

# Molecular Study of FMF Patients in Armenia

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**Abstract:** Familial Mediterranean Fever (FMF, MIM 249100), or Periodic disease, is a recessively transmitted and ethnically restricted condition prevalent in population from the Mediterranean decent. FMF notoriously has been hard to diagnose until mutations in the MEFV gene have been identified and as a tremendous help are used for the diagnosis of difficult cases. Since FMF can be controlled by medication, it is extremely desirable to have a firm diagnosis. The aim of this study was to establish the frequency of the most common mutations and genotypes in Armenian population. Molecular analysis of MEFV gene mutations in 3000 Armenian patients has demonstrated direct correlation between the clinical severity and the molecular diagnostic criteria of the disease, including the development of renal amyloidosis with MEFV genotypes. MEFV genotyping performed in the framework of a genetic counseling may reveal and identify affected individuals in presymptomatic phase, providing the possibility of a precocious start of the therapy.

**Keywords:** FMF, MEFV gene mutations, genotype and phenotype correlations.

## INTRODUCTION

FMF as a separate nosological form was established by Siegal in 1945 [17]; its familial cases and lethal renal complications were described by Cattani and Mamou in 1951 [3]. FMF is widely spread in Armenian, Arab, Jewish, Greek, Turkish and Italian populations. First information about recurrent inflammatory syndrome in Armenians was reported in ancient manuscripts of XII century. Nowadays more than 30000 FMF patients in Armenia are registered with high prevalence in highlanded regions. The daily treatment with colchicine was established by Goldfinger in 1972 [8]. Renal amyloidosis (RA), the most severe complication of FMF, leads to the progressive renal failure if the therapeutic treatment absent in affected individuals.

FMF is a genetic autoinflammatory disorder characterized by self-limited recurrent episodes of fever and localized serosal inflammation. Main publications showed that FMF is a prototype for several hereditary periodic fever syndromes, which are a group of rare Mendelian disorders of the inflammatory response. FMF is caused by the mutations in the chromosome 16p13.3 mapped by positional cloning [10, 11]. The 60 kb transcriptional unit in MEFV interval was identified on the basis of genomic sequence analysis and exon trapping. Haplotype and mutational analyses showed ancestral relationships among carrier chromosomes that have been separated for centuries [12]. It was showed by Kastner that FMF is the first disorder of different periodic inflammatory fevers caused by MEFV gene as one of attractive targets in Human Genome project, due to its defined regulatory role in the inflammatory response [12]. Touitou [18] presented the complex data of spectrum of 29 MEFV mutations, (26 missense and 1 nonsense mutations and 2 small deletions). The main mutational "hotspots" were identified at codons 148, 694, 680 [18]. Mansfield *et al.* [15] proposed that the coding protein, pyrin or marenostin, which is constituted of 781 amino acids and is a member of nuclear factors family homologous to the Ro52 antigen, regulates the inflammatory responses at the level of leukocyte cytoskeletal organization. This protein normally acts as a mediator in controlling inflammation.

We report the results of screening of MEFV mutations in group of 3000 Armenian patients suffering from FMF. Mutational study has lead to the observation of the correlations between the clinical severity and diagnostic criteria of the disease, including development of renal amyloidosis in individuals with MEFV genotypes. MEFV mutations in FMF patients (homozygotes and compound heterozygotes) in comparison with healthy carriers of mutations were investigated. The molecular analysis of MEFV mutations in a group of healthy individuals was performed to reveal the frequency of total carriers in Armenian population.

## MATERIAL AND METHODS

Present study provides the identification of seven MEFV gene mutations in independent alleles of 3000 probands suffered from FMF from different regions of Armenia. An ongoing survey gives the information of frequency of MEFV mutations carriers and reveals genotype-phenotype correlation. The average age of the patients was 30.5 years. The male/female ratio was 1.16 which indicated the existence of

reduced penetrance in females. The diagnosis of FMF was performed according to the established clinical criteria by Livneh *et al.* in 1997 [14]. In order to determine the clinical differences between patients the criteria of severity scores were used.

Molecular testing is used to screen the MEFV gene mutations in patients with the clinical suspicion of FMF. For this purpose genomic DNA was isolated from peripheral blood using "Puregene kit" (Gentra System, USA). DNA was amplified by PCR technique with specific primers for MEFV gene region and the screening of MEFV gene mutations was realized by mutation-specific restriction-endonuclease assay for seven MEFV mutations from exons 2, 3, 5, 10.

Mutational analysis was realized on 250 DNA samples obtained from control group of asymptomatic individuals.

