families. Table 1 depicts the distribution of 356 mutant alleles among 178 unrelated patients. M694V accounted for the majority of FMF chromosomes (224/356; 62.9%). Mutations V726A, M680I, E148Q, M694I and the complex V726A–E148Q allele, accounted for 13.8, 7,6, 5,3, 5.3 and 5.1% of FMF chromosomes, respectively.

Among North-African Jews, mutations M694V and E148Q accounted for 95.6 and 3.3% of FMF chromosomes, respectively. Mutation M694I, mainly restricted to Arab patients was observed, in combination with either M694V or E148Q, in three non-Ashkenazi Jewish individuals.

Among Arab patients, all major mutations were identified. V726A and M680I accounted for 26.7 and 22.5% of FMF chromosomes, respectively. In most cases, mutation V726A was associated with either M694V or M680I on the other chromosome. V726A homozogotes were rare. M694V, M694I and the V726A–E148Q alleles, equally distributed among Arab patients, accounted for 16.7, 13.3 and 15% of FMF chromosomes, respectively. E148Q was rare and M680I was exclusive to Muslim Arabs. The complex V726A–E148Q allele, preponderant among patients of Druze origin, accounted for 50% of FMF chromosomes in this ethnic group.

The distribution of genotypes among 220 FMF patients, stratified according to ethnicity, is shown in Table 2. Nearly half (105/220; 47.7%) were M694V homozygotes. Of these, the vast majority were North-African Jews. Two common genotypes, M694V/V726A (*n*=25) and M694V/E148Q (*n*=12), were observed more frequently among Jews. The M680I/V726A (*n*=12) and the V726A–E148Q/V726A–E148Q genotypes (*n*=12), were restricted to individuals of Arab descent.

Phenotype-genotype correlation

Clinical manifestations, mean age at disease onset and severity score, associated with the different genotypes, are provided in Table 3. M694V homozogotes (*n*=105) scored higher ($P<0.0001$), manifested more frequently articular ($P<0.0001$) and renal manifestations ($P0.005$), and consumed higher doses of colchicine to control their attacks ($P<0.0001$), compared to compound heterozygotes for the M694V mutation with either of mutations V726A, M680I or E148Q (*n*=39) and compared to V726A or M680I homozogotes (*n*=15). However, compared to V726A–E148Q homozygotes, M694V homozogotes did not differ on any of these effects other than the frequency of arthritis that was higher among M694V homozogotes. No statistical difference was observed when compound heterozygotes for the M694V mutation with either of mutations V726A, M680I or E148Q were compared with compound heterozygotes for the V726A–E148Q allele with either of mutations V726A, M680I or E148Q (Table 4). The severity score associated with M694I was slightly lower than that associated with M694V but the small number of individuals bearing the M694I mutation does not allow statistical evaluation.

Amyloidosis

Of the 42 patients with amyloidosis (34 non-Ashkenazi Jews, eight Arabs), 32 were M694V homozygotes and four were V726A–E148Q homozygotes. The remaining six were compound heterozygotes for one of these alleles with either the V726A (*n*=4), the M694I (*n*=1) or the E148Q mutant allele

Table 1 Distribution of predominant carrier chromosomes among 178 unrelated FMF patients of Jewish and Arab descent

Ethnic background	*M694V*	*M694I*	*V726A*	*M680I*	*E148Q*	*V726A/E148Q*	*Total*
North African Jews	175	2	0	0	6	0	183
Iraqi Jews	12	0	6	0	4	0	22
Non-Ashkenazi Jews*	17	1	3	0	1	0	22
Ashkenazi Jews	0	0	8	0	1	0	9
Total Jewish alleles	*204*	*3*	*17*	*0*	*12*	*0*	*236*
Muslim Arabs	19	6	23	27	2	7	84
Christian Arabs	0	7	7	0	0	0	14
Druze	1	3	2	0	5	11	22
Total Arab alleles	*20*	*16*	*32*	*27*	*7*	*18*	*120*
Grand total	**224**	**19**	**49**	**27**	**19**	**18**	**356**

*Includes patients from Iraq, Eqypt, Greece.

