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Prevalence of known mutations in the MEFV gene in a population screening with high rate of carriers

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Abstract The Familial Mediterranean Fever (FMF) shows an autosomal recessive pattern of inheritance and affects certain ethnic groups. Disease is caused by mutations in MEFV gene and more than 180 mutations have been defined in affected individuals. Current study aimed to determine the frequency-type of the mutations for MEFV gene in Sivas—middle Anatolian city. The cohort was composed of 3340 patients. MEFV gene mutations were studied by multiplex PCR based reverse hybridization stripAssay method. Patients' clinical features were; family history: 68%, erysipelas-like erythema: 17.6%, fever: 89.9%, abdominal pain: 84.2%, peritonitis: 90.2%, arthritis: 33%, pleuritis: 14.2%, parental consanguinity: 21.2%. Current results revealed that M694V is the most frequent mutation (43.12%), followed by E148Q (20.18), M680I(G/C) (15.00%) and V726A (11.32%). The study population has a high rate of carriers and the E148Q mutation frequency was found to be highest when compared to the other regions of Turkey and other Mediterranean groups.

Keywords FMF · Allelic frequencies · Sivas population · Turkey

Introduction

Familial Mediterranean fever is the prototypic recessively inherited autoinflammatory disease, prevalent among multiple populations from the eastern Mediterranean basin, particularly Jews, Armenians, Turks and Arabs. FMF, a disease of innate immunity, is characterized by seemingly unprovoked episodes of recurrent fever, serositis, arthritis

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and erysipelas-like erythema [1, 2]. Symptoms start in the first decade of life in about 50% of cases. Eighty to 90% of cases are diagnosed before the age of 20 years [3]. Amyloidosis is the most significant complication of FMF and may result in progressive renal failure. Before the advent of colchicine, amyloidosis was the main cause of death in 60% of affected patients who were over 40 years of age [4]. Colchicine is the major therapeutic agent of FMF both for the prophylaxis of attacks and amyloid deposition since 1970s [5] nevertheless new therapeutic options such as low-dose methotrexat, infliximab [6], anakinra [7], thalidomide and etanercept [8] were reported for therapy in colchicine resistant patients.

The gene MEFV is responsible for FMF and has been mapped to short arm of chromosome 16p13.3. MEFV gene is consists of 10 exons and encodes a 781 amino acid protein called pyrin which is expressed in polymorphonuclear cells, cytokine activated monocytes, dendritic cells, and synovial fibroblasts [9]. Due to the restricted expression of pyrin in innate immune cells, the major role of pyrin appears to be in the regulation of inflammation [1]. Mutations interfere with the role of the pyrin domain, allowing an uninterrupted inflammatory cascade. Syndrome includes high levels of ESR, CRP and SAA but still there is no case specific consensus on phenotype and genotype relation in FMF patients. To date more than 180 mutations associated with FMF have been identified [10]. The majority of cases are caused by four mutations located on exon 10: M694V, V726A, M680I(G/C) and M694I, the prevalence of which varies according to the population studies [11]. The carrier frequency of *MEFV* mutations is quite high in the four classically affected populations, ranging from 37 to 39% in Armenians and Iraqi Jews to 20% in Turks, North African and Ashkenazi Jews, and Arabs [12–15].

To date a relatively small number of FMF studies were reported from different regions of Turkey but FMF database for Turkish population still needs a new nationwide studies on different regions including large patients and/or carriers number. Here we report a MEFV gene mutations spectrum data belong to 3340 patients that analysed in our laboratory over 4 years due to their some minor and/or major clinical FMF findings.

Materials and methods

In the current study the molecular test results of 3340 patients (1822 male, 1518 female) that referred to our department for MEFV mutation analysis between November 2005 and June 2009 were evaluated. The origin of all patients was Turkish and mainly living in Sivas—central Anatolian city. An informed consent was obtained from all adults and parents of child cases with the approval of the local ethical committee.

