



An analysis of the most common MEFV gene variants found in Egyptian patients with familial Mediterranean fever

Noha A. Abd El Atty^{1,2*}, Khalid S. Hashem³, AbdAllah Y. Mandouh⁴, and Amr E. Ahmed¹

¹ Department of Biotechnology and life sciences, Faculty of postgraduate studies for advanced sciences, Beni-Suef University, Egypt.

² Department of Molecular Biology and Cytogenetic, Armed Forces Central Laboratories and Blood Bank, Egypt.

³ Department of Biochemistry and chemistry of nutrition, faculty of veterinary medicine, Beni-Suef University, Beni-Suef, Egypt.

⁴ Egypt Center of Research and Regenerative Medicine, Egypt.

*Corresponding author: Noha A. Abd El Atty, noharefai2019@gmail.com

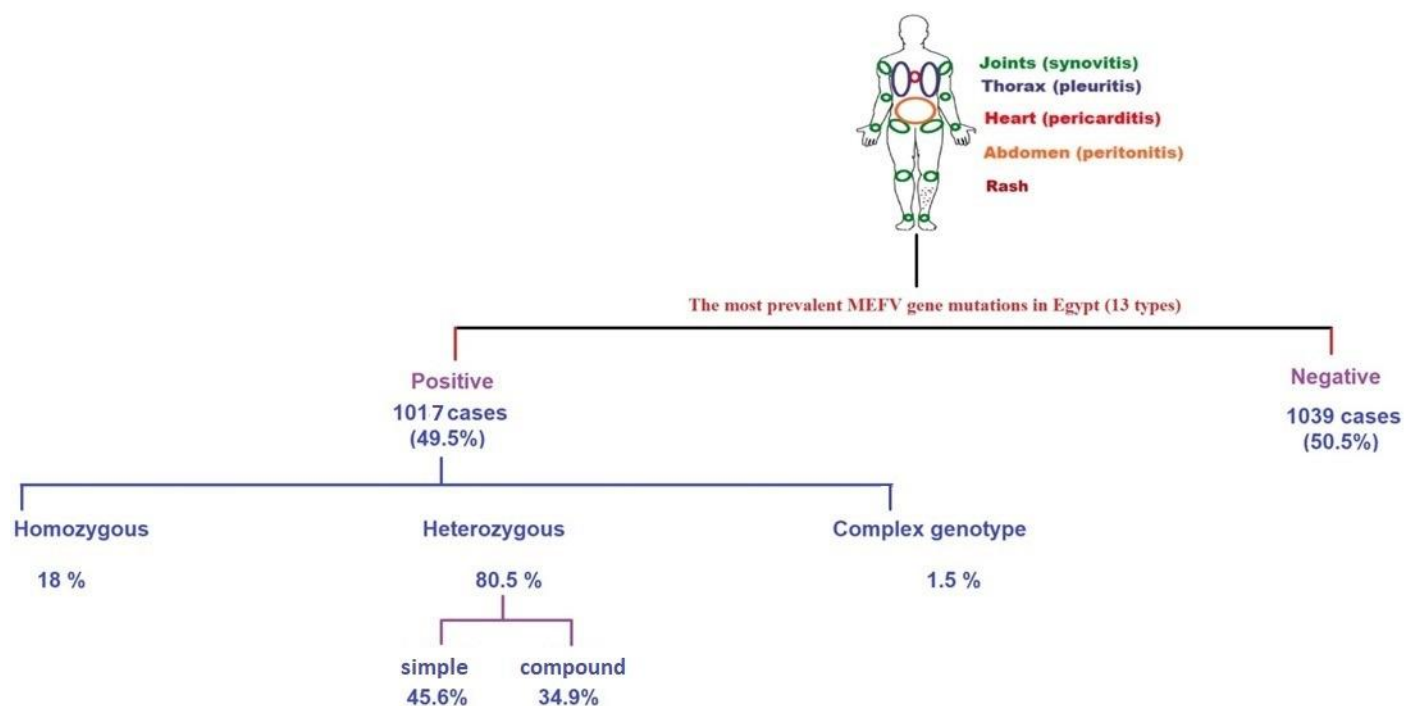
Abstract

Familial Mediterranean is a genetic health problem inherited through recessive autosomal transmission. It may cause serious long-term morbidity and mortality. The goal of this research is to survey the most widespread MEFV gene mutations in a large population of Egyptian patients suspected of having FMF disease. 2056 patients were tested for the 13 most prevalent types of MEFV mutations found in the basin of the Mediterranean using real-time PCR.

