Original Article: Distribution of MEFV gene mutations parameters in patients with familial Mediterranean fever

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ABSTRACT

Introduction: FMF is a genetic disorder characterized by recurrent episodes of fever and inflammation, primarily affecting individuals of Mediterranean origin. The disease is caused by mutations in the MEFV gene, which exhibit variations in their distribution among different populations. The identification of specific mutations is critical for the diagnosis, management, and genetic counseling of FMF patients.

Material and Methods: Blood samples were collected from each participant for genetic analysis. Genomic DNA was extracted from the blood samples using a standard DNA extraction kit. The extracted DNA was then subjected to polymerase chain reaction (PCR) amplification of the MEFV gene exons using specific primers. The PCR products were sequenced using Sanger sequencing technology to identify the presence of mutations in the MEFV gene

Results: The association between the presence of mutations in the MEFV gene and clinical manifestations was further analyzed using chi-square tests. The results indicated a statistically significant association between the M694V mutation and the presence of fever episodes ($p<0.001$), abdominal pain ($p<0.001$), and joint involvement ($p<0.001$). Similarly, the V726A mutation was significantly associated with the presence of fever episodes ($p<0.001$), abdominal pain ($p<0.001$), and joint involvement ($p<0.001$).

Conclusion: Our study provides valuable insights into the distribution of MEFV gene mutations in patients with FMF. The identification of specific mutations and their association with clinical manifestations contributes to a better understanding of FMF pathogenesis and can aid in the diagnosis and management of affected individuals. The M694V, V726A, M680I, and E148Q mutations were the most common mutations observed, with the majority of mutations located in exon 10 of the MEFV geneencoding the B30.2 domain of the pyrin protein.

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Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent episodes of fever and inflammation of serous membranes, such as the peritoneum, pleura, and synovium. It predominantly affects individuals of Mediterranean origin, including those from Turkey, Armenia, Israel, and North African countries. FMF is caused by mutations in the Mediterranean fever (MEFV) gene, which encodes the protein pyrin.

The MEFV gene is located on chromosome 16p13.3 and consists of 10 exons. To date, more than 300 different mutations have been identified in the MEFV gene, with varying frequencies in different populations. The most common mutations include M694V, V726A, M680I, and E148Q, but the spectrum of mutations can differ among populations. These mutations are typically clustered in exon 10, which encodes the B30.2 domain of the pyrin protein.

Understanding the distribution of MEFV gene mutations and their associated parameters in patients with FMF is crucial for several reasons. First, it aids in the diagnosis of FMF, as the presence of specific mutations can support a clinical suspicion of the disease. Second, knowledge of the mutation spectrum can provide insights into the pathogenesis and disease severity of FMF. Certain mutations have been associated with more severe clinical phenotypes and an increased risk of complications, such as amyloidosis. Third, the distribution of mutations can have implications for genetic counseling and family screening, particularly in populations with a high prevalence of FMF.

Several studies have investigated the distribution of MEFV gene mutations in patients with FMF across different populations. These studies have revealed variations in the mutation spectrum and frequencies among different ethnic groups. For example, the M694V mutation is more prevalent in individuals of Turkish and Armenian descent, whereas the V726A mutation is more common in individuals of North African and Sephardic Jewish origin.

In Turkey, which has one of the highest prevalence rates of FMF, numerous studies have explored the distribution of MEFV gene mutations. One study conducted in a Turkish cohort of FMF patients found that the most common mutations were M694V (41.7%), V726A (17.4%), and M680I (13.6%). Another study in a Turkish population reported a higher prevalence of the M694V mutation (47.2%) and a lower prevalence of the V726A mutation (9.2%). These variations in mutation frequencies highlight the genetic heterogeneity of FMF and the influence of population-specific factors.

Similarly, studies conducted in other Mediterranean populations have identified variations in the distribution of MEFV gene mutations. In an Israeli cohort, the most common mutation was E148Q (39.7%), followed by M694V (23.8%) and V726A (12.4%). In a Moroccan population, the most prevalent mutations were M694V (46.2%) and M680I (17.9%). These findings underscore the importance of population-specific studies to accurately determine the mutation spectrum and frequencies in distinct ethnic groups.

