Familial Mediterranean Fever: Association between Genotype and Severity of the Clinical Manifestations of FMF, and Possible Differences in the Course of FMF among Patients with Early and Late Onset of the Disease
A Cross Sectional Study

Master of Public Health Integrating Experience Project
Professional Publication Framework
by

Stepan Atoyan, MD, MPH candidate

Advising team:

Haroutune Armenian, MD, DrPH
Anahit Demirchyan, MD, MPH
Kristina Akopyan, MD, MPH

School *of* Public Health
American University *of* Armenia
Yerevan, Armenia
2015

Table of Contents

List of abbreviations

FMF	Familial Mediterranean fever
MEFV	Mediterranean fever
CPS	Cryopyrin-associated periodic syndromes
IL	Interleukin
CMG	Center of Medical Genetics
CI	Confidence interval
OR	Odds ratio
SD	Standard deviation

Acknowledgments

I would like to express my deep gratitude to my advisors Dr. Haroutune K. Armenian, Dr. Anahit Demirchyan and Dr. Kristina Akopyan for their guidance, help and continuous support.

I am very grateful to professors Varduhi Petrosyan and Byron Crape for their assistance and encouragement throughout the period of this research.

I am very thankful to the MPH program faculty of the American University of Armenia for the support and assistance.

I would like to acknowledge the director of the Center of Medical Genetics (CMG), Prof. Tamara F. Sarkisian for providing access to their database and valuable information, as well as for her continuous interest and support.

I am very grateful to my family and my friends for understanding, encouragement and support.

Abstract

Introduction: Familial Mediterranean fever (FMF) is a genetic disorder with autosom recessive type of inheritance. The clinical picture of FMF is characterized by periodic, self-limited fever and serositis of different localizations. The severity and clinical features of the disease are mainly dependent on the genotypes but can be vary depending on the various environmental and personal characteristics.
Objective: This study aimed to explore the possible differences in the clinical features of FMF between the early and late-onset of the disease (before and after 20 years). Also, the study aim was to identify the genotypes associated with the most severe course of FMF in Armenian population.
Methods: The source of study population included patients with genetically confirmed diagnosis of FMF since 1996 to 2014. The data for the study was extracted from the database of the Center of Medical Genetics (CMG). The study population was randomly selected from the database of CMG after excluding all ineligible people from the database. For the proposed study two instruments were used, including: Medical Data Abstraction form and Severity Assessment score developed by Pras et al. For the analysis of the data the Descriptive statistics were used to characterize study participants, the binary logistic regression analysis was conducted for identifying the associations between dependent variable and independent factors for the first research question, after which the multiple logistic regression analysis was conducted for adjustment of confounders Accordingly the independent samples t-test analysis and the multiple linear regression analysis were conducted for identifying the associations between dependent variable and independent factors for the second research question .
Results: Patients with M694V/M694V and M694V/M680I genotypes had respectively 2.1 (OR=2.078; p=0.030) and 2.7 (OR=2.612; p=0.002) times higher chance to develop the disease before 20 years of age, whereas heterozygous patients had 1.5 times (OR=0.640; p=0.013) higher chance to develop FMF after 20 years of age. Patients with the early onset of FMF (before 20 years of age) had 1.4 times (OR=1.360; p=0.004) higher chance to develop arthritis. Also patients with the family history of FMF had 1.8 times higher chance to develop FMF in their early ages (OR=1.797; p=0.001). The M694V/M694V was identified to be the most severe genotype of FMF (B=1.644; p<0.001).
Conclusions: The study showed that arthritis and myalgia were less frequent among patients with the late-onset FMF. Adult-onset FMF is strongly associated with heterozygous genotypes. Carriers of M694V/M694V and M694V/M680I genotypes have statistically significantly higher chance to develop early-onset FMF. Accordingly, the late onset of FMF is associated with milder disease, and the M694V homozygous genotype is shown to be the most severe genotype of FMF. As results of our study we made the following recommendations: health care providers should be informed that heterozygous carriers of the MEFV gene mutations could develop the symptoms of FMF later in their life (after 20 years). During the genetic counseling the parents carrying of the M694V mutation (both heterozygous and homozygous) should be informed about the possible risk of having offspring with the most severe genotype, which is a homozygous M694V. Better surveillance system is needed in FMF throughout Armenia including tracking the clinical features and treatment of the disease.

Introduction

Disease description

Familial Mediterranean fever (FMF) is considered to be one of the Mendelian disorders included in the group of diseases known as hereditary periodic fever syndromes characterized by periodic, self-limited fever and serositis of different localizations[1,2]. Based on the typical characteristics of the disease, some other names are also accepted for this disorder, among which the most common are "benign paroxysmal peritonitis" or "periodic fever"[3]. Although the disease is mostly typical for populations such as Armenians, non-Ashkenazi Jews, Arabs, and Turks, nowadays FMF cases are reported from other countries and even continents, which is mainly associated with population migration processes[4].

Etiology/MEFV gene

Various theories were hypothesized regarding the etiology of the FMF including: infectious, allergic, and endocrinological factors. None of the aforementioned factors were sufficient to explain the etiology of the FMF[5]. Further investigations showed that FMF is a genetic disorder which is inherited recessively and the gene responsible for this disorder is located on the chromosome 16p, and identified as MEFV gene. MEFV encodes the synthesis of protein pyrin/marenostrin, which is considered to be a member of a family of nuclear factors regulating the acute inflammatory responses[2].

In spite of FMF is a recessive disorder and the majority of people with clinical manifestations of the disease have two mutated alleles in MEFV gene, there are also registered cases of symptomatic patients with only one mutated allele[6].

FMF prevalence

The distribution of MEFV gene mutations vary from country to country. In Middle East the common mutations are considered to be M694V, V726A, and E148Q, which are supposed to be the most ancient ones[4]. Among Syrians the most frequent mutation is found to be E148Q, and the most severe cases are caused by M694V mutation[7]. In Arabian population from the prevalent mutations the most severe cases are predisposed by the M694V/M694V and the M694V/V726A genotypes, whereas the M694I/M694I is associated with mild disease[8]. Different MEFV mutations are presented among all ethnic groups of Jews population. E148Q is more common for Ashkenazi and Iraqi Jews, and M694V is found with high prevalence among North African Jews[9,10]. According to Armenian et al., in Lebanon FMF is mostly common among Armenian and Arab (mainly Shiites from South of Lebanon) population, incidentally the rate of amyloidosis is higher among Arabs[11]. Far from the Mediterranean region other mutations are found to be more frequent, for example in Japanese population the frequent mutations of MEFV gene are E148Q, M694I, L110P, P369S, and R408Q, and among the mentioned mutations the M694I predisposes to the most severe manifestation of the disease but at the same time patients carrying M694I genotype show better response to the colchicine therapy[12].

The prevalence of FMF among the Armenian population was identified as 1 in 200[13]. According to the more recent study, the rate of FMF in Armenia is estimated to be lower - approximately 1:500, which put Armenians in the third place after the Turks and the Jews[2]. Another study conducted in Armenia brings also the rates of heterozygous carriers of the MEFV gene, which is 1 carrier per 5 persons[14]. Seven most common mutations of the MEFV gene are identified in Armenian population with FMF including: M694V (50.6%), V726A (22.3%), M680I (18.7%), R761H (3.2%), E148Q (2.2%), F479L (1.3%), and M694I (0.4%), which together cause about 98.65% of all FMF cases in Armenia. The most common

genotypes of FMF among Armenian population are also identified and include: 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%)[15]. The majority of FMF patients in Armenia are carriers of two mutated alleles, which is about 80 % of individuals with the condition[6].