Results: Our results showed that fever was reported as the most prevalent clinical feature (94.5%), followed by abdominal pain (90.0%) and arthritis (59.4%). Only 1017 patients (49.5%) had positive MEFV gene mutations (754 males and 263 females), while 1039 patients (50.5%) showed negative mutations for the 13 most prevalent types of MEFV gene mutations in Egypt.

Conclusion: We suggested that F479L mutation analysis shouldn't be added to the routine molecular diagnosis of FMF patients. Depending on screening of the most prevalent mutations of MEFV gene in Egypt is not a sufficient confirmatory test for diagnosis of FMF in suspected patients and sequencing the entire MEFV gene is recommended. Most positive patients for the MEFV prevalent mutations were heterozygous.

Keywords: Familial Mediterranean fever, MEFV gene mutations



1. Introduction

Familial Mediterranean is a genetic health problem inherited through recessive autosomal transmission; it is one of the more common hereditary diseases. Classically characterized by irregular recurring episodes of fever, stomach ache, pain in the chest, arthritis pain, and skin eruption, it may cause serious long-term morbidity and mortality because of the development of severe complications, amyloidosis, and renal failure [1]. The majority of patients with attacks present in childhood or early adulthood; they commonly last three days or less and repeat every few months or couple of weeks; in ninety percent of cases, the first symptoms appear before the age of 20, and in seventy-five percent before the age of 10. The disease's late onset, defined as manifestation after the age of 40, is uncommon and occurs in less than 1% of patients [2].

FMF is the result of aberrant mutations in the MEFV gene, which is found on the short arm of chromosome 16p13.3. Pyrin, also known as marenostrin, is a protein that is encoded by this gene and is responsible for controlling inflammation in the body. This protein is primarily expressed in neutrophils, eosinophils, monocytes, dendritic cells, and fibroblasts. Pyrin's primary function is to participate in the inflammatory response and to resolve inflammation by cleaning out damaged cells of innate immune regulators [3].

In 1997, the Mediterranean fever gene "MEFV" was discovered. A pyrin protein has 781 amino acids and is made up of 10 exons; the majority of the mutations occur in exon 10 between amino

acids 680 and 761, as well as in exons 2, 3, and 5 [4]. The majority of the mutations are substitution mutations, such as p.M694V in exon 10, which replaces methionine at position 694 with valine; this is the most common Mediterranean mutation with a severe clinical picture; another well-known mutation is p.V726A, which is common in Ashkenazi Jews [5, 6]. Small deletions are exceedingly uncommon; two small in-frame deletions and three mutations that result in the creation of a stop codon (p.I692del, p.M694del, and p.Y688*) have been reported [7]. The Infevers Database currently contains 391 sequence variants for the MEFV gene [8].

FMF has a global prevalence, with carrier frequencies of 1:5, 1:7, 1:5, and 1:16, respectively, in nations originating from the Mediterranean Sea, particularly North African Jews, Armenians, Turks, and Arabs. FMF has spread throughout the Mediterranean Sea, taking advantage of ancient seafarers' travels and migrations. Nowadays, because the possibility of travelling has increased, we will see the disease spread to geographical regions other than the traditional Eastern Mediterranean basin [9].

Familial Mediterranean fever disease is classified clinically into three phenotypes: type I, type II, and type III.

-FMF type I is characterised by recurrent episodes of inflammation and serositis, such as fever, peritonitis, synovitis, pleuritis, and, in rare cases, pericarditis and meningitis. The severity and frequency of symptoms vary from person to person and even within families. The most serious complication is amyloidosis, which can lead to renal failure if left untreated.

- FMF type II is a rare form of FMF characterised by amyloidosis as the first clinical manifestation of FMF in otherwise asymptomatic people [10].