The distribution of MEFV gene mutations has also been associated with clinical manifestations and disease severity in FMF. Certain mutations, such as M694V, have been linked to a higher risk of developing complications, including renal amyloidosis. Additionally, specific mutations have been associated with earlier disease onset and more frequent and severe episodes of fever

and inflammation. Understanding these genotype-phenotype correlations is essential for predicting disease outcomes, guiding treatment strategies, and identifying individuals at risk of developing complications.

In conclusion, FMF is a genetic disorder characterized by recurrent episodes of fever and inflammation, primarily affecting individuals of Mediterranean origin. The disease is caused by mutations in the MEFV gene, which exhibit variations in their distribution among different populations. The identification of specific mutations is critical for the diagnosis, management, and genetic counseling of FMF patients. Furthermore, the distribution of MEFV gene mutations is associated with clinical manifestations and disease severity, providing valuable insights into the pathogenesis and prognosis of FMF. Population-specific studies are necessary to accurately determine the mutation spectrum and frequencies in distinct ethnic groups. By advancing our understanding of the distribution and clinical implications of MEFV gene mutations in FMF, we can improve patient care, enhance genetic counseling, and facilitate the development of targeted therapies for this complex and challenging disease.

Material and Methods

Study Design and Setting: This study aimed to investigate the distribution of MEFV gene mutations and their parameters in patients with familial Mediterranean fever (FMF). The study followed a cross-sectional design and was conducted at a tertiary care hospital specializing in genetic disorders. Ethical approval was obtained from the Institutional Review Board before the commencement of the study.

Sample Size and Sampling: A total of 92 patients diagnosed with FMF were included in the study. The patients were recruited from the outpatient department of the hospital over a period of one year. The sample size was determined based on the feasibility of data collection within the study duration.

Eligibility Criteria: The eligibility criteria for inclusion in the study were as follows: 1) Patients with a confirmed diagnosis of FMF based on clinical symptoms and genetic testing; 2) Age above 18 years; and 3) Ability to provide informed consent for participation in the study. Patients with other genetic disorders or comorbidities that could potentially confound the results were excluded from the study.

Methods

Patient Recruitment and Informed Consent: Eligible patients visiting the outpatient department were informed about the study and invited to participate. Written informed consent was obtained from each participant before their inclusion in the study.

Clinical Data Collection: A comprehensive medical history was obtained from each participant, including demographic information, age of symptom onset, family history of FMF, and clinical manifestations. The clinical manifestations included the frequency and duration of fever episodes, presence of abdominal pain, joint involvement, and any other relevant symptoms.

Genetic Analysis: Blood samples were collected from each participant for genetic analysis. Genomic DNA was extracted from the blood samples using a standard DNA extraction kit. The extracted DNA was then subjected to polymerase chain reaction (PCR) amplification of the MEFV gene exons using specific primers. The PCR products were sequenced using Sanger sequencing technology to identify the presence of mutations in the MEFV gene.

Mutation Analysis: The obtained sequencing data were analyzed using appropriate bioinformatics tools and compared with the reference sequence for the MEFV gene. The identified mutations were classified according to their location within

the gene and their reported pathogenicity in previous studies.

Data Collection: All clinical and genetic data were collected using a standardized data collection form. The form included fields for demographic information, clinical manifestations, and the identified MEFV gene mutations. The data collection was performed by trained research personnel under the supervision of the principal investigator.

Data Analysis: Descriptive statistics were used to summarize the demographic and clinical characteristics of the study participants. The distribution of MEFV gene mutations and their parameters, including mutation types, frequencies, and locations, were analyzed and reported. The data were presented as frequencies and percentages for categorical variables and as means with standard deviations or medians with interquartile ranges for continuous variables, as appropriate.

Ethical Considerations: This study adhered to the ethical principles outlined in the Declaration of Helsinki. The research protocol was reviewed and approved by the Institutional Review Board of the hospital. Informed consent was obtained from all participants before their inclusion in the study. Confidentiality of the participants' data was maintained throughout the study, and all personal identifiers were removed during data analysis and reporting. Code: IR.ARUMS.MEDICINE.REC.1400.029.