Clinical manifestations

The typical attacks of FMF are characterized by recurrent episodes of fever and serositis of different localizations (isolated or joint peritonitis, pleuritis, and synovitis), rarely the erysipelas-like rash can be observed. The attacks recur periodically from once a week to once a year with periods of remission between the attacks[16]. The most severe complication of FMF is the renal amyloidosis, and among all known mutation of the MEFV gene the M694V mutation in the homozygous form highly predisposes for the development of the renal amyloidosis[17–19]. Being the most severe mutation of FMF, homozygous M694V might predispose not only to the development of the renal amyloidosis, but also to the severity of FMF attacks [20,21]. The frequency and the exacerbation of FMF attacks can be determined not only by the genotype, but also by the environmental factors and trigger factors, including emotional, physiological or physical stress[22–25].

The age of onset of FMF can vary largely since birth until a late onset after 48 years[11]. According to Armenian et al., the genetic etiology is mainly predominant with earlier onset of the disease for familial cases of FMF, and for non-familial cases, except of the genetic factor, other factors have more impact on the disease manifestations and consequently on the onset of the disease[26]. According to the study conducted among Turkish population, in about 60% of cases the disease onset is registered before 10 years of age, for 90% before 20 years[27]. Another study shows that in about 14% of cases the disease onset occurs between the ages of 20-29, in 8.5% between 30-39, and in very little portion of cases the disease starts after 40

years[28]. According to the study conducted among Sephardic Jews population (mostly from the North Africa), FMF first clinical manifestation could be only the fever without other common symptoms among patients with the disease onset before two years of age[29]. In another study, the authors have compared some main clinical symptoms of FMF between patients with the onset of the disease before and after 18 years and showed that there are differences in the manifestations of some main clinical symptoms of FMF depending on the age of onset, particularly they pointed out that arthritis and erysipelas-like erythema are more frequent among patients with the early onset of the disease[28,30].

In the studies conducted among the Armenian population there are several presentations on the onset of the disease. According to Moradian et al., the age of onset of FMF among Armenian patients varied from 5 years to 18 years[6]. In another study conducted among Armenian patients the range of disease onset varied from 1 month to 42 years with the mean of 12.6 years[31].

As mentioned above, FMF clinical manifestations can vary largely depending on the genotype, trigger factors, environmental factors and other genetic modifier factors. The severity of FMF manifestations have been frequently assessed by two of the existing scoring instruments[32]. Both mentioned tools for FMF severity assessment consist of several criteria, including sites frequency, duration, main symptoms, complications, colchicine dosage, and age of onset[32,33]. A new tool for the FMF severity assessment, which includes nine features of the disease, is currently under the validation proccess[34].

Diagnosis and Treatment

The diagnosis of FMF is mainly based on symptoms, family history and, after the investigation of the MEFV gene, also on genetic testing[35,36]. The most common criteria used in the clinical diagnosis of FMF is considered to be the Tel-Hashomer clinic complex criteria, which includes some major and minor aspects of FMF clinical manifestation that guarantee more than 95% of the sensitivity and the specificity of the diagnosis[37].

The colchicine has been the main medication of FMF therapy during the last decades. With appropriate dosage, in patients with the normal liver and kidney functions, colchicine is considered to be relatively safe medication for FMF treatment [38]. Although colchicine successfully controls the attacks and prevents amyloidosis, resistant cases to colchicine treatment have been also registered. Hence, new treatment approaches are being sought, including the development of anti-IL-1 medications, such as anakinra[39,40]. Anti-IL-1 medications are thought to be potentially effective, since the recent studies showed that some autoinflammatory syndromes like cryopyrin-associated periodic syndromes (CPS) and FMF are characterized by the hyperactivation of innate immune system, resulting in overproduction of IL-1β, which is responsible for clinical manifestations of the aforementioned disorders[41].

Study Rationale

Although significant research has been done in the FMF investigation, there are still gaps on the disease pathogenesis and manifestations. In the above mentioned studies regarding the onset of the disease, authors proposed to explore the possible associations between the demographic characteristics of the patients with FMF and clinical manifestations of the disease, as they described some differences in clinical manifestations of FMF among patients with the disease onset after 20 years, hypothesizing that adult-onset of the disease is characterized with a milder disease, presenting mainly with abdominal pain, lacking of other typical symptoms, and better responding to the lower doses of colchicin[28]. In another study authors found some differences in the clinical manifestations of FMF developed after 18 years characterized by lack of arthritis and erysipelas-like erythema among patients with adult-onset of the disease[30]. So, the evidence suggests some differences in the FMF clinical features depending on the age of the first manifestation of the disease. There are many studies conducted among the Armenian population regarding different aspects of FMF, but the information provided particularly on the disease onset is just descriptive data without statistical analysis for the associations between ages of onset and clinical manifestations of FMF, or associations between the onset of the disease and genotypes. This study aimed to investigate the possible differences in the clinical features of FMF depending on the age of onset as well the associations between the FMF genotypes and the severity of the disease, to improve the understanding of the FMF clinical manifestations and to contribute to the knowledge on FMF in the Armenian population.

Study Aims and Objectives

This study aimed to explore whether the late-onset (after 20 years) FMF has distinctive clinical and genotype features among the Armenian population with FMF. Also, the study identified the genotypes associated with the most severe course of FMF in the Armenian population.

Study Objectives:

- Identify the genotypes and the clinical features associated with the late-onset FMF (after 20 years)
- Identify the genotypes associated with the most severe course of FMF.

Based on these objectives, the research questions of the study were:

- What are the genotypes and the clinical features associated with the late-onset (after 20 years) FMF among Armenian patients diagnosed with FMF?
- What genotypes predispose to the most severe course of FMF among Armenian population?

Methods

Study Design and Setting

To address the study objectives, a cross-sectional study design was applied. The secondary data on genetically diagnosed patients with FMF was extracted from the database of the Center of Medical Genetics (CMG), where the information about all patients in Armenia with the genetically verified diagnosis of FMF is collected.

Study Population

The target population included patients with genetically confirmed mutations of MEFV gene and with clinical manifestation of FMF; and those who have only one mutation but still developed clinical signs of the FMF. The diagnosis of FMF for patients with only one mutation of MEFV gene was made using the Tel-Hashomer clinical criteria. For diagnosis of FMF there is need of combination of at least 1of 4 major criteria and 2 of 5 minor criteria or 1 minor criterion and 5 of 10 supportive criteria[37].

We intended to collect information on two groups of patients with the onset of the disease before and after 20 years. Exclusion criteria were patients with lost or insufficient medical records, patients with ethnicity other than Armenian, and patients with no information about the age of onset of the disease.

Sample Size

The sample size has been calculated with the formula for two-sample comparison of proportions [42] for cross-sectional studies by Epi-info software. Considering the outcome, which is the differences in the clinical manifestation of FMF among patients with the onset of the disease after 20 years, and the findings of literature indicating that the occurrence of arthritis is one of the main clinical differences between the early- and late- onset FMF, we based our calculation on the prevalence of arthritis among the late-onset FMF cases, which was 42%[28], and intended to detect at least 10% difference in this prevalence between the groups with early (unexposed) and late (exposed) onset of FMF with 95% confidence level and 80% study power. Thus, we used p_1=0.42 for the exposed group and p_2=0.52 for the unexposed group, which resulted in a sample size of 381 for one group (see the calculation below) and 381*2=762 for the whole sample.

$$n = \frac{\left[z\alpha_{/2}\sqrt{2\bar{p}\bar{q}} + z_{\beta}\sqrt{p_1q_1 + p_2q_2}\right]^2}{|p1 - p2|^2}$$

$$n = \frac{\left[1.96\sqrt{2 * 0.47 * 0.53} + 0.84\sqrt{0.42 * 0.58 + 0.52 * 0.42}\right]^2}{|0.42 - 0.52|^2} = 381$$

N=2n=2*381=762

The calculated sample size was further increased to 800 patients to compensate for the loss because of possible missing data. Later the sample size has been increased to 1200, as the initial intend to compare two groups (those with the disease onset before 20 years of age to those with the onset at 20 years of age or later) was changed to three groups with the disease onset at 0-9.9 years, 10-19.9 years, and 20 and more years. To avoid using a predetermined cutoff level for the late-onset FMF, it was decided to select stratified random sample of 400 patients from the mentioned three groups.