- FMF type III is distinguished by the presence of two MEFV mutations in the absence of FMF or AA amyloidosis symptoms in a 'silent' homozygous or compound heterozygous state [11].

FMF is diagnosed using the Tel-Hashomer criteria for major and minor features:

- Symptoms include fever, abdominal pain, chest pain, joint pain, and skin rashes.

-Characteristics that are minor include an increase in inflammatory markers such as C-reactive protein, erythrocyte sedimentation rate (ESR), and white blood cell count.

FMF clinical symptoms are nonspecific and difficult to differentiate from those resulting from other diseases, which are frequently misdiagnosed as appendicitis, so by combining genetic

testing with clinical criteria, MEFV gene sequencing is assisting in the diagnosis of illness in a large number of patients [12].

Long-term colchicine prophylaxis can result in excellent disease control for the vast majority of FMF patients. Colchicine has an anti-inflammatory effect that is:

a-At the transcriptional level, it may suppress pro-inflammatory genes while enhancing anti-inflammatory genes.

b- Changes L-selectin surface expression on leukocytes.

c-Could obstruct NF- κ B activation

d- Microtubule self-assembly processes are inhibited, such as ASC-NALP3 approximation and neutrophil chemotaxis [13].

Despite the fact that FMF knowledge is rapidly expanding, as well as FMF mutations are common in the Egyptian population, as previously described. However, because data is still poor, the purpose of this study was to identify the most prevalent MEFV gene mutations in a large population of Egyptian FMF patients

2.Methods

A retrospective cohort analysis was carried out at the department of molecular biology and cytogenetics, Armed Forces Central Laboratories, and Blood Bank on a large series of 2056 unrelated Egyptian patients. Tel Hashomer criteria were used to diagnose all patients [14]. All patients with FMF symptoms and signs were referred for genetic testing by doctors from various military hospital clinics in the Cairo governorate. The study followed the Helsinki Declaration's principles and was approved by the Egypt Centre for Research and Regenerative Medicine Ethics Committee (2022-05/1), and all participants gave their informed consent and the consent of the guardian of any child patients; patients having concurrent diagnoses of another chronic illness unrelated to FMF; acute or chronic infection; and the study excluded people with chronic liver or renal illness.

Questionnaire including the applicant's age and sex. Clinical history considering the onset of the disease, symptoms, and treatment were registered. Laboratory tests included the complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum amyloid A, liver, and kidney functions, which were also estimated but not mentioned due to space

limitations, and not all results could be included in this article. Additional results are available upon request.

2.1. DNA extraction and purification

3 ml from peripheral venous Blood samples from each patient were taken in Ethylene diamine tetra acetic acid tubes (EDTA) for the extraction of genomic DNA. The purification and extraction of DNA were done using a commercially available PREP-RAPID GENETICS DNA Extraction Kit (DNA-technology, Russian).

2.2. Mutational analysis

After DNA extraction, a kit based on real-time polymerase chain reaction with melting curve analysis is available, and the DT Prime real-time PCR instrument (DNA-technology, Russian) was applied to detect MEFV gene mutations.

The patients were tested for the 13 most prevalent MEFV mutations found in the Mediterranean basin: E148Q in exon 2, P369S and R408Q in exon 3, F479L in exon 5, M680I (G/A), M680I (G/C), I692del, M694V, M694I, K695R, V726A, A744S, and R761H in exon 10.

2.3. Statistical analysis

All statistical calculations were performed using the Statistical Package for SPSS Inc., Chicago, IL, USA. Social Science Version 26.0 Numbers and percentages were used to represent qualitative factors. Variables having a mean and standard deviation (SD) value.

3. Results

The most frequently reported complaints were fever, abdominal pain, and arteritis (Fig. 1). Of the 2,056 patients (65.9% males and 34.1% females) suspected to have FMF, the population investigated ranged in age from four to forty-one years old, with a mean age of 22.3 ± 13.4 years.