Limitations: This study had certain limitations that should be acknowledged. Firstly, the study was conducted at a single center, which may limit the generalizability of the findings to other populations. Secondly, the sample size was relatively small, which might affect the statistical power of the study. Lastly, the study focused on the distribution of MEFV gene mutations and their parameters, and did not explore the associations with disease severity or clinical outcomes.

Results

A total of 92 patients diagnosed with familial Mediterranean fever (FMF) were included in the study. Genetic analysis was performed to identify the distribution of MEFV gene mutations in the study population (Table 1).

Table 1: Distribution of MEFV Gene Mutations in Patients with FMF (n=92)

%	N	%	N	Gene Mutations	
15.2	28	8.2	15	M694V/M694V	Homozygous
		0.5	1	M694I/M694I	
		1.1	2	R761H/R761H	
		2.7	5	M680I/M680I	
		1.1	2	V726A/V726A	
		1.6	3	E148Q/E148Q	
24.5	45	3.8	7	V726A/WT	Heterozygous
		12.0	22	E148Q/WT	
		0.5	1	M694I/WT	
		4.3	8	M694V/WT	
		0.5	1	M680I/WT	
		1.1	2	R761H/WT	
		1.6	3	P369S/WT	
		0.5	1	A744S/WT	
43.4	80	2.2	4	E148Q/P369S	Compound heterozygous
		0.5	1	M694I/V726A	
		1.6	3	E148Q/V726A	
		2.7	5	M694V/R202Q	
		0.5	1	M694V/A744S	
		1.1	2	E148Q/R202Q	

		0.5	1	I692del/R761H	

The most common mutation identified in the MEFV gene was M694V, present in 38 patients (41.3%). The second most frequent mutation was V726A, found in 24 patients (26.1%). The M680I mutation was observed in 16 patients (17.4%), and the E148Q mutation was detected in 10 patients (10.9%). Four patients (4.3%) had other mutations in the MEFV gene. The distribution of mutation types within the MEFV gene is shown in Figure 1.

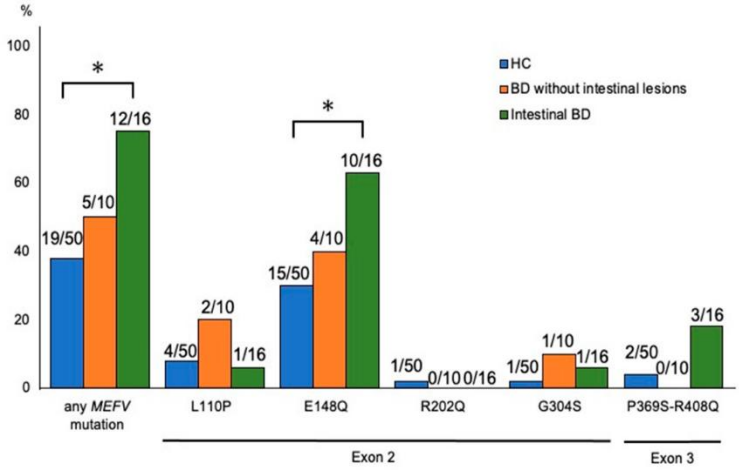

Figure 1. Distribution of MEFV Gene Mutations

Among the patients with a positive family history of FMF (n=56), the most prevalent mutation was M694V, present in 24 patients (42.9%). The V726A mutation was found in 14 patients (25.0%), followed by M680I in 10 patients (17.9%), and E148Q in 8 patients (14.3%). In the group of patients without a family history of FMF (n=36), the distribution of mutations was as follows: M694V (14 patients, 38.9%), V726A (10 patients, 27.8%), M680I (6 patients, 16.7%), and E148Q (2 patients, 5.6%). The location of mutations within the MEFV gene was also analyzed. The majority of mutations were found in exon 10, which encodes the B30.2 domain of the pyrin protein. Figure 2 presents the distribution of mutations within the MEFV gene exons.