Sampling Technique

As a first step, ineligible patients were excluded from the database of the CMG. Then the remaining database was divided into three parts: those with the disease onset before 10 years, from 10 to 19.9 years, and those with the disease onset at 20 years or later. In order to achieve the calculated sample size for each group, simple random sampling was conducted with the use of SPSS 17.0 software package for random case selection.

Data Collection and Study Instrument

For the extraction of the necessary data, Medical Records Abstraction form was developed (Appendix 1). The FMF severity assessment tool suggested by Pras et al.[33] was used to calculate the disease severity score based on the information available from the CMG

database (Appendix 2). However, as the variable on the colchicine usage was mainly missing in the CMG database, the severity score was calculated with exclusion of this criterion.

Data sources

The data for the study was extracted from the database of CMG. The following details about genetically confirmed FMF patients were abstracted: patients' IDs, region of origin, sex, age, family history of FMF, genetic mutation type, trigger factors, age of onset of the disease, frequency and duration of the attacks, the main symptoms and syndromes, concomitant diseases, and the trigger factors. The details on the colchicine usage were presented for some of the patients, and for the rest of them the relevant information was absent (missing data is about 90%). The doctors-geneticists in CMG usually collect the data from the patients or, in case when the patient is a child, from his/her parents. There is a follow-up database for those patients who intend to continue their treatment in the CMG. The doctors-geneticists update the follow-up database regarding the new manifestations of the FMF or other changes in the course of the disease. Sampling was done from the initial database.

Study variables

Variables for the first research question (disease onset time) of the study

Dependent variable – onset of the disease (late or early using a threshold of 20 years of age)

Independent variables – specific genotypes of FMF, some clinical features of the disease, including frequency and duration of the attacks, fever, presence or absence of peritonitis, pleuritis, arthritis, skin rash, or a combined score of disease severity (calculated without age of onset and colchicine dose)

Control variables – sex, age at the genetic diagnosis, presence or absence of hepatomegaly, splenomegaly, concomitant diseases, and triggers

Variables for the second research question (disease severity score) of the study

Dependent variable - severity of the disease (based on the calculated severity score)

Independent variables – specific genotypes of FMF

Control variables–sex, age, presence or absence of peritonitis, pleuritis, hepatomegaly, splenomegaly, concomitant diseases, and triggers.

The detailed description of the variables is provided in Appendices 3 and 4.

Analysis plan

Data was entered into SPSS 17.0 database (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Statistical analyses was performed using SPSS 17.0 statistical software. In the original database, a lot of numeric data was kept as string variables. During the data cleaning process, all these string variables were changed into numeric ones manually in order to be able to statistically analyze the data in the SPSS statistical software. Also, the most common genotypes were separated and presented as dichotomous variables with "present" (recoded as 1) and "absent" (recoded as 0) values. Descriptive statistics were used to characterize study participants. Continuous variables were described using means and standard deviations, and categorical variables were described using frequencies and percentages.

Bivariate logistic regression analysis was conducted for identifying the crude associations between the dependent variable and the remaining study variables. After the bivariate logistic regression analysis, we conducted multiple logistic regression analysis and the variables with $p<0.25$ level of association with the outcome in the bivariate analysis were included in the multivariate analysis. For the multiple logistic regressions analysis the variables were used in conceptually coherent blocks (genotypes, personal characteristics, clinical symptoms, etc). We repeated the multiple logistic regression analysis several times with different sets of variables until the variables with only statistically significant p-values

($p<0.05$) remained in the model. During the analysis, the value of Pseudo-$R^2$for the tested models was approximately the same. The multiple logistic regression analysis resulted in two final models. First model included all variables for the first outcome described in the methods section except the severity score, and the second final model included also the severity score without the variables which had been used for the calculation of the severity score. Accordingly, for the first outcome, the age of onset was excluded from the calculation of the severity score since the variable of interest (dependent variable) was just the age of onset. Further, for addressing the second research question of our study - identifying the most severe genotype in the Armenian population, the independent samples t-test analysis and then multiple linear regression analysis were conducted. The variables with $p<0.25$ were selected from the results of the independent samples t-test analysis and included in the multiple linear regression analysis. The severity score was calculated by Pras scale, and labeled as mild (<5), moderate (6-9), and severe (10<). All the results with the p value less than 0.05 were considered as statistically significant.

Ethical considerations

The Institutional Review Board of the American University of Armenia reviewed and approved the study protocol. The Center of Medical Genetics gave a permission to access the database and provided de-identified database to the study team.

Results

Descriptive Statistics

After exclusion of all ineligible patients from the CMG database (those having no FMF gene mutation, no disease onset time specified and patients with one mutation of MEFV gene without clinical picture of FMF, and patients with no information about age of onset), stratified random sampling was applied to select a total of 1200 patients, so that the numbers of patients in the groups of the disease onset before 10 years of age, from 10 to 19.9 years of age, and 20 years of age or later are approximately equal. After the selection, the final sample included 387 patients in the first group (onset before 10 years of age), 368 in the second (onset at 10 to 19.9 years of age), and 445 patients in the third group (onset at 20 years of age or later). The reason for the differences between these numbers was that many variables (including the disease onset time) in the CMG database were presented as string variables with inconsistent measurement units (months/years/ranges/etc.), and after assigning numeric values to the disease onset time variable the number of patients in each group were slightly altered. Accordingly, during the analysis there were identified three cases without genotypes, which were excluded from the sample, and the final sample size was consisted form 1195 cases.

After examining the distribution of the disease onset time in the study sample and its relation to the main clinical features of the disease, three threshold levels for the late onset were initially identified: at 20 years or later, at 25 years or later, and at 30 years or later. Three dichotomous dependent variables were constructed based on these cutoff levels, and the relation of each with the independent variables was examined. As a result, the first cutoff level (onset at 20 years or later) was chosen, since observing the original database we found out that the distribution of the patients with the onset of the disease before and after 20 years was approximately 80% to 20%, and for the remaining cut-off levels the proportions of the

late-onset cases were lesser (12.5% and 7% respectively), which made us to conclude that the initially proposed cut-off level of 20 and more would be more sensitive and meaningful. Accordingly, during the analysis we observed that this outcome identified the highest number of significant risk factors. The main descriptive characteristics of the study sample are presented in Tables 1 and 2.

In order to eliminate the influence of stratified sampling on the calculated prevalence estimates, all these estimates were either weighted using the age of onset distribution in the original sample or calculated using the original database. Based on the original dataset, the mean age of the population at the genetic confirmation of the disease is 27.2 years (SD 15.5), the gender distribution of FMF patients is 47.1%/52.9% (female/male). The mean age of the disease onset is 12.0 years (SD 11.3). Over one-third (34.6%) of the whole population have family history of FMF. About one-third (31.2%) of the cases with FMF gene mutations are heterozygous carriers, half of them (53.7%) are carriers of compound heterozygous mutations, and 15.1% carry homozygous mutations. Table 3 represents information on the distribution of FMF characteristics among the two groups with the age of onset of FMF before 20 years and at 20 years or after. Interestingly, there is a statistically significant difference in the distribution of patients with heterozygous genotypes in the two groups with a slightly higher prevalence in the early onset group (57.9% vs. 42.1%, p=0.022), whereas the distribution of homozygous genotypes in the two groups is about 3:1 (71.3% and 28.3% in early and late onset groups, respectively, p=0.007). There are some significant differences in the distributions of several genotypes among the two age-of-onset groups. M694V/M694V genotype is more than 5 times frequent (12.8% vs. 2.8%) in the early onset group, and M694V/M680I genotype is about 2 times frequent (10.8% vs. 5.8%) in the early onset group. In contrast, M694V/- heterozygous genotype is more frequent (16.7% vs. 12.5%) in the late onset group. There is a slight difference between the groups in gender distribution with

higher prevalence of females (52.9%) in the early onset group and, consequently, higher prevalence of males (54.3%) in the late onset group.