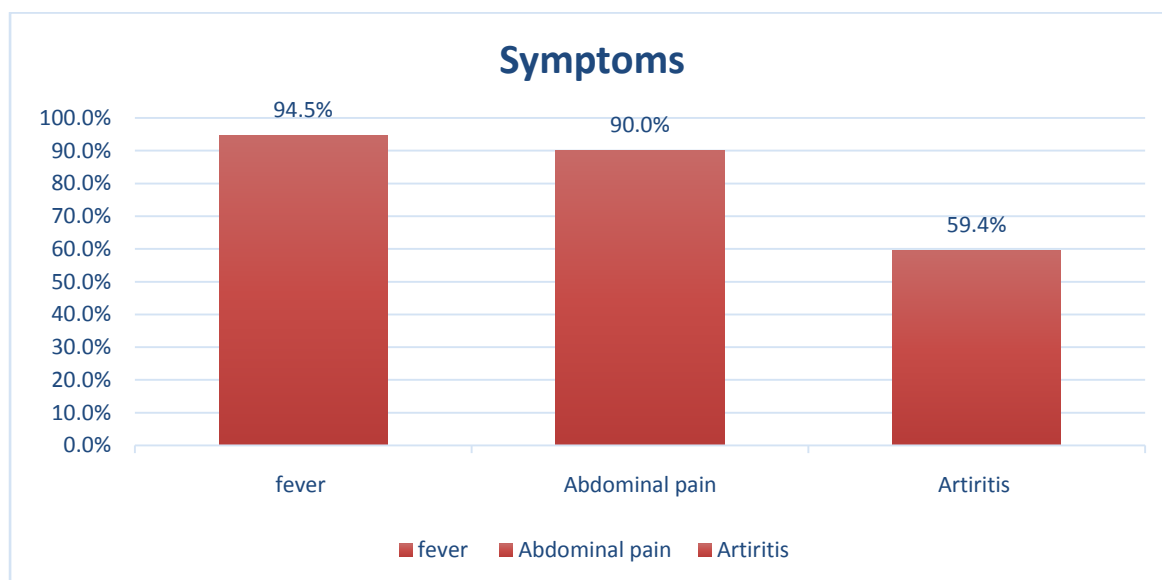


Figure 1:The frequency of the most common symptoms among FMF suspects as a percentage.

From all 2056 patients, 1017 (49.5%) had positive MEFV gene mutations (754 males and 263 females), while 1039 (50.5%) indicated negative mutations for the examined 13 MEFV gene mutations. Simple heterozygous mutations were found in 464 of the 1017 patients with positive MEFV gene mutations (45.6%), and in 354 patients (34.9%), compound heterozygous mutations were observed as two different combinations of alleles. Homozygous mutations were two identical combinations of alleles, observed in 184 patients (18.1%), and complex genotype mutations were more than two alleles found in 15 patients (1.5%), of whom 5 were female and 10 were male. Fig 2

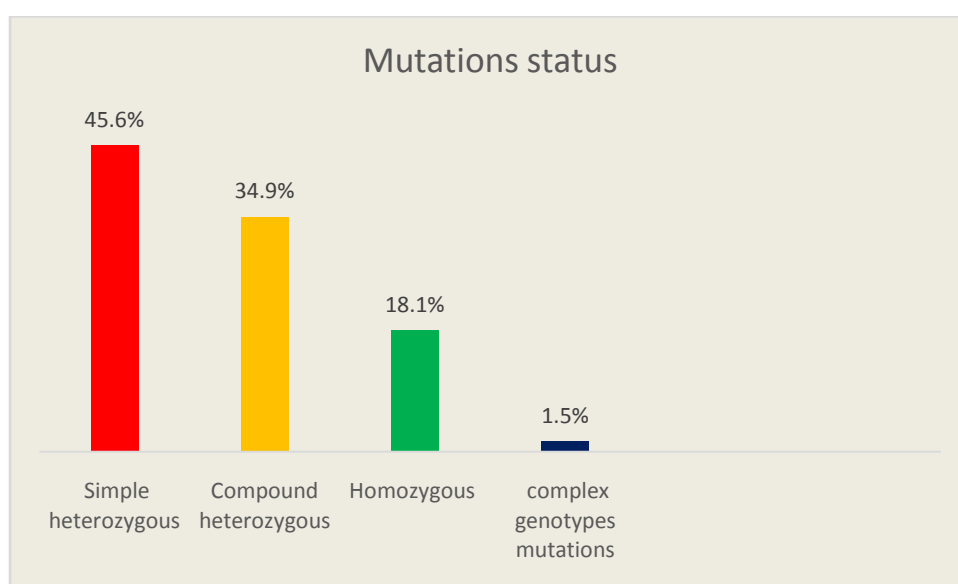


Figure 2: MEFV gene mutation distribution in the positive studied population.