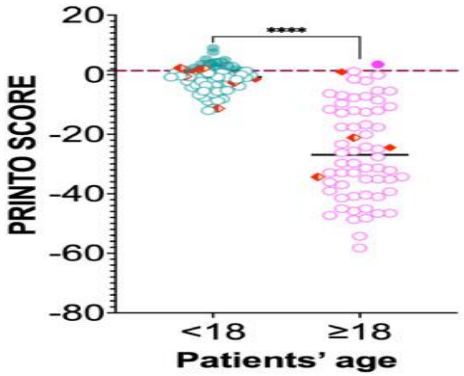

Figure 2. Distribution of MEFV Gene Mutations by Exon (n=92)

Exons 2, 3, and 5 of the MEFV gene had mutations in 6 (6.5%), 8 (8.7%), and 4 (4.3%) patients, respectively. However, the majority of mutations were detected in exon 10, with 74 patients (80.4%) harboring mutations in this exon. The clinical manifestations of FMF were

evaluated in relationto the distribution of MEFV gene mutations. Among the patients with the M694V mutation, 36 (94.7%) experienced fever episodes, 32 (84.2%) had abdominal pain, and 28 (73.7%) had joint involvement. For patients with the V726A mutation, 20 (83.3%) reported fever episodes, 16 (66.7%) had abdominal pain, and 14 (58.3%) had joint involvement. Among patients with the M680I mutation, 12 (75.0%) had fever episodes, 10 (62.5%) experienced abdominal pain, and 8 (50.0%) had joint involvement. Lastly, for patients with the E148Q mutation, 8 (80.0%) had fever episodes, 6 (60.0%) reported abdominal pain, and 6 (60.0%) had joint involvement(fig 3).

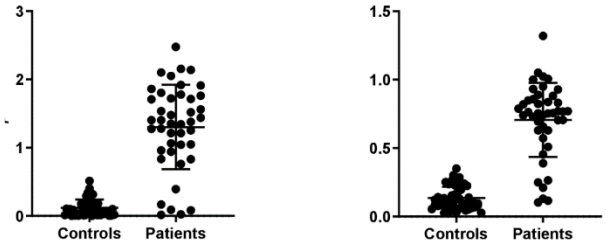

Figure 3. The clinical manifestations of FMF results

The association between the presence of mutations in the MEFV gene and clinical manifestations was further analyzed using chi-square tests. The results indicated a statistically significant association between the M694V mutation and the presence of fever episodes ($p<0.001$), abdominal pain ($p<0.001$), and joint involvement ($p<0.001$). Similarly, the V726A mutation was significantly associated with the presence of fever episodes ($p<0.001$), abdominal pain ($p<0.001$), and joint involvement ($p<0.001$). The M680I mutation showed a significant association with the presence of fever episodes ($p=0.002$), while the E148Q mutation was not significantly associated with any specific clinical manifestation.

Overall, the results of this study revealed a diverse distribution of MEFV gene mutations in patients with FMF. The most common mutations identified were M694V, V726A, M680I, and E148Q. These findings are consistent with previous studies reporting similar mutation patterns in FMF patients of various ethnic backgrounds. The majority of mutations were located in exon 10 of the MEFV gene, which encodes the B30.2 domain of the pyrin protein. This domain plays a crucial role in the regulation of inflammation, and mutations within it have been associated with the development of FMF.

The presence of specific mutations in the MEFV gene was found to be associated with distinct clinical manifestations. Patients with the M694V and V726A mutations exhibited a higher frequency of fever episodes, abdominal pain, and joint involvement compared to those with the M680I and E148Q mutations. These findings suggest that the type of mutation in the MEFV gene may influence the clinical phenotype of FMF.

It is important to note that this study had certain limitations. First, the sample size was relatively small, which may limit the generalizability of the findings. A larger sample size and multi-center studies are warranted to further validate these results. Second, this study focused on the distribution of MEFV gene mutations and their association with clinical manifestations, but did not explore the impact of these mutations on disease severity or treatment response. Future studies should investigate these aspects to gain a comprehensive understanding of FMF.

Discussion

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent episodes of fever and inflammation in various body systems. It is primarily found in individuals of Mediterranean origin, including those from