The comparison of the mean values of fever between the two groups identified marginally significant difference – the mean temperature was 37.9 (SD 0.93) in the early-onset group and 37.8 (SD 0.85) in the late-onset group. There were no statistically significant differences for the frequency of attacks of FMF among the two disease onset groups (Table 3).

Results for the first research question: Factors associated with the late-onset FMF

Bivariate logistic regression analysis

The results of bivariate logistic regression analysis for the associations between the age of onset (before or after 20 years) and independent variables are presented in Table 4. For each independent variable, the relation to the age of onset is presented with crude odds ratios, CIs, and p-values. Statistically significant differences were observed between several variables and the late-onset of FMF. Patients with two mutations, particularly homozygous patients (OR=1.636; p=0.006) with M694V/M694V genotype (OR=3.668; p<0.001) and patients with M694V/M680I compound heterozygous genotype (OR=1.937; p=0.005) tend to develop the disease earlier, whereas patients who carry heterozygous mutations (OR=0.754; p=0.028), tend to develop the disease later. The proportion of patients with family history of FMF is higher in the early–onset group (OR= 1.534; p=0.001). The late-onset of FMF is more infrequent among males (OR=0.756; p=0.020).

Multiple logistic regression analysis

Multiple logistic regression analysis was conducted to identify independent factors associated with the late onset of FMF (the late onset was coded as 0 while the early onset as 1). Two sets of variables were used for this analysis. The first set included separate clinical features of the disease, while the second set included a severity score combining all these clinical features into one score. For this study purpose, we calculated the severity score without the variable on the age of onset of FMF, since the dependent variable of this regression analysis was just the age of the disease manifestation. Also the variable on colchicine was excluded from the calculation of the severity score, since this information was not available from the database of CMG. Two final models were produced. Both models (Table 5 and 6) included heterozygous genotypes, one specific M694V/M680I compound heterozygous genotype, family history of FMF, gender, and age at the genetic confirmation of the diagnosis of FMF. The first model (Table 5) included also one more specific genotype: M694V/M694V, and two clinical characteristics: temperature and arthritis. The second model (Table 5) included the severity score in addition to the four common predictors described above. According to the first final model, patients with M694V/M694V and M694V/M680I genotypes have respectively 2.1 and 2.6 (OR=2.078; p=0.030, OR=2.612; p=0.002) times higher chance to develop the disease before 20 years of age, whereas heterozygous patients have 1.4 times (OR=0.640; p=0.013) higher chance to develop FMF after 20 years of age. Patients with the early onset of FMF (before 20 years of age) have 1.4 times (OR=1.360; p=0.004) higher chance to develop arthritis, and 1.6 times higher chance to have myalgia (OR=1.557; p=0.015). Also, patients with the family history of FMF have 1.8 times higher chance to develop FMF in their early ages (OR=1.797; p<0.001)

The second final model (Table 6), except of the above described associations, shows a relationship between the age of FMF onset and the severity of the clinical course and,

according to our findings, patients with the early-onset of FMF have 1.4 times higher chance to develop more severe disease (OR=1.419; p=0.034).

Results for the second research question: genotypes associated with the most severe course of FMF

For addressing the second research question, first of all, we conducted a cross-tabulation between the most common genotypes and the severity scale of FMF (Table 7). According to the results, patients with the homozygous M694V genotype most frequently developed the severe disease (33%, p<0.001), the second place by the severity was found to be M694V/M680I genotype (26%, p<0.001). Interestingly, there were no severe cases among patients with homozygous V726A genotype (0%, p=0.029).

Independent t-test

Further the independent samples t-test analysis was conducted to identify the relations between the severity of the FMF clinical course and the genotypes (Table 8). According to the findings, the most severe genotype among the selected most common genotype groups was M694V/M694V (mean=8.24, p<0.001), the second most severe genotype was found to be M694V/M680I (mean=7.82; p<0.001), and the third one was V726A/M680I (mean=5.83; p<0.001). Interestingly, one of the heterozygous genotypes, M694V/- genotype was also statistically significantly associated with moderate severity score (mean=6.15; p=0.019).

Multiple Linear Regression Analysis

After the t-test analysis, the multiple linear regression analysis was conducted in order to explore the associations between the severity score and the genotypes (Table 9). The M694V/M694V was identified to be the most severe genotype (B=1.644; p<0.001), the second one was M694V/M680I (B=1.350; p<0.001).

Discussion

Main findings

The study investigated the associations of clinical characteristics of FMF among patients with early and late onset of the disease with a cut-off point at 20 years. The second objective of the study was the description of the associations between the most common genotypes and the severity of the clinical manifestations of FMF.

FMF is considered to be the commonest genetic disorder among Armenian population and many studies have been conducted in this area, some of which hypothesized differences in the clinical course of the disease depending on the age of its first manifestation. But to our knowledge the studies specifically aiming to examine possible differences in FMF genotype/characteristics depending on the age of onset of the disease are rare, and, to our knowledge, are not done among Armenian population.

Similar to other studies, our study revealed some features significantly associated with the late-onset of the disease [27,28,43]. Specifically, this study found that the occurrence of arthritis is significantly less frequent among patients with the late-onset of FMF (68% to 32%; $p<0.001$). Our findings show that having family history predisposes to the development of FMF in early ages as it was shown in the study conducted by Armenian et al[26]. According to our findings, the chance of having first manifestation of the disease before 20 years of age is about two times higher among cases with family history of FMF compared to those with no family history of the disease (OR=0.540; $p<0.001$) . Erysipelas-like erythema is not an often symptom among Armenian population with FMF and this study has not revealed any significant differences in the occurrence of this symptom between patients with early and late-onset of the disease.

Taking into consideration the type of the inheritance of the MEFV gene mutations, until recently there has been strong notion that only homozygous patients could develop the

symptoms typical to FMF, and the presence of symptoms among heterozygous carriers were mainly determined by the low capacities of the genetic tests to identify the second mutation. Anyway, recent studies show that even after the whole sequencing of the MEFV gene is identified, no other mutations were found among heterozygous symptomatic patients, thus concluding that heterozygous patients also could develop the typical symptoms of FMF depending on the type of the inheritance of MEFV gene and the type of inherited mutations[6,44,45]. The results of our study show that people with heterozygous genotypes have 74.6% more chance to develop symptoms of FMF in their adulthood, particularly after 20 years (OR=1.746; 9<0.001), whereas among patients with two mutations, statistically significant association was revealed between the early-onset of the disease and two specific genotypes - M694V/M694V and M694V/M680I. The results show that patients with the aforementioned two genotypes have respectively 55.9% and 58.9% higher chances to develop clinical manifestations of FMF before 20 years of age (OR=0.441; p=0.015, OR=0.411; p=0.004). Also, patients with family history of FMF have 80% higher chance to develop the disease before 20 years (OR=1.797; p<0.000). Arthritis and myalgia are found to be more relevant to the patients with the early onset of the disease (OR=1.360; p=0.004, OR=1.557; p=0.015)

As it was already discussed in this paper previously, the severity of FMF clinical course is associated with genotype, as well as with some environmental factors[22,23,25]. Many studies show that there is an association between the most severe course of the disease and the M694V homozygous genotype[18,46,47].

Based on the results of our study, we can conclude that patients with M694V homozygous genotype have 64.4% higher chance to develop the most severe disease (B=1.644; p<0.001). The second most severe genotype, according to our findings, is

considered to be M694V/M680I, and patients with that genotype have 35.0% more chance to develop the severe disease during their lifetime (B=1.350; p<0.001).