In the simple heterozygous mutation group, there were 159 (34.3%) females and 305 (65.7%) males; in total, the most observed mutations were M694I (35.8%), V726A (18.1%), E148Q (13.4%), A744S (11.9%), M680I (G/A) (10.1%), and M694V (6.5%), with M680I (G/C) and K695R showing low distribution (2.2%) and (1.7%), respectively. The I692del and R761H were relatively rare (0.3%), while the F479L, P369S, and R408Q were not seen. Fig 3

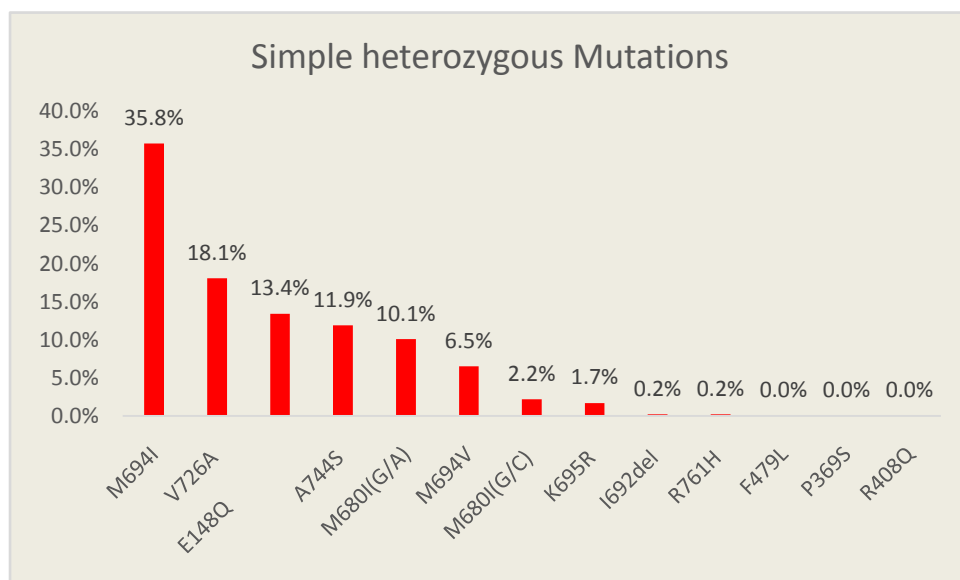


Figure 3:The frequency of observed MEFV gene mutations in the simple heterozygous group.

In the compound heterozygous mutation group, there were 61 (17.2%) females and 293 (82.8%) males. In the total group, the most five genotypes were M694I, V726A with a percentage of 30.4%, M680I (G/A), V726A, and M680I (G/A), M694I with a percentage of 12.7%, and