A questionnaire including main clinical data concerning the age, sex, consanguinity, symptoms related with attack (fever, abdominal pain, arthritis, chest pain, erysipelas-like erythema, positive family history) registered on a standard form. Total genomic DNA was extracted from peripheral blood samples using DNA isolation kit according to manufacturer's instructions (Invitek Invisorb Spin Blood Kit, Germany). Patients were screened for 12 MEFV gene mutations (E148Q, P369S, F479L, M680I(G/A), M680I(G/C), I692del, M694V, M694I, K695R, V726A, A744S, R761H) using reverse hybridization stripAssay (Vienna Lab, FMF StripAssay, GMBH, Austria) according to the manufacturer' instructions:

- Multiplex PCR amplification of the target exons by using biotinylated primers
- Incubation of PCR products to nitrocellulose strips for revers hybridisation
- Color development and detection of the signals (Auto-LIPA-Innogenetics).

Statistical analysis was performed using SPSS 14.0 program (SPSS Inc., Chicago, IL, USA) for the evaluation of the mutated and non-mutated signals that received in the current results.

Results

The cohort was composed of 3340 patients with a male:female ratio of 1.2:1 (1822 male, 1518 female; mean age, 23.0 ±13.33 year; range, 5–71 year). The main clinical characteristics of the patients were as follows; peritonitis was observed in 90.2% of the patients, fever in 89.9%, abdominal pain: 84.2%, arthritis in 33%, pleuritis in 14.2%, rash and eryspilas-like erythema in 17.6%. Sixty-eight percent of patients had positive family history of FMF. Parental consanguinity was also questioned for all patients and 21.2% of patients were found to have parental consanguinity. MEFV mutations were studied by multiplex PCR based reverse hybridisation stripAssay method. No mutations were detected in 1548 (46.4%) patients but one and/or compound mutations were detected in 1792 (53.6%) patients (Table 1). A thousand two hundred thirteen patients (67.68%) were heterozygous, three hundred ninety-five (22.04%) were compound heterozygous, six patients (0.31%) had complex alleles and a hundred eighty-four (10.27%) patients were in homozygous mutation in out of 1792 mutated patients (Table 1). Current results of 3340 FMF patients revealed that M694V was the most frequent mutation (43.12%), followed by E148Q (20.18), M680I(G/C) (15.00%) and V726A (11.32%) in Sivas-central

Table 1 Genotype and frequency of detected mutations in the current population

Mutation type	Genotype	Patients	
		n	%
Heterozygous	M694V	500	27.90
	E148Q	327	18.25
	M680I(G/C)	132	7.37
	V726A	124	6.92
	A744S	51	2.85
	P369S	35	1.95
	R761H	26	1.45
	F749L	7	0.39
	M694I	7	0.39
	K695R	4	0.22
	Total	1213	67.68
Compound heterozygous	M694V/M680I(G/C)	94	5.24
	M694V/V726A	71	3.96
	M694V/E148Q	61	3.40
	E148Q/P369S	35	1.95
	M680I(G/C)/V726A	34	1.89
	M694V/R761H	22	1.23
	E148Q/M680I(G/C)	14	0.78
	E148Q/V726A	8	0.45
	E148Q/A744S	8	0.45
	M680I(G/C)/R761H	8	0.45
	P369S/M649V	7	0.39
	M649V/A744S	5	0.28
	F749L/V726A	4	0.22
	M680I(G/C)/A744S	3	0.17
	E148Q/F749L	3	0.17
	V726A/A744S	2	0.11
	V726A/R761H	2	0.11
	M694V/F749L	1	0.05
	E148Q/R761H	1	0.05
	F749L/M680I(G/C)	1	0.05
	M694V/K695R	1	0.05
	P369S/F749L	1	0.05
	M694I/E148Q	1	0.05
	M694V/M694I	1	0.05
	K695R/A744S	1	0.05
	M694V/E148Q/P369S	2	0.11
	M680I(G/C)/E148Q/P369S	1	0.05
	M694V/E148Q/V726A	1	0.05
	M694V/E148Q/M680I(G/C)	1	0.05
	M694V/M680I(G/C)/V726A	1	0.05
	Total	395	22.04
Homozygous	M694V	127	7.08
	M680I(G/C)	34	1.90
	V726A	11	0.61
	E148Q	9	0.50
	A744S	1	0.05
	R761H	1	0.05