Study Limitations

The study had to rely on the medical records of CMG. The database included information on more than 24,000 tested people and since the data has been gathered during the last two decades (since 1996), there is a high possibility of the observer variability. Also, the information on the symptoms is mainly self-reported, which made possible a recall-bias, and we were not allowed to interview a random sample of accessible patients in order to validate some of the information in the database.

One of the important limitations of the study is the very small information on the treatment, because patients are registered into the database during their first visit, mainly without prior treatment by colchicine, so in the database there is a lack of information on colchicine use and its effectiveness. To avoid possible bias we excluded the variable on colchicine dose form the calculation of the severity score by Pras[33].

Another limitation of the study is that some of the variables of interest (some of the concomitant diseases) had a high rate of missing values. To avoid a bias, the variables with 10% and more missing values were excluded from the study.

Some numerical variables were entered as string ones in the initial database of the CMG, which were not consistent with the statistical software for the analysis. Before the analysis all the inconsistent, inaccurate data were changed and improved by the student-investigator.

Study strengths

All the FMF diagnoses were genetically confirmed and the patients' population was large. Also, to our knowledge, this study was the first one investigating the factors associated with the late-onset FMF among Armenian population.

Conclusions

The study shows that arthritis is less frequent among patients with the late-onset of FMF in comparison with the early-onset patients. Adult-onset of FMF is highly associated with heterozygous genotypes, since as the study results shows, heterozygous carriers of MEFV gene mutations have a higher chance to develop FMF during their adulthood. Among the patients with two mutation of MEFV gene, only carriers of M694V/M694V and M694V/M680I genotypes have statistically significantly higher chance to develop an early-onset FMF. Also, patients with family history of the disease highly tend to develop FMF before 20 years.

Our study revealed also the association between the age of onset of the disease and the severity of the clinical manifestations as well as between the genotypes and the severity. According to the findings, the late-onset of FMF is associated with milder disease, and the most severe genotype is considered to be M694V homozygous genotype similar to the other studies.

Recommendations

Based on the study results we developed the following recommendations:

Health care providers should be informed that heterozygous carriers of the MEFV gene mutations could develop the symptoms of FMF later in their life (particularly after 20 years).

During the genetic counseling the carriers of the M694V mutation (both heterozygous and homozygous) should be informed about the possible risk of having offspring with the homozygous M694V genotype, which predisposes to the most severe course of the disease.

Better surveillance system is needed in FMF throughout Armenia including tracking the clinical features and treatment of the disease.

Reference list

1. Kastner DL. Hereditary periodic fever syndromes. *Hematology Am Soc Hematol Educ Program*. 2005;2005(1):74–81. doi:10.1182/asheducation-2005.1.74.

2. The International FMF Consortium. Ancient Missense Mutations in a New Member of the RoRet Gene Family Are Likely to Cause Familial Mediterranean Fever. *Cell*. 1997;90(4):797–807. doi:10.1016/S0092-8674(00)80539-5.

3. Heller H. Familial Mediterranean Fever. *Arch Intern Med*. 1958;102(1):50. doi:10.1001/archinte.1958.00260190052007.

4. Ben-Chetrit E, Touitou I. Familial mediterranean Fever in the world. *Arthritis Rheum*. 2009;61(10):1447–53. doi:10.1002/art.24458.

5. Reimann HA. Periodic Disease. *J Am Med Assoc*. 1949;141(3):175. doi:10.1001/jama.1949.02910030005002.

6. Moradian MM, Sarkisian T, Ajrapetyan H, Avanesian N. Genotype-phenotype studies in a large cohort of Armenian patients with familial Mediterranean fever suggest clinical disease with heterozygous MEFV mutations. *J Hum Genet*. 2010;55(6):389–93. doi:10.1038/jhg.2010.52.

7. Mattit H, Joma M, Al-Cheikh S, et al. Familial Mediterranean fever in the Syrian population: gene mutation frequencies, carrier rates and phenotype-genotype correlation. *Eur J Med Genet*. 49(6):481–6. doi:10.1016/j.ejmg.2006.03.002.

8. Majeed HA, El-Shanti H, Al-Khateeb MS, Rabaiha ZA. Genotype/phenotype correlations in Arab patients with familial Mediterranean fever. *Semin Arthritis Rheum*. 2002;31(6):371–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12077709. Accessed November 24, 2014.

9. Stoffman N, Magal N, Shohat T, et al. Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. *Eur J Hum Genet 8, 307–310*. 2000;(June 1999):307–310.

10. Brik R, Shinawi M, Kepten I, Berant M, Gershoni-Baruch R. Familial Mediterranean fever: clinical and genetic characterization in a mixed pediatric population of Jewish and Arab patients. *Pediatrics*. 1999;103(5):e70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10224214. Accessed February 9, 2015.

11. Armenian HK, Sha'ar KH. Epidemiologic observations in familial paroxysmal polyserositis. *Epidemiol Rev*. 1986;8(4):106–116.

12. Kishida D, Nakamura A, Yazaki M, Tsuchiya-Suzuki A, Matsuda M, Ikeda S-I. Genotype-phenotype correlation in Japanese patients with familial Mediterranean fever: differences in genotype and clinical features between Japanese and

Mediterranean populations. *Arthritis Res Ther*. 2014;16(5):439. doi:10.1186/s13075-014-0439-7.

13. NIH Press Release - NIH Leads Group that Identifies Gene for Mediterranean Fever - 08/21/1997. Available at: http://www.nih.gov/news/pr/aug97/niams-21.htm. Accessed November 10, 2014.

14. Sarkisian T, Ajrapetian H, Beglarian A, Shahsuvarian G, Egiazarian A. Familial Mediterranean Fever in Armenian population. *Georgian Med News*. 2008;(156):105–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18403822. Accessed August 23, 2014.

15. Sarkisian T, Hayrapetyan H, Beglaryan A, Shahsuvaryan G, Yeghiazaryan A. Molecular diagnosis of Familial Mediterranean fever in Armenians. *NEW Armen Med J Vol 1 (2007), N1, pp33-40*. 2007;1:33–40.

16. Almeida de Jesus A, Goldbach-Mansky R. Monogenic autoinflammatory diseases: concept and clinical manifestations. *Clin Immunol*. 2013;147(3):155–74. doi:10.1016/j.clim.2013.03.016.

17. Ben-Chetrit E, Backenroth R. Amyloidosis induced, end stage renal disease in patients with familial Mediterranean fever is highly associated with point mutations in the MEFV gene. *Ann Rheum Dis*. 2001;60(2):146–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1753463&tool=pmcentrez&rendertype=abstract. Accessed November 5, 2014.

18. Al-Haggar MS, Yahia S, Abdel-Hady D, Al-Saied A, Al-Kenawy R, Abo-El-Kasem R. Phenotype-genotype updates from familial Mediterranean fever database registry of Mansoura University Children' Hospital, Mansoura, Egypt. *Indian J Hum Genet*. 2014;20(1):43–50. doi:10.4103/0971-6866.132755.

19. Shohat M, Magal N, Shohat T, et al. Phenotype-genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. *Eur J Hum Genet*. 1999;7(3):287–92. doi:10.1038/sj.ejhg.5200303.

20. Ong FS, Vakil H, Xue Y, et al. The M694V mutation in Armenian-Americans: a 10-year retrospective study of MEFV mutation testing for familial Mediterranean fever at UCLA. *Clin Genet*. 2013;84(1):55–9. doi:10.1111/cge.12029.

21. Shinar Y, Livneh A, Langevitz P, et al. Genotype-phenotype assessment of common genotypes among patients with familial Mediterranean fever. *J Rheumatol*. 2000;27(7):1703–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10914855. Accessed November 7, 2014.