M694V, V726A with a percentage of 9.6%. Fig 4

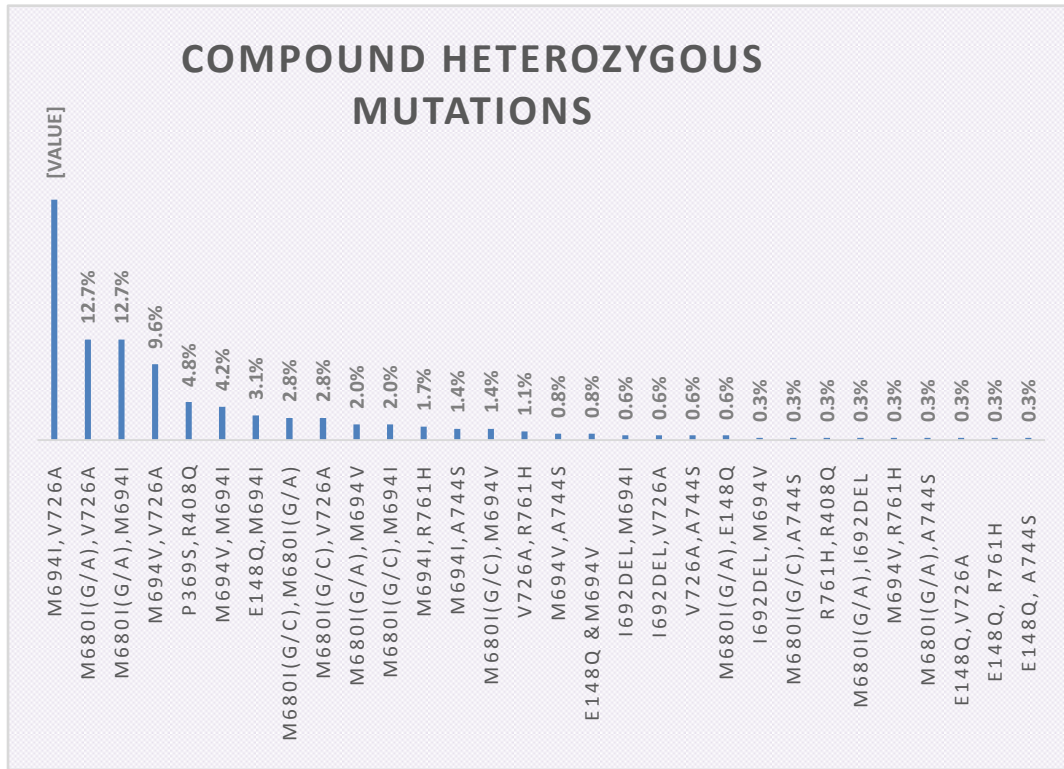


Figure4:The proportion of MEFV mutations found in the compound heterozygous group.

In homozygous group mutations, there were 38 (20.7%) females and 146 (79.3%) males; the most observed mutations were M694I with a percentage of 53.8%, V726A (19.0%), M680I (G/A) (13.6%), and M694V (9.8%), while E148Q showed a low distribution with a percentage of 1.6%. The A744S, R761H, M680I (G/C), and I692del were relatively rare (0.5%), while the F479L, K695R, P369S, and R408Q were not seen. Fig 5

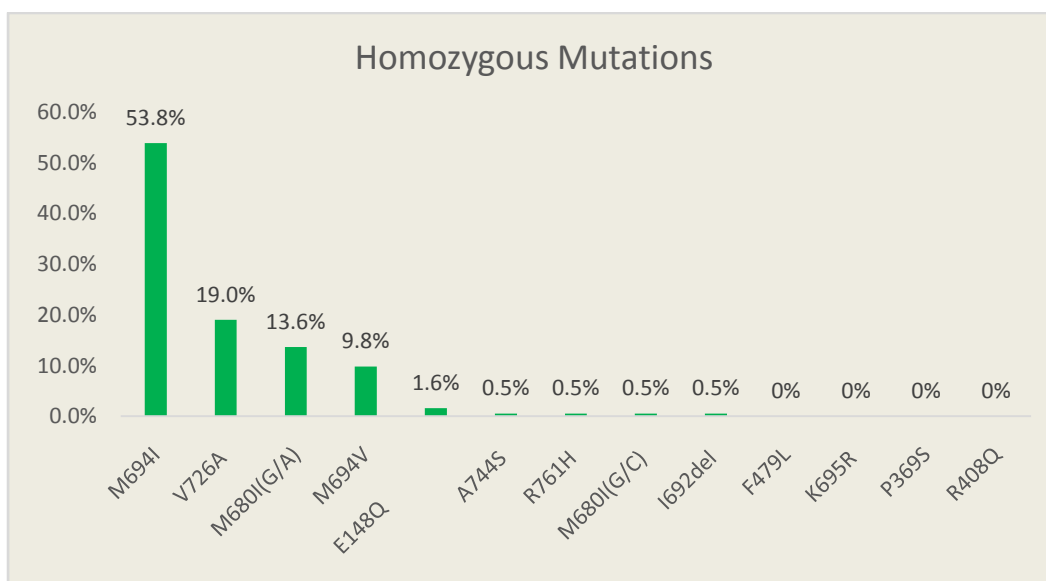


Figure5: The frequency of MEFV gene mutations observed in the homozygous group.