The statistical analysis was performed using  $\chi^2$  and Fisher's tests.

## RESULTS AND DISCUSSION

The first molecular-genetic analysis of Armenian FMF patients (since 1997) was performed in collaboration with Prof. S. Amselem and Dr. C. Cazeneuve in The Hospital Henri Mondor (France). We have demonstrated that both diagnostic and prognostic values of MEFV analyses and particular cases of inheritance should lead to a new ways for the management of FMF, including genetic counseling and therapeutic decisions in affected families [4].

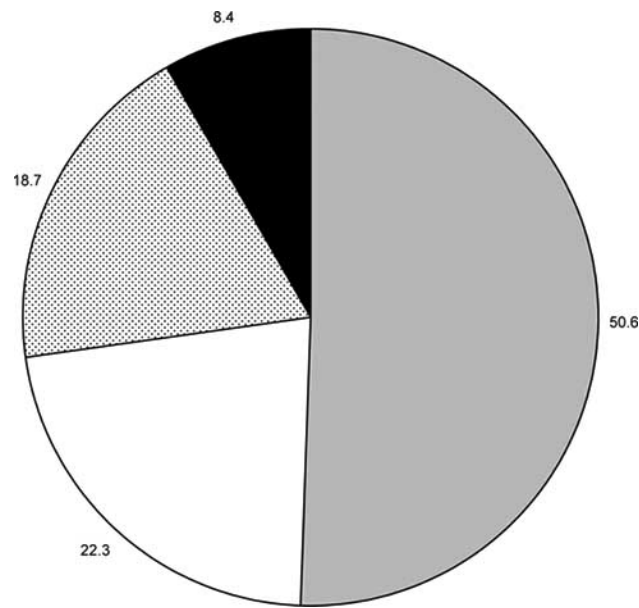
Our previous data obtained in the Lab of Prof. S. Amselem, which provided new insights into the pathophysiology of FMF, demonstrated that susceptibility to renal amyloidosis in FMF patients is influenced by at least two MEFV-independent factors of genetic origin – SAA 1 gene and the sex of patients – that act independently each other [5].

Investigation of the group of 250 healthy individuals helped to estimate, that the overall carrier rate of FMF mutations was 0.21 (1:5) which is extremely high in healthy Armenian population. We compared our data with the frequency of particular MEFV mutations and their distribution in different populations of the Mediterranean region [1, 6, 13, 19]. The rate of carriers of FMF mutations in Armenians was as high as in North African and Iraqi Jews, Turks, but lower than in Ashkenazi Jews (1:4.5), Moroccan Jews (1:3.5) and Muslim Arabs (1:4.3), [7].

Knowledge of the geographical patterns of distribution of mutations implicated in FMF is crucial to an understanding of how this disease became established and what kind of selection pressures may have acted on these mutations to account for present day distributions. The distribution of the most common mutations of MEFV gene among healthy individuals, including M694V (4.7%); V726A (4.6%); M680I (1.8%); R761H (0.2%) in exon 10; F479L (0.4%) in exon 5; P369S (4.9%) in exon 3; E148Q (3.4%) in exon 2 was revealed. The mutational rate in the group of healthy persons is significantly different from the data in the group of FMF patients, where the most common mutations were M694V (50.6%), followed by V726A (22.3%), M680I (18.7%), R761H (3.2%), M694I (0.4%); E148Q (2.2%), F479L (1.3%). 98.65% of Armenian FMF patients had the seven most common MEFV mutations. Approximately in 77% of these patients both alleles are mutated in exon 10. The distribution of MEFV gene mutations in Armenian patients is presented in Fig. (1).

The results of this study allowed to identify specific MEFV mutations associated with severe or mild phenotypes of the disease. The diagnostic

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**Fig. (1).** Distribution of MEFV mutations in 3000 Armenian FMF patients.

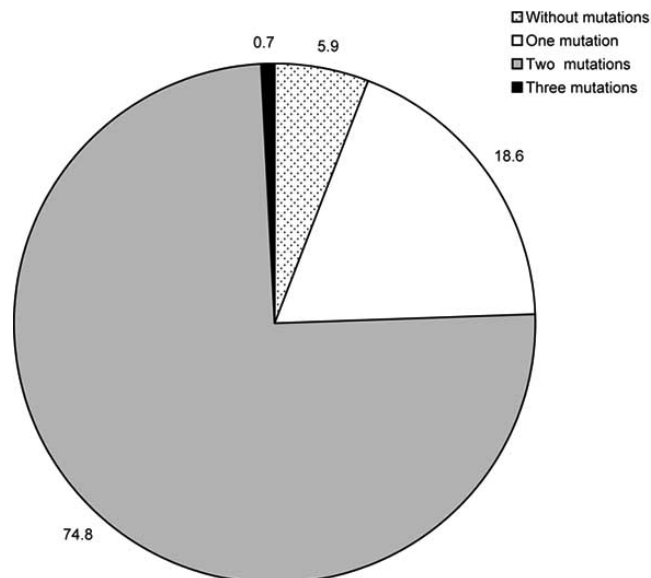
value of MEFV gene analysis was revealed on the bases of investigations of associations between mutations' spectrum and clinical features. From this data it becomes obvious that the most part of FMF patients carry two mutations of MEFV (74.8%) compared with patients carrying only one mutation (18.6%) and patients with three mutations (0.7%). And only a small number of patients without any MEFV mutation (5.9%) were detected. These results are presented in Fig. (2).

The most frequent genotypes presented in Fig. (3) were M694V/M694V (20.9%), M694V/V726A (18%), M694V/M680I (12.7%), M680I/V726A (9.8%), M680I/M680I (3.4%), V726A/V726A (2.8%), and M694V/R761H (2.8%).

Some rare common genotypes were found in only one patient (homozygous for F479L/F479L; V726A/R42W). Estimation of the differences between frequency of various mutations among FMF patients and healthy controls in Armenian population suggests that penetrance depends on the type of mutations involved in the pathogenesis of FMF. These suggestions were confirmed after three mutant alleles had been found in 10 FMF patients in comparison with a group of 11 healthy individuals. However, Armenians have a relative milder mutation E148Q, characterized by reduced penetrance, and associated with a mild phenotype, as well P369S, which was found in asymptomatic carriers.

Based on these data we could suggest that the genotypes with P369S mutation are not associated with clinical features of FMF (Table 1).

Because MEFV genotyping can be done in presymptomatic phase of the disease, it's a valuable means to reveal affected individuals, especially with M694V/M694V homozygous genotype, which is strongly associated with renal amyloidosis [4, 5]. In collaboration with Prof. S.Amselem and Dr. C. Cazeneuve we investigated a relatively homogeneous population of Armenian FMF patients with or without renal amyloidosis (RA). We have carried out the molecular analysis of the SAA1 and SAA2 genes coding serum amyloid proteins. We also showed that the frequency of the M694V homozygous genotype in the group of patients with RA (51.1%) was significantly higher than it was observed in the group of patients without RA (18.9%,  $p=0.0001$ ) [3]. The risk of male patients to develop renal amyloidosis was four times higher than that of female patients. The results demonstrated that SAA  $\alpha/\alpha$  homozygous genotype was associated with a seven-fold increased risk for renal amyloidosis, compared with other SAA1 genotypes (OR=6.9, 95% CI=2.5-19.0). The presence of only one SAA1- $\alpha$  allele did not suggest an increased susceptibility to RA. There were no significant differences in the frequency of other common genotypes between two groups of patients with and without RA. Our data are correlated to Booth's data [2] (1998) where was shown that the risk



**Fig. (2).** Distribution of Armenian FMF patients according to number of MEFV mutations.



disease phenotype is explained by the heredity and mutational spectrum proved to be autosomal recessive in 91.5% and pseudo-dominant in 8.49% of all families.

## CONCLUSIONS

Our study demonstrates the evidence, that MEFV gene analysis provides the first objective diagnostic criterion for FMF. It was also revealed that M694V homozygous genotype has an unfavorable prognostic value for development of renal amyloidosis. The analysis of genotype-phenotype correlations also provides the molecular evidence for some cases characterised by incomplete penetrance and pseudo-dominant type of inheritance. The differences between frequency of mutations in FMF patients and healthy controls suggest that penetrance depends on the type of mutations. The various spectrums of MEFV mutations correlate with severe to mild phenotypes of disease. 74.8% of patients have two mutated alleles, mostly of exon 10.

Our results suggest that the frequency of the mutations of MEFV gene is extremely high in Armenian population, and the genotyping is recommended for all siblings of FMF patients. In addition, our investigations lead to the identification of the first MEFV-independent modifying genetic factors for this disease. The results of this study allowed to identify specific MEFV mutations associated with severe or mild phenotypes of the disease. Overall, these data, which confirm the involvement of MEFV gene in FMF, is essential in clinical practice for the faster diagnosis and better management of patients with FMF.

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