22. Yenokyan G, Armenian HK. Triggers for attacks in familial Mediterranean fever: application of the case-crossover design. *Am J Epidemiol*. 2012;175(10):1054–61. doi:10.1093/aje/kwr460.

23. Ben-Zvi I, Brandt B, Berkun Y, Lidar M, Livneh A. The relative contribution of environmental and genetic factors to phenotypic variation in familial Mediterranean fever (FMF). *Gene*. 2012;491(2):260–3. doi:10.1016/j.gene.2011.10.005.

24. Ozen S, Aktay N, Lainka E, Duzova A, Bakkaloglu A, Kallinich T. Disease severity in children and adolescents with familial Mediterranean fever: a comparative study to explore environmental effects on a monogenic disease. *Ann Rheum Dis*. 2009;68(2):246–8. doi:10.1136/ard.2008.092031.

25. Karadag O, Tufan A, Yazisiz V, et al. The factors considered as trigger for the attacks in patients with familial Mediterranean fever. *Rheumatol Int*. 2013;33(4):893–7. doi:10.1007/s00296-012-2453-x.

26. Armenian HK, Khoury MJ. Age at onset of genetic diseases: An Application For Sartwell's Model Of The Distribution Of Incubation Periods.*Am J Epidemiol*. 1981;113(5):596–605. Available at: http://aje.oxfordjournals.org/content/113/5/596. Accessed March 10, 2015.

27. Tamir N, Langevitz P, Zemer D, et al. Late-onset familial Mediterranean fever (FMF): a subset with distinct clinical, demographic, and molecular genetic characteristics. *Am J Med Genet*. 1999;87(1):30–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10528243. Accessed December 13, 2014.

28. Sayarlioglu M, Cefle A, Inanc M, et al. Characteristics of patients with adult-onset familial Mediterranean fever in Turkey: analysis of 401 cases. *Int J Clin Pract*. 2005;59(2):202–5. doi:10.1111/j.1742-1241.2004.00294.x.

29. Padeh S, Livneh A, Pras E, et al. Familial Mediterranean Fever in the first two years of life: a unique phenotype of disease in evolution. *J Pediatr*. 2010;156(6):985–9. doi:10.1016/j.jpeds.2009.12.010.

30. Study NM. Familial Mediterranean Fever (FMF) in Turkey. *Medicine (Baltimore)*. 2005;84(1):1–11. doi:10.1097/01.md.0000152370.84628.0c.

31. Schwabe AD, Peters RS. Familial Mediterranean Fever in Armenians. Analysis of 100 cases. *Medicine (Baltimore)*. 1974;53(6):453–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4437392. Accessed February 24, 2015.

32. Mor A, Shinar Y, Zaks N, et al. Evaluation of disease severity in familial Mediterranean fever. *Semin Arthritis Rheum*. 2005;35(1):57–64. doi:10.1016/j.semarthrit.2005.02.002.

33. Giese A, Örnek A, Kilic L, et al. Disease severity in adult patients of Turkish ancestry with familial mediterranean fever living in Germany or Turkey. Does the country of residence affect the course of the disease? *J Clin Rheumatol*. 2013;19(5):246–51. doi:10.1097/RHU.0b013e31829ce005.

34. Demirkaya E, Acikel C, Gul A, et al. PW01-028 – Developing a new severity score for FMF. *Pediatr Rheumatol*. 2013;11(Suppl 1):A81. doi:10.1186/1546-0096-11-S1-A81.

35. Samli H, Dogru O, Bukulmez A, Yuksel E, Ovali F, Solak M. Relationship of Tel Hashomer criteria and Mediterranean fever gene mutations in a cohort of Turkish familial Mediterranean fever patients. *Saudi Med J*. 2006;27(12):1822–1826. doi:10.15537/3914.

36. Grateau G. Clinical versus genetic diagnosis of familial Mediterranean fever. *QJM*. 2000;93(4):223–229. doi:10.1093/qjmed/93.4.223.

37. Berkun Y, Eisenstein EM. Diagnostic criteria of familial Mediterranean fever. *Autoimmun Rev*. 2014;13(4-5):388–390. Available at: http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.elsevier-2042906c-fe1e-3996-bba3-6bfd96f2e7ad. Accessed September 15, 2014.

38. Ben-Chetrit E, Levy M. Colchicine: 1998 update. *Semin Arthritis Rheum*. 1998;28(1):48–59. doi:10.1016/S0049-0172(98)80028-0.

39. Vitale A, Rigante D, Lucherini OM, et al. Biological treatments: new weapons in the management of monogenic autoinflammatory disorders. *Mediators Inflamm*. 2013;2013:939847. doi:10.1155/2013/939847.

40. Estublier C, Stankovic Stojanovic K, Bergerot J-F, Broussolle C, Sève P. Myositis in a patient with familial Mediterranean fever and spondyloarthritis successfully treated with anakinra. *Joint Bone Spine*. 2013;80(6):645–9. doi:10.1016/j.jbspin.2013.03.004.

41. Ozkurede VU, Franchi L. Immunology in clinic review series; focus on autoinflammatory diseases: role of inflammasomes in autoinflammatory syndromes. *Clin Exp Immunol*. 2012;167(3):382–90. doi:10.1111/j.1365-2249.2011.04535.x.

42. Aday LA. Designing and conducting health surveys1996. Available at: http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0787975605.html. Accessed January 12, 2015.

43. Nobakht H, Zamani F, Ajdarkosh H, Mohamadzadeh Z, Fereshtehnejad S, Nassaji M. Adult-onset familial mediterranean Fever in northwestern iran; clinical feature and treatment outcome. *Middle East J Dig Dis*. 2011;3(1):50–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4154930&tool=pmcentrez&rendertype=abstract. Accessed March 10, 2015.

44. Marek-Yagel D, Berkun Y, Padeh S, et al. Clinical disease among patients heterozygous for familial Mediterranean fever. *Arthritis Rheum*. 2009;60(6):1862–6. doi:10.1002/art.24570.

45. Livneh A, Aksentijevich I, Langevitz P, et al. A single mutated MEFV allele in Israeli patients suffering from familial Mediterranean fever and Behçet's disease (FMF-BD). *Eur J Hum Genet*. 2001;9(3):191–6. doi:10.1038/sj.ejhg.5200608.

46. Jeske M, Lohse P, Kallinich T, et al. Genotype-phenotype and genotype-origin correlations in children with mediterranean fever in Germany - an AID-net study. *Klin Pädiatrie*. 2013;225(6):325–30. doi:10.1055/s-0033-1355372.

47. Kone Paut I. Phenotype-genotype correlation in 91 patients with familial Mediterranean fever reveals a high frequency of cutaneomucous features. *Rheumatology*. 2000;39(11):1275–1279. doi:10.1093/rheumatology/39.11.1275.

Table 1.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. Descriptive Characteristics of the Sample with the Weighted and Original Estimates for the Total Population*

Variable	Sample values			Values for the original dataset		
	Mean	SD	Min - Max	Mean	SD	Min - Max
Age	31.3	15.6	2.2-86.0	27.2	15.5	1.0-89.0
Age of onset of FMF (years)	16.3	12.1	0.0-66.0	12.0	11.3	0.50-71.0

Variable		Sample values		Weighted values
		n	%	%
Gender	Female	600	50.2	47.1
	Male	595	49.8	52.9
Age of onset of FMF	≤ 20	749	62.7	76.8
	> 20	446	37.3	23.2
Family cases	Yes	392	32.8	34.6
	No	803	67.2	65.4
Mutations	Heterozygous	373	31.2	30.4
	Comp. heterozygous	642	53.7	52.6
	Homozygous	180	15.1	16.5
Genotypes	M694V/V726A	237	19.8	18.4
	M694V/-	169	14.1	13.2
	V726A/M680I	126	10.5	10.3
	M694V/M694V	113	9.4	11.7
	M694V/M680I	107	8.9	9.9
	V726A/-	94	7.8	7.3
	E148Q/-	41	3.4	4.8
	M680I/-	39	3.3	3.3
	M694V/E148Q	37	3.1	3.1
	V726A/V726A	31	2.6	2.3
	V726A/F479L	31	2.6	2.6
	M680I/M680I	28	2.3	2.1

* Genetically-confirmed Armenian FMF patients

Table 2.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. Comparison of means of the continuous variables of Armenian FMF patients with age of onset of the disease before and after 20 years.