In the complex genotypes group, triple complex heterozygous mutations were found in six patients (2 male and 4 female); compound homozygous mutations were observed in five male patients. Both homozygous and heterozygous for different mutations were observed in 4 patients, 3 males and 1 female. Table 1

Table 1: The distribution of MEFV mutations in a complex genotype population.

Mutation	n	%
I692del, heterozygous, M694I heterozygous, K695R heterozygous	1	0.07
V726A, heterozygous, E148Q heterozygous, I692del heterozygous	1	0.07
M694I, heterozygous, I692del heterozygous, E148Q heterozygous	1	0.07
I692del heterozygous, V726A heterozygous, E148 Q heterozygous	1	0.07
M680I(G/A) heterozygous, I692del heterozygous, E148Q heterozygous	1	0.07
P369S heterozygous, R408Q heterozygous, E148Q heterozygous	1	0.07
M680I(G/A) homozygous, M680I(G/C) homozygous	2	0.13
M694V homozygous, M694I homozygous	3	0.2
M694I heterozygous, V726A homozygous	2	0.13
M694V homozygous, K695R heterozygous	1	0.07
I692del homozygous, M694I homozygous, E148Q heterozygous	1	0.07

The distribution of the 13 MEFV gene mutations discovered in 1017 positive FMF patients resulted in 1576 alleles, which are presented in table 2.

Table 2: Distribution and frequency of the 13 MEFV alleles in the studied population.

Mutations	Number of alleles	Frequency %
M694I	563	35.7
V726A	366	23.3
M680I(G/A)	213	13.5
M694V	143	9.1
E148Q	94	6.0
A744S	70	4.4

M680I(G/C)	51	3.2
R408Q	19	1.2
P369S	18	1.1
R761H	16	1.0
I692del	13	0.8
K695R	10	0.6
F479L	0	0

4. Discussion

In this work, we looked at the frequency and prevalence of 13 MEFV mutations in 2056 Egyptian patients suspected to have FMF disease. Among this large series of suspected patients, fever was reported as the most prevalent clinical feature (94.5%), followed by abdominal pain (90.0%) and arthritis (59.4%). Our clinical data was similar to a study by Nazif et al. [15], which revealed that the most common symptoms were fever (89.8%), followed by abdominal pain (88.6%), while contradictory to Beshlawy et al. [16]. They discovered that abdominal pain is the most common clinical sign, followed by fever, with percentages of 90.8% and 88.5%, respectively.

Our data revealed that there is a gender difference in our studied population, with 65.9% males and 34.1% females in a ratio of 1.9:1. This outcome was mainly in line with earlier research by Ali et al. [17], who reported a closed ratio of 1.7:1. In contrast with Nazif et al. [15] and Zarouk et al. [18], who showed that there is no sex difference, our data support the suggestion that FMF may have partial penetrance in women.

There was no mutation detected in one thousand thirty-nine (50.5%) of two thousand fifty-six patients in the study population; this large series of negative mutation identification accounted for a relatively close relationship with Mansour et al. (19), who showed that 57.2% had no MEFV gene mutations. This issue could be possible due to the occurrence of other uncommon or unknown mutations. From this finding, we suggested that we could screen for more mutations by sequencing all 10 exons.

Screening 13 common mutations of the MEFV gene, we found that 1017 patients (49.5%) of 2056 patients had positive MEFV gene mutations, varying according to the number of alleles between simple heterozygous (45.6%), compound heterozygous (34.9%), homozygous mutations (18.1%), and complex genotype mutations (1.5%). These findings are congruent with the findings of Cekin et al.'s study [20], which reported a similar result with a different percentage, and previous Egyptian studies by Mansour et al. [19] and Ali et al. [17]. All of these findings support

the high mutational heterogeneity of FMF in Egypt. This high proportion of simple heterozygosity may be explained by the existence of at least one modifying allele in associated genes or by other environmental variables.

