Familial Mediterranean fever gene mutation frequencies in a sample Turkish population

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ABSTRACT

Objective. *Our knowledge about the frequencies of mutations in the Turkish population is based on the studies on the affected patients and hospital-based control groups. We aimed to determine the frequencies of* MEFV *gene mutations in a population-based field study in Turkey.*

Methods. *Turkish citizens aged between 5 and 65 years were included in the study. Cities from seven regions of Turkey were studied. Blood samples were obtained from individuals who gave permission for laboratory experiments, and they were analysed for 10* MEFV *gene mutations.*

Results. *Among 500 participants,* MEFV *mutations were found in 74 (14.8%). Sixty four (12.8%), 7 (1.4%), and 3 (0.6%) participants were heterozygous, compound heterozygous, and homozygous, respectively. Among inhabitants with heterozygous mutations, the most common heterozygous mutations were E148Q/- and M694V/-. Sixteen participants were found to be heterozygous for M694V, 2 were compound heterozygous for M694V/E148Q, and one was homozygous for M694V/M694V mutation; in total, the frequency of M694V allele was 4% (n=20). Twenty-three (4.6%) individuals were heterozygous for common mutations (M694V, M680I, V726A). Total allelic frequency was 8.4%.*

Conclusion. *Our study, which describes the* MEFV *mutational spectrum and distribution in a healthy Turkish population, found a carrier rate that is much higher than expected.*

Introduction

Familial Mediterranean fever (FMF) is a hereditary inflammatory disease with an autosomal recessive trait, which is characterised by episodic, self-limiting attacks of fever, along with abdominal pain, chest pain, and arthralgia (1). The responsible gene for FMF is mapped on chromosome 16p13.3 and encodes a 15 kb region of 781 amino acids, a protein known as pyrin which is primarily expressed in polymorph nuclear cells (2). This protein plays an important role in the regulation of inflammation, and mutated pyrin leads to heightened potential for inflammation characterised by excessive Il-1β secretion in FMF (3).

The cloning of the gene (designated as *MEFV*) broadened our understanding of FMF population genetics. Four missense mutations (M680I, M694V, M694I, and V726A) are responsible for a large percentage of mutations; especially the M694V mutation is usually associated with the most severe phenotype (4). FMF is common in Middle Eastern populations, including Sephardic and Ashkenazi Jews, Turks, Armenians, and Arabs (5). The carrier rate varies among these populations and the disease prevalence reaches frequencies of 1/500–1 / 1000 (6, 7).

The Turkish population numbering more than 75 million inhabitants has a large proportion among FMF cases worldwide. Recent FMF studies from various regions of Turkey have been reported, but their study populations were relatively small (8, 9). To date a relatively small number of FMF studies were reported from different regions of Turkey. Our knowledge about the frequencies of mutations in the Turkish population is based on the studies on the affected patients and hospital-based control groups. We aimed to determine the frequencies of *MEFV* gene mutations in a population-based field study in Turkey.

Materials and method

This is a population-based field study in which individuals were accessed by house visits throughout Turkey. Turkish citizens aged between 5 and 65

years who gave informed consent (parents in the name of paediatric subjects) were included in the study. The participants who resided for less than a year in their place of residence were excluded. Information about the participants was collected through home visits and interviews carried out by specially trained field study teams. During the interviews, all participants were asked to complete the study questionnaire, which also included questions for symptoms and signs of FMF. Participants with signs and symptoms that were suspicious of FMF were excluded.

Sample size

The expected prevalence of FMF *MEFV* gene is approximately 20% in Turkey. Based on this knowledge, the required sample size was calculated as 459 subjects to reach a study power of 95% confidence and ±5% sensitivity. With the anticipation of possible loss of data and participants during the study period, it was planned to include 500 individuals. The study field was selected according to the 12 Local Administrative Units (LAUs) compatible with the nomenclature of territorial units for statistics (EUROSTAT NUTS 1). House visits in villages and towns were randomly performed. Care was taken that the sampling process would represent Turkish population in terms of region, gender, and age groups. The number of clusters in the regions and provinces was determined according to the distribution of the subjects among regions, as well as the gender- and age-based distribution of the subjects in these regions and provinces (2007 Address-based Population Registry System of The Turkish Statistical Institute). By this way, cities were selected from seven regions of Turkey. Blood samples were obtained from the individuals who gave permission for laboratory experiments, and were analysed for 10 *MEFV* gene mutations (A761H, A744S, V726A, K695R, M694V, M694I, M680I (G>A) on exon 10, F479L on exon 5, P369S on exon 3, and E148Q on exon 2) by using Qiagen type FMF sequence kits.

The study was approved by the Ethics Committee of the Gazi University Medical Faculty.

Table I. Patients with heterozygous mutations.

Alleles of heterozygous mutations	n	% (n/500)
E148Q/-	24	4.8
M694V/-	16	3.2
A744S/-	8	1.6
V726A/-	6	1.2
P369S/-	5	1
R761I/-	4	0.8
M680I/-	1	0.2
Total	**64**	**12.8**

Table II. Allele number, frequency, and distribution for the described mutations in the study group.

Mutation	n	% (n/1000)	Homozygous allele (n)	Compound heterozygous allele (n)	Heterozygous allele (n)
E148Q	35	3.5	4	7	24
M694V	20	2.0	2	2	16
P369S	8	0.8		3	5
A744S	8	0.8			8
V726A	7	0.7		1	6
R761H	4	0.4			4
M680I	2	0.2		1	1
Total	**84**	**8.4**	**6**	**14**	**64**

Results

Among 500 participants, 74 (14.8%) had *MEFV* mutations. Sixty-four (12.8%), 7 (1.4%), and 3 (0.6%), participants were heterozygous, compound heterozygous, and homozygous, respectively. *MEFV* mutation carrier rates by of the geographical region were as follows: Marmara:16.1%, Mid-Anatolian: % 20.2, Mediterranean: 6.4%, Aegean: 12.1%, South-east Anatolian 15.9%, North Anatolian 11.8%, East Anatolian 25.9%.