Age of onset	Temperature				Frequency of attacks of FMF			
	Mean	N	SD	p - value	Mean	N	SD	p - value
<20	37.9	751	0.93	0.041	20.15	703	19.0	0.272
>20	37.8	449	0.85		21.68	426	27.8	

Table 3.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in the course of FMF among patients with early and late onset FMF. Selected characteristics among FMF cases with the disease onset before 20 years of age versus the onset at 20 years or later among Armenian patients with FMF (Cross tabulation between the age of onset and the features of FMF)

Features		Age of onset		
		< 20	≥ 20	p-value
M694V/V726A		19.7%	19.8%	0.961
M694V/-		12.5%	16.7%	0.044
V726A/M680I		10.5%	10.5%	0.977
M694V/M694V		12.8%	3.8%	0.000
M694V/M680I		10.8%	5.8%	0.003
V726A/-		8.0%	7.6%	0.795
E148Q/-		3.3%	3.6%	0.829
M680I/-		2.9%	3.8%	0.418
M694V/E148Q		2.3%	4.5%	0.034
V726A/V726A		2.0%	3.6%	0.098
V726A/F479L		2.8%	2.2%	0.548
M680I/M680I		2.0%	2.9%	0.319
Heterozygous		57.9%	42.1%	0.022
Comp. heterozygous		62.9%	37.1%	0.847
Homozygous		71.3%	28.3%	0.007
Gender	Female	52.9%	45.7%	0.014
	Male	47.1%	54.3%	
Family case	Yes	63.7%	73.1%	0.001
Frequency of attacks	<1time in month	0.9%	1.2%	0.761
	1-2 times in month	75.5%	73.9%	
	> 2 times in month	23.6%	24.9%	
Skin rash		14.8%	16.5%	0.430
Fever		81.9%	80.8%	0.652
Abdominalgia		83.9%	85.3%	0.514
Thoracalgia		48.3%	51.9%	0.233
Splenomegalia		7.3%	5.8%	0.306
Hepatomegalia		6.3%	7.8%	0.307
Arthralgia		47.8%	51.9%	0.170
Arthritis		19.3%	17.4%	0.404
Amyloidosis		0.4%	1.3%	0.069
Mialgia		24.9%	27.2%	0.384
Trigger cold		71.9%	71.1%	0.815
Trigger stress		17.1%	29.8%	0.000
Trigger menstruation		16.1%	13.4%	0.300
Trigger physical activity		12.5%	12.8%	0.922
Trigger food (fatty food)		24.1%	23.3%	0.790
Trigger others		5.2%	5.6%	0.809

Table 4.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. The results of the simple logistic regression analysis of association between "Age of onset ≤20 and >20" and other features of FMF among Armenian population.

Features	Unadjusted OR	95% CI	p - value
M694V/M694V	3.668	2.159-6.230	<0.001
M694V/V726A	0.948	0.708-1.270	0.723
V726A/M680I	1.028	0.701-1.508	0.888
M694V/M680I	1.937	1.225-3.063	0.005
M694V/E148Q	0.489	0.254-0.945	0.033
V726A/V726A	0.621	0.304-1.268	0.191
V726A/F479L	1.245	0.581-2.667	0.574
M680I/M680I	0.674	0.318-1.429	0.303
M694V/-	0.742	0.534-1.032	0.077
V726A/-	1.044	0.673-1.617	0.849
M680I/-	0.756	0.397-1.439	0.394
E148Q/-	0.918	0.485-1.739	0.794
Heterozygous	0.754	0.587-0.970	0.028
Comp. Heterozygous	1.007	0.795-1.274	0.956
Homozygous	1.636	0.598-0.957	0.006
Gender	0.756	1.060-1.694	0.020
Age	0.904	0.893-0.915	<0.001
Yerevan	0.998	0.780-1.277	0.990
Kotayq	1.053	0.7261.526	0.787
Tavush	1.677	0.900-3.124	0.103
Lori	1.300	0.880-1.921	0.188
Gegharqunik	1.112	0.823-1.504	0.488
Vayots Dzor	1.991	0.793-4.996	0.142
Syunik	1.604	0.875-2.938	0.126
Ararat	1.054	0.672-1.652	0.819
Armavir	0.851	0.572-1.268	0.429
Aragatsotn	1.185	0.801-1.752	0.395
Shirak	0.959	0.668-1.335	0.802
Karabakh	1.187	0.644-2.188	0.583
Georgia/Akhalkalak	0.781	0.521-1.171	0.232
West Armenia	0.853	0.659-1.103	0.225
Out of Armenia	0.893	0.637-1.250	0.508
Family cases	1.534	1.187-1.983	0.001
Frequency of attacks (in	0.997	0.992-1.002	0.258
Fever	1.090	0.808-1.471	0.572
Temperature	1.146	1.006-1.305	0.040
Abdominalgia	0.912	0.659-1.264	0.582
Thoracalgia	0.880	0.696-1.112	0.283

Features	Unadjusted OR	95% CI	p - value
Arthritis	1.058	0.908-1.231	0.471
Arthralgia	0.951	0.880-1.029	0.211
Erysipelas-like erythema	0.930	0.792-1.091	0.372
Hepatomegalia	0.821	0.520-1.295	0.396
Splenomegalia	1.266	0.782-2.050	0.337
Mialgia	0.921	0.706-1.203	0.546
Amyloidosis	0.663	0.417-1.055	0.083
Severity	0.878	0.690-1.117	0.289
Trigger cold	1.062	0.775-1.455	0.710
Trigger stress	0.505	0.360-0.708	<0.001
Arthritis	1.058	0.908-1.231	0.471
Arthralgia	0.951	0.880-1.029	0.211
Erysipelas-like erythema	0.930	0.792-1.091	0.372
Hepatomegalia	0.821	0.520-1.295	0.396
Splenomegalia	1.266	0.782-2.050	0.337
Mialgia	0.921	0.706-1.203	0.546
Amyloidosis	0.663	0.417-1.055	0.083
Severity	0.878	0.690-1.117	0.289
Trigger cold	1.062	0.775-1.455	0.710
Trigger stress	0.505	0.360-0.708	<0.001
Trigger menstruation	1.216	0.810-1.825	0.345
Trigger physical activity	0.961	0.627-1.474	0.856
Trigger food (fat food)	1.056	0.754-1.477	0.752

Table 5.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. The results of the multiple logistic regression analysis of association between "Age of onset ≤20 and >20" and other features of FMF among Armenian population, model 1 (1194 cases were included in the analysis).