Table 2 Distribution of genotypes in 220 patients of Jewish and Arab descent

			Jews			*Arabs*		
Genotype	*Total*	*NA*	*NAJ*	*Mixed*	*Mixed/Ashk*	*Muslim*	*Christian*	*Druze*
M694V/M694V	105	85	5	8	0	7	0	0
M694V/M694I	4	1	1	0	0	2	0	0
M694I/M694I	5	0	0	0	0	0	4	1
M694V/E148Q	12	5	2	3	1	0	0	1
M694I/E148Q	5	0	0	1	0	2	0	2
M694V/V726A	25	0	4	5	8	7	1	0
M694I/V726A	4	0	0	0	0	3	1	0
M694V/M680I	2	0	0	0	0	2	0	0
M680I/M680I	7	0	0	0	0	7	0	0
V726A/V726A	8	0	0	0	0	6	1	1
M680I/V726A	12	0	0	0	0	12	0	0
Complex/M680I	4	0	0	0	0	4	0	0
Complex/V726A	6	0	0	0	0	6	0	0
Complex/E148Q	9	0	0	0	0	1	0	8
Complex/Complex	12	0	0	0	0	8	0	4
Total	220[a]	91	12	17	9	67	7	17

NAJ=non-Ashkenazi Jews; NA=north African Jews; mixed=patients of mixed North African and non-Ashkenazi Jewish descent; mixed/Ashk=patients of both Ashkenazi and either North African or non-Askenazi Jewish descent. [a]Includes 178 independent patients and 44 affected relatives.

(*n*=1) (Table 3). Altogether, M694V and the complex V726A–E148Q alelles accounted for 81 and 11.9% of carrier chromosomes, respectively.

Discussion

To better delineate the phenotypic profile associated with the various genotypes, we investigated 220 manifesting FMF patients in whom both MEFV alleles have been identified. The results of this study agree with the observation that M694V homozygotes have a more severe form of disease, manifested by earlier age of onset, higher frequency of attacks, prevalence of arthritis and a higher severity score, compared to compound heterozygotes for this mutation and either of V726A, M680I and E148Q and to those who carry these three mutations in any combination.

In Ashkenazi Jews the FMF carrier rate was previously estimated at 1/135.[13] Recent population based studies have, surprisingly, derived a prevalence of more than 1/5, in Ashkenazim, for the E148Q and the V726A mutations taken together,[4–6] validating the reduced penetrance of these two mutations. The small number of V726A homozygotes and the absence of V726A/ E148Q compound heterozygotes, among our patients, strengthen the view that these two alleles have reduced penetrance. Otherwise, mutations V726A and E148Q were associated, as expected, with a mild form of disease.

Muslim Arabs harbor all the predominant MEFV mutations and exclusively display the M680I mutation. The relatively high number of individuals, either homozygous or compound heterozygous for M680I, included in this study, allows to better delineate the clinical manifestations associated with this mutation, found to be associated with a moderate form of disease. Mutations M680I, V726A and M694V, account for the majority of FMF chromosomes (32.1, 27.4 and 22.6%, respectively) in this ethnic group. We have previously

Table 3 Clinical features associated with MEFV genotypes in 220 FMF patients

			Frequencies				Mean ± S.D.			
Genotype	*No.*	*M/F*	*Arthritis*	*Renal amyloidosis*	*Peritonitis*	*Fever*	*Onset*	*Attacks*	*Colchicine*	*Severity*
M694V/M694V	105	59/46	76	32	93	94	6.2±6.7	1.8±1.5	1.7±0.6	9.3±2.9
Complex/complex	12	1.4	4	4	12	12	6.8±3.8	3±2.0	1.8±0.6	8.8±2.5
M694V/M694I	4	3/1	2	1	3	3	8.6±10.3	2.6±1.2	1.5±0.4	8.7±4.1
M694I/M694I	5	1/4	4	0	5	5	5.8±3.1	1.7±0.9	1.1±0.2	7.2±1.9
M694I/E148Q	5	1/4	3	0	5	4	5.4±2.9	1.5±0.6	1.5±0.6	7.2±1.8
M680I/M680I	7	6/1	1	0	7	7	4±3.6	2.5±2.0	1.3±0.3	7±1.8
M694V/M680I	2	2/0	2	0	2	2	6±2.8	0.65±0.5	1.0±0.0	7±0.0
Complex/V726A	6	3/3	5	1	5	5	20±13.5	1.6±1.6	1.5±0.5	6.5±1.2
M694V/V726A	25	14/11	5	3	24	23	8.8±8.3	2.4±3	1.2±0.3	6.1±2.3
M680I/V726A	12	6/6	0	0	12	12	9.2±7.5	2±1.2	1.4±0.4	5.7±1.7
Complex/M680I	4	1/3	0	0	4	4	5.7±5.6	2.1±1.4	1±0.0	5.7±1.9
V726A/V726A	8	5/3	1	0	8	8	18.5±14.7	2±1.5	1.2±0.3	5.4±2.6
Complex/E148Q	9	6/3	1	1	9	9	18±8.6	1.3±1.1	1.3±0.5	5.1±6.5
M694V/E148Q	12	6/6	0	0	10	10	13.7±10.6	1.8±1.7	1.0±0.7	4.8±1.8
M694I/V726A	4	1/3	1	0	4	4	17.8±5.7	0.95±0.8	1.0±0.0	3.5±1
Total	220	124/96	105	42	202	201	8.6±8.5	1.9±1.8	1.5±0.6	7.7±3.1