The frequency of inhabitants with 2 mutant alleles was 2% (n=10); none of them had symptoms of FMF during the investigation.

Among inhabitants with heterozygous mutations, the most common heterozygous mutations were E148Q/- and M694V/- (Table I).

Sixteen inhabitants were found to be heterozygous for M694V; 2 inhabitants were compound heterozygous for M694V/E148Q; and one inhabitant was homozygous for M694V/M694V mutation; in total, the frequency of M694V allele was 4% (n=20). Twenty-three (4.6%) individuals were heterozygous for common mutations (M694V, M680I, V726A).

Table II presents the allelic frequencies of the identified mutations; the total allelic frequency was 8.4%.

Discussion

To our knowledge, this is the first comprehensive study on FMF in the Turkish population, which examined the spectrum and distribution of *MEFV* mutations among a large national cohort of healthy inhabitants. Population characteristics, such as familial history, parental consanguinity, migration, population type (small or closed type) and the presence of heterozygous carriers may affect the frequencies *of MEFV* gene mutations. Kohei Fujikura's epidemiological meta-analysis (10) of full FMF mutations from 28 ethnic groups in 19 countries showed an unexpectedly high carrier rate for FMF in Europeans and Asians; therefore, it raised a strong possibility that some *MEFV* mutations may be benign variants with few or no clinical significance. Although we found a mutation carrier rate of 14.8% (n/N=74/500), the FMF carrier rate can be as high as 1 in 3 in the commonly affected ethnic groups, raising the possibility of selec-

tive heterozygote advantage (11, 12). A similar study in healthy 250 Armenian individuals has shown an overall carrier rate of FMF mutations as 21% (13, 14). The overall carrier frequency of all MEFV mutations was higher than expected (9.3%) from different in central and southeastern European countries as follows: Macedonia 16%, Serbia 11%, Bosnia and Herzegovina 8%, Slovenia 6% and Hungary 5% (15). Field *et al*. found a high *MEFV* mutation allelic frequency among Bucharian Jew, Georgian and Bulgarian origin (20%), whereas a study with 160 adults detected intermediate and low rates in Jews of Turkish and Yemenite extraction (14% and 8%, respectively) (16). In a screening sample (n=100) of nonconsanguineous parents of children presenting to various clinics, Yılmaz *et al*. (17) found a high frequency of carrier rate in a healthy Turkish population (20%). Our results show a lower mutation rate at 1/12 (8.4%).

Despite the established clinical criteria for FMF in adults and children (18, 19), many patients remain undiagnosed. One of the most significant complications of FMF is amyloidosis that characteristically develops after the age of 15 years in untreated individuals. Amyloidosis may even develop in those who do not have a history of recurrent inflammatory attacks; a favourable response to continuous colchicine treatment; a first-degree relative with FMF; and who are not a member of an at-risk ethnic group (20). Several studies have shown that patients with M694V mutation exhibit a more severe disease expression, increased susceptibility to amyloidosis, and unresponsiveness to colchicine therapy (21, 22). It is known that exon 10 variations are increasing the risk of FMF-phenotype, and a "multifactorial" form of FMF can be observed in heterozygous carriers with the contribution of genetic and environmental factors such as severe infections (23). Moreover, clinical characteristics of patients carrying a single mutated *MEFV* allele might be as severe as patients carrying two-mutant alleles (24). It was showed that rare mutations (A744S, P369S) may also lead to a similar disease severity as heterozygote common mutations (M694V, V726A, and M680I) (25). 3.8% (n=19) of our study population had at least one M694V; indicating that some 2.850.000 members of Turkish population carry a significant mutation (M694V), suggesting that the Turkish population is a high risk ethnic group.

In a field study carried out by Ozdemir O *et al*. that involved 3340 patients referred for *MEFV* mutation analysis, the rate of compound heterozygosity was found 22.04 % and the rate of heterozygosity 67.68% (26). Our study showed a relatively higher rate of heterozygosity (86.4%) among healthy inhabitants who carried a mutation. We thought that our study population was composed of asymptomatic or minimally symptomatic individuals; therefore the compound /homozygous mutation rate was lower and the heterozygous mutation rate was higher in our study group than those reported by the studies mentioned above.

Studies from different regions of Turkey have demonstrated that M694V is the most frequent mutation followed by E148Q although the remainder mutations differ from one region to another (27-29). Our population-based field study in healthy individuals identified E148Q (4.8%), M694V (3.2%), A744S (1.6 %), V726A (1.2%), and P369S (1.0%) as the most frequent mutations. Because carrier frequencies are far higher among healthy carriers than among patients with FMF, it has been proposed that E148Q is a polymorphism, not a disease causing mutation, and has a low penetrance (12, 30, 31). However, Topaloglu *et al*. shown that a patient population homozygous for E148Q had a heterogeneous clinical presentation with some subjects being symptomatic and requiring colchicine treatment (32). Hence, patients with E148Q/E148 should be closely monitored for disease onset, as was the case in our two participants.

The aim of the study was to determine the mutation frequencies for MEFV gene by analysing 10 different mutations in a population based field study in Turkey and we described a higher carrier rate which is much higher than expected. Physicians must pay attention to this higher rate of carriers in Turkey and consider the diagnosis of FMF with clinical symptoms.

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