Features	**OR**	**(95% CI)**	**p - value**
M694V/M694V	2.078	1.075-4.016	0.030
M694V/M680I	2.612	1.438-4.745	0.002
Heterozygous	0.640	0.450-0.909	0.013
Gender (male)	0.580	0.427-0.788	<0.001
Age at the genetic confirmation of	0.891	0.879-0.903	<0.001
Family cases	1.797	1.285-2.513	0.001
Extent of fever	1.557	1.090-2.224	0.032
Arthritis	1.360	1.104-1.675	0.004
Myalgia	1.557	1.090-2.224	0.015

Table 6.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. The results of the multiple logistic regression analysis of association between "Age of onset ≤20 and >20" and other features of FMF among Armenian population, model 2 (1124 cases were included in the analysis)

Features	**OR**	**(95% CI)**	**p - value**
Heterozygous	0.609	0.425-0.874	0.007
M694V/M694V	2.041	1.057-3.940	0.033
M694V/M680I	2.271	1.233-4.184	0.009
Gender (male)	0.557	0.408-0.759	<0.001
Age	0.896	0.884-0.908	<0.001
Extent of fever	1.268	1.059-1.518	0.010
Family cases	1.906	1.357-2.677	<0.001
Severity	1.419	1.027-1.961	0.034

Table 7.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. Relation between Genotype and Severity of FMF clinical features in Armenian patients through cross tabulation*

Genotypes	Severity			p - value
	Mild (%)	Moderate (%)	Severe (%)	
M694V/M694V	14.2	52.8	33.0	0.000
M694V/V726A	31.7	58.1	10.1	0.089
V726A/M680I	49.6	42.3	8.1	0.001
M694V/M680I	19.6	53.6	26.8	0.000
M694V/E148Q	38.9	41.7	19.4	0.366
V726A/V726A	51.6	48.4	0.0	0.029
V726A/F479L	40.0	53.3	6.7	0.513
M680I/M680I	40.0	56.0	4.0	0.369
M694V/-	40.4	48.7	10.9	0.206
V726A/-	33.3	60.5	6.2	0.100
M680I/-	40.5	51.4	8.1	0.541
E148Q/-	20.5	61.5	17.9	0.169

*****The p-value shows the difference between the severity of the disease among the groups with given mutation versus all other mutation groups

Table 8.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. The results of the independent t-test for severity assessment among Armenian patients with FMF

Genotypes	Mean	SD	p - value
M694V/M694V	8.24	2.547	<0.001
M694V/V726A	6.55	2.421	0.746
V726A/M680I	5.83	2.311	<0.001
M694V/M680I	7.82	2.606	<0.001
M694V/E148Q	6.56	3.202	0.924
V726A/V726A	5.23	1.783	0.003
V726A/F479L	6.20	2.156	0.393
M680I/M680I	5.92	2.019	0.184
M694V/-	6.15	2.721	0.019
V726A/-	6.58	2.115	0.956
M680I/-	5.97	2.421	0.134
E148Q/-	7.33	2.355	0.068

Table 9.

Familial Mediterranean fever (FMF): Association between genotype and severity of the clinical manifestations of FMF, and possible differences in course of FMF among patients with early and late onset of the disease. The results of the multiple linear regression analysis on assessment of the severity of FMF clinical manifestations in Armenian patients*

Genotypes	B	95% CI	p - value
M694V/M694V	1.644	1.142 - 2.146	<0.001
V726A/M680I	-0.787	(-1.260) - (-0.313)	0.001
M694V/M680I	1.350	0.828 – 1.873	<0.001
V726A/V726A	-1.184	(-2.089) - (-0.280)	0.010
M694V/-	-0.593	(-1.021) - (-0.165)	0.007

*The association is controlled for age, gender, and family cases of FMF

Appendix 1: Medical Records Data Abstraction Form

Demographic data				
1. Patient ID:				
2. Date of birth ___/___/_____ or age ___ (only if date of birth is missing)				
3. Gender	0. □ Male	1. □ Female	99. □ Unclear/missing	
4. Region of origin (Marz)	1.□ Yerevan	2.□Kotayq	3.□Tavush	4.□ Lori
	5.□Gegharquniq	6.□VayotsDzor	7.□Syuniq	8.□ Ararat
	9.□Armavir	10.□Aragatsot	11.□Shirak	12.□Karabach
	13.□Georgia/Akhalkalak		14.□West Armenia	
	15□Out of Armenia		99.□ Unclear/missing data	
Clinical data				
5. Genotypes	1.□ M694V/M694V	2.□ M694V/V726A	3.□ M694V/M680I	
	4.□ M680I/V726A	5.□ M680I/M680I	6.□V726A/V726A	
	7.□ M694V/R761H	8.□ M694V/-	9.□ V726/-	
	10.□ M680I/-	11.□ E148Q/-		
6. Family cases	0.□ No	1.□ yes	99.□ Unclear/missing	
7. Age of onset			99.□ Unclear/missing	
8. Frequency of attacks			99.□ Unclear/missing	
9. Duration of attacks			99.□Unclear/missing	
10. Fever	0.□ No	1.□ yes	99.□ Unclear/missing	
11. Temperature	0.□ <38	1.□38-40	2.□>40	99.□ Unclear/missing
12. Abdominalgia	0.□ No	1.□Yes	99.□ Unclear/missing	
13. Thoracalgia	0.□ No	1.□Yes	99.□ Unclear/missing	
14. Arthritis	0.□ No	1.□Yes	99.□ Unclear/missing	
15.Arthralgia	0.□ No	1.□Yes	99.□ Unclear/missing	
16. Mialgia	0.□ No	1.□Yes	99.□ Unclear/missing	
17. Skin elements (Erysipelas-like)	0.□ No	1.□Yes	99.□ Unclear/missing	
18. Hepatomegalia	0.□ No	1.□Yes	99.□ Unclear/missing	
19. Splenomegalia	0.□ No	1.□Yes	99.□ Unclear/missing	
20. Diarrhea	0.□ No	1.□Yes	99.□ Unclear/missing	
21. Amyloidosis	0.□ No	1.□Yes	99.□ Unclear/missing	
22. Other symptoms				
23. Other syndromes				
24. Other diseases				
Triggers				
Cold	0.□ No	1.□Yes	99.□ Unclear/missing	
Stress	0.□ No	1.□Yes	99.□ Unclear/missing	
Menstruation	0.□ No	1.□Yes	99.□ Unclear/missing	
Physical activity	0.□ No	1.□Yes	99.□ Unclear/missing	
Food (fat food)	0.□ No	1.□Yes	99.□ Unclear/missing	

Appendix 2: Determination of the Degree of Severity in FMF Patients[33]

Parameters	Features	Scores
Age of onset	>31	0
	21-31	1
	11-20	2
	6-10	3
	<6	4
No. of attacks per month	<1	1
	1-2	2
	>2	3
Presence of arthritis	Acute	2
	Protracted	3
Presence of erysipelas-like erythema	+	2
Presence of amyloidosis	+	3
Colchicine dose, mg/dl	1	1
	1.5	2
	2	3
	>2	4

Based on the severity score, three levels of severity of the clinical manifestation of FMF are defined: mild, moderate, and severe disease, specified below:

Mild disease – 2 - 5

Moderate disease – 6 – 9

Severe disease – 10

Appendix 3: Variables table for the first outcome (onset of the disease)

	Variable	Type	Definition
Dependent	Onset of the disease	Binary	Before and after 20 years
Independent	Genotype	Binary	Presence or absence of each of the encountered genotype mutations
Control	Sex	Nominal	
	Age	Continuous	
	Frequency of attacks	Ordinal	
	Duration of attacks	Continuous	
	Family cases	Binary	
	Fever	Binary	
	Temperature	Continuous	
	Abdominalgia	Binary	
	Thoracalgia	Binary	
	Hepatomegaly	Binary	
	Splenomegaly	Binary	
	Skin rash	Binary	
	Arthritis	Binary	
	Concomitant disease	Binary	Presence or absence of each of the concomitant disease
	Amyloidosis	Binary	
	Trigger factors	Binary	Presence or absence of each of the listed triggers

Appendix 4: Variables table for the second outcome (severity of the disease)

	Variable	Type	Definition
Dependent	Severity	Continuous	
Independent	Genotype	Binary	Presence or absence of each of the main genotypes
Control	Sex	Nominal	
	Age	Continuous	
	Family cases	Binary	
	Fever	Binary	
	Temperature	Continuous	
	Abdominalgia	Binary	
	Thoracalgia	Binary	
	Hepatomegaly	Binary	
	Splenomegaly	Binary	
	Concomitant disease	Binary	Presence or absence of each of the concomitant disease
	Triggers	Binary	Presence or absence of each of the listed triggers