Table 4 Clinical features associated with selected genotypes in FMF patients

		Frequencies				Mean ± S.D.			
Genotype	*No.*	*Arthritis*	*Renal amyloid*	*Peritonitis*	*Fever*	*Onset*	*Attacks*	*Colchicine*	*Severity*
M694V/M694V	105	76	32	93	94	6.2±6.7	1.8±1.5	1.7±0.6	9.3±2.9
V726A-E148Q/V726A-E148Q	12	4	4	12	12	6.8±3.8	3±2.0	1.8±0.6	8.8±2.5
P value[b]		0.006	0.839	0.216	0.239	0.999	0.232	1.0	0.847
M694V/other[a]	39	7	7	35	35	10.2±9.6	2.1±2.6	1.1±0.5	5.6±2.2
P value[c]		<0.0001	<0.005	0.842	0.969	0.084	0.871	<0.0001	<0.0001
Other/other[a]	27	2	0	27	27	10.2±10.6	2.1±1.5	1.3±0.3	5.9±2.1
P value[d]		<0.0001	0.001	0.065	0.275	0.106	0.927	0.011	<0.0001
V726A-E148Q/other[a]	19	6	2	18	18	16±10.6	1.96±1.3	1.3±0.5	5.7±2.1
P value[e]		0.919	0.117	0.419	0.419	0.02	0.233	0.034	0.003
P value[f]		0.245	0.718	0.525	0.525	0.078	0.798	0.880	1.0

[a]Mutations E148Q, M680I and V726A. [b]*P* value applied to genotypes M694V/M694V and V726A–E148Q/V726A–E148Q. [c]*P* value applies to genotypes M694V/M694V and M694V/other. [d]*P* value applies to genotypes M694V/M694V and other/other. [e]*P* value applies to genotypes V726A–E148Q/V726A–E148Q and V726A–E148Q/other. [f]*P* value applies to genotypes M694V/other and V726A–E148/other.

investigated the carrier rates of these mutations, in the general healthy Muslim population, and found them to be 3, 8 and 1%, respectively.[4] Taking into account these numbers we can deduce that M694V is twice as penetrant as M680I, and that M680I is at least twice as penetrant as V726A.

This study, conducted on the largest cohort of FMF patients in whom both FMF alleles have been characterised, helps to define the phenotypic profile associated with the V727A–E148Q allele. Homozygotes for the complex V726A–E148Q allele are as severely affected as M694V homozygotes and often endure renal amyloidosis. Finally, the morbidity conferred by the V726A–E148Q allele by far outweighs that associated with the V726A allele, bearing evidence to the fact that the E148Q mutation is not, as previously suggested,[14] a benign polymorphism.

Patients with amyloidosis were randomly, yet deliberately, recruited by us and do not reflect the prevalence of this manifestation among our FMF patients. The majority were M694V homozygotes. V726A–E148Q was the second most frequent allele observed among such patients. Four, of the 12 individuals, homozygous for the V726A–E148Q allele, had renal amyloidosis. Of the remaining eight, four were under 13 years of age and two were under 25 years of age. We deduce that the complex V726A–E148Q allele strongly predisposes to renal amyloidosis.

The wide clinical variability of the disease seems to be partly affected by allelic heterogeneity and partly by additional genetic and/or environmental modifiers. Sex is considered as one likely modifier factor.[15] Consistent with this observation, our study group includes more males than females (ratio 1.31). Considering, first, the vast intra-familial and interfamilial variability among individuals with identical genotypes (data not shown) and second, the fact that among individuals who carry 'mild' mutations (V726A and M680I) some remain symptom free while others manifest a variable disease, the role of additional, still unknown, genetic and/or environmental modifiers remains to be elucidated.

The results of this study reinforce the view that M694V homozygotes have a severe form of disease[7,8] and do not support the notion that mutations M680I, V726A, and

E148Q are equally contributive to disease severity.[9,16] Our observation, that the V726A – E148Q allele is associated with a severe disease and strongly predisposes to renal amyloidosis, highlights the need to prescribe colchicine, as early as possible, to patients who carry the complex allele. Since amyloidosis, which is preferentially associated with either the M694V or the V726A – E148Q allele, may develop in FMF patients who do not have attacks of serositis (phenotype II), the need to screen asymptomatic family members, for mutations in the MEFV gene, is underlined.

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