Table 1 continued

Mutation type	Genotype	Patients	
		n	%
	K695	1	0.05
	Total	184	10.27
	Patients with MEFV mutations	1792	53.6
	Patients without MEFV mutations	1548	46.4
	Total	3340	100

Table 2 The frequency of the 12 studied mutations in 2359 detected mutated alleles in the current population

Mutation type	Number of alleles	Allele frequency (%)
M694V	1017	43.12
E148Q	476	20.18
M680I(G/C)	354	15.00
V726A	267	11.32
P369S	78	3.30
A744S	72	3.05
R761H	61	2.59
F749L	17	0.72
M694I	9	0.38
K695R	8	0.34
M680I(G/A)	0	0.0
I692del	0	0.0
Total	2359[a]	100

[a] Complex heterozygous alleles (n = 6) genotypes were excluded because of unclear allelic state

Anatolian population (Table 2). The K695R was the rarest mutation and the frequency of the rare mutations were K695R (0.34%), M694I (0.38%), F749l (0.72%), R761H (2.59%), A744S (3.05%) and P369S (3.30%) respectively. Most of those rare mutations were in combine heterozygous state. No mutation were detected in M681I(G/A) and I692del alleles in that cohort. Four common mutations (M694V, E148Q, M680I(G/C) and V726A) were found in 89.62% in patients of current population (Table 2).

The study population has a high rate of carriers and E148Q mutation frequency was found the highest when current results compared to the other regions of Turkey (Tables 2, 3).

Discussion

Common 12 MEFV gene mutations and clinical features among FMF patients was studied. In the current results, peritonitis was the most common clinical feature (90.2%) followed by fever (89.9%), abdominal pain (84.2%), family history (68%), arthritis (33%), erysipelas-like erythema (17.6%), pleuritis (14.2%) and parental consanguinity (21.2%). Peritonitis was also reported as a most common clinical feature by Tunca et al. in another large series of Turkish patients [16]. The consanguinity as well as positive family history should be reconsidered due to recessive inherited pattern of FMF. Because of high carrier frequency and recessive inheritance pattern of FMF in addition to family history parental consanguinity should be reconsidered in such a current population which have high parental consanguinity rate. The parental consanguinity and familial history of FMF findings were detected as 21.2 and 68% in the current population screening with high rate of carriers respectively. Parental consanguinity and positive familial history were also reported by some other researchers in different ethnic groups varying between 20 and 60% [17, 18]. Analyzing twelve common mutations of FMF, we found that 53.6% of cases had positive mutations varying between simple heterozygous (67.8%), compound heterozygous (22.04%) and homozygous (10.27%) (Table 2; Fig. 1). Current analysis of twelve common mutations in FMF patients were resulted in 53.6% of cases had positive mutations varying between simple heterozygous (67.8%), compound heterozygous (22.04%) and homozygous (10.27%) (Table 1; Fig. 1). Current results of 3340 FMF patients revealed that M694V mutation was the most frequent mutation (43.12%), followed by E148Q (20.18), M680I(G/C) (15.00%) and V726A (11.32%) in Sivas-central Anatolian population (Table 2). Studies from different regions of Turkey have demonstrated that M694V is the most frequent mutation and followed by E148Q but remaining mutations differs from one region to another. When in Aegean and southeast region of Turkey M694V and E148Q were followed by V726A mutation [19–21], in Mediterranean (south) region of Turkey M694V and E148Q mutations were followed by M680I(G/C) mutation. In a field study carried out by Onen et al. indicated that the prevalence of FMF in Sivas may be higher than that in general Turkish population, which has been reported to be 0.1% [20]. Gkretsi et al. claimed that the carrier rate for the MEFV mutations of M694V and V726A is extremely low in the general Greek population [16]. Rate of compound heterozygosity in the current population (22.04%) was compatible to the southeast region (21.84%), but lower than Aegean region (26.51%). Rate of heterozygosity