Current analysis of 1.017 positive patients revealed that M694I was the most prevalent mutant allele (35.7%), followed by V726A (23.3%), M680I (G/A) (13.5%), M694V (9.1%), E148Q (6.0%), and A744S (4.4%). These results were similar to those of Ali et al. [16] and Zarouk [17], who demonstrated that M694I was the most common mutation in the Egyptian population. Contrary to other Egyptian researchers, Nazif et al. [15] and Mansour et al. [19], who described E148Q as being the most frequently detected mutation, discrepancies in the results can be traced to population disparities in the sample sizes analyzed.

In our study, V726A is the second most common mutant allele with a percentage of 23.3%; these results go with those of Talaat et al. [21], who reported that V726A is the second most widespread mutation with a nearly similar percentage (20%) in Egypt, and Jarjour et al. [22], who stated that V726A is the second most common mutant allele in the Syrian population. While V726A was the first most common mutation in a study by Farag et al. [23].

In the present study, M680I (G/A) was the third most common mutant allele, accounting for 13.5 percent of the mutant alleles, similar to a prior study on 818 Egyptian FMF patients by Zarouk et al. [18]. Nevertheless, in a previous study on the north region of Turkey, nouns of the studied population had the M680I (G/A) mutation, as reported by Celep et al. [24].

With a frequency of 9.1%, M694V was the fourth most prevalent mutation in our cohort. M694V has previously been identified as the most prevalent mutation in distinct ethnic groups, as recorded in recent studies conducted in several regions of Turkey by Güneş-Yılmaz et al. [25], ztürk et al. [26], and BLGE et al. [27]. In southern Lebanon and Jordan, M694V was the most frequently encountered mutation, with a frequency of 20.7 percent by El Roz et al [28] and 49.7 percent by Alzyoud et al [29].

In our cohort, A744S was found as the sixth common allele with a percentage of 4.4%. These results were contrary to other Egyptian research by Mansour et al. (19), who described A744S as the fourth most common mutation reported in 9.3% of cases.

We also found that M680I (G/C), R408Q, P369S, R761H, I692del, and K695R were the rarest mutations. In a local Iranian study by Salehzadeh et al [30]. There were no M680I (G/C) or

I692del mutations found, while P369S, R761H, and K695R were documented with frequencies of 3.1%, 0.8%, and 0.4%, respectively.

In our study, we also screened for the rare F479L mutation, but it was not detected as mentioned in previous study of Mansour et al. [19]. While in research that was conducted in Armenia, F479L mutation frequency was documented as 2.59% by Kriegshäuser et al [31], As a result, we proposed that F479L mutation analysis not be included in the routine molecular diagnosis of FMF patients because it would be more economically feasible.

The differences in results and mutation carriage rate could be attributed to ethnic differences as well as differences in the studied sample size. Only 15 patients in our study have more than two alleles (three or more), which is contradictory to a previous Egyptian study that didn't detect any complex genotypes, Zarouk et al. [18].

5. Conclusion

We suggested that F479L mutation analysis shouldn't be added to the routine molecular diagnosis of FMF patients, because it would be more economically feasible. Depending on the prevalence of the most prevalent mutations of the MEFV gene in Egypt, screening is not a sufficient confirmatory test for the diagnosis of FMF in suspected patients, and sequencing the entire MEFV gene is recommended. Most positive patients for the MEFV prevalent mutations were heterozygous, the heterogeneity of the allele frequency is high in the Egyptian population. There are some limitations to our study. Because sequencing was not performed on the complex genotype group, we recommend including it in future studies to ensure the presence of these complex mutations.

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Authors' contributions: Khalid S. Hashem, N. A. did the experiment, analysis of data and writing the manuscript. A.E.A. helped in the experiment and the analysis of data and shared in the methodology of the experiment and writing processes. A.E.A and A. M. made the proofreading.

Compliance with ethical standards:

Conflict of interests: The authors declare that they have no competing interests.

Ethical approval:The study was approved by the Egypt Centre for Research and Regenerative Medicine Ethics Committee (2022-05/1), and all participants gave their informed consent, and the guardian of child patients completed a questionnaire that included the patient's age, gender, and symptoms.

Consent to publish: All authors agreed with the submission and approved the publication.

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Availability of data and materials:Supplemental data and materials are available to authorised users.

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