Table 3 The comparation of the allele frequency of the four commonest and other MEFV mutations in different regions of Turkey

Region (studied patients)	M694V	V726A	M680I(G/C)	E148Q	Others	Reference
Current results (3340)	43.12	11.32	15.00	20.18	10.38	
Kirikkale (126)	31.80	9.10	13.60	13.60	31.90	Gunel-Ozcan et al. [13]
Aegean (1.201)	47.60	12.95	11.94	16.75	10.76	Akin et al. [20]
Diyarbakir (119)	28.57	9.60	7.56	12.18	42.09	Pasa et al. [22]
Turkish FMF consortium (1.090)	51.40	8.60	14.40	–	25.60	Tunca et al. [24]
Gulhane military hospital (330)	50.00	9.70	14.10	1.37	24.83	Demirkaya et al. [27]
Black sea (625)	33.90	4.90	15.80	3.50	41.90	Yiğit et al. [28]

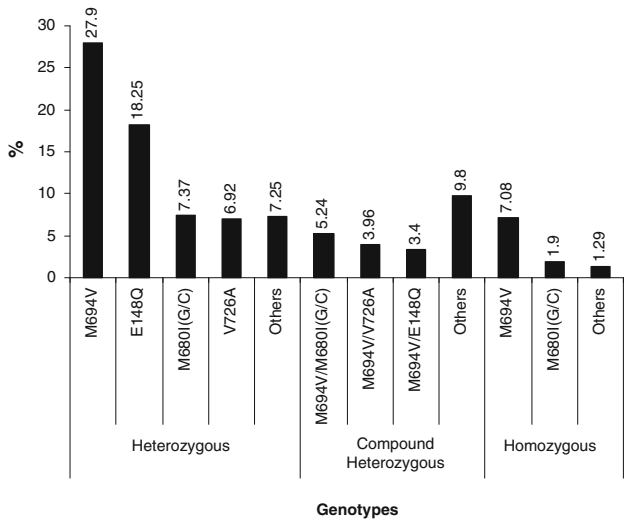

Fig. 1 Frequencies of most frequent genotypes in the Sivas-central Anatolian population

(67.68%) was higher than Aegean (54.11%) and southeast region (53.26%) of Turkey (Table 3). FMF is a hereditary disease which has excessive compound mutation frequency. In case of compound heterozygosity it is important whether these mutations are in located in same chromosome or different chromosomes. If mutations are located in same chromosome patient who carry them has one wild type allele otherwise patient has two mutant alleles and it is possible to consider as homozygous patients. This is very imported for the transmission of the mutation to the next generations. Most of the methods that determined MEFV gene mutation are not give the information about allelic state of compound mutations. Data on genotype phenotype correlation in FMF is generally agree with the presence of M694V homozygosity correlates with most severe FMF phenotypes and amiloidosis [22–26]. The most serious complication of those patients is chronic renal failure that highly associated with point mutations in the MEFV gene. The study population has a high rate of carriers and E148Q mutation frequency after M694V was found the highest when current results compared to the other regions of Turkey and other Mediterranean groups (Tables 2, 3) [15]. Therefore it is expected that M694V mutation which cause the worst pathologic phenotype must be the less penetrated mutation in patients with FMF. According to the preliminary results of our another study that conducted on dialysis depended chronic renal failure patients in the same population; patients have a high rate of MEFV gene mutations as a heterozygous profiles (data not presented). This study and almost all of the studies were shown that M694V is the most frequent mutation in FMF patients from different ethnic groups. On the other hand a significant association is reported between the presence of E148Q mutation toad increased rheumatoid factor levels in elderly population by Ozturk et al. [14].

In conclusion, the MEFV gene mutations are varying according to the population characteristics, such as familial history, parental consanguinity, migration, population type (small and close type) and the presence of heterozygous carriers. All above characteristics are fit to our study population that caused to increased number of mutated carriers. Such a populations should be examine for mutation type and frequency for the healthy next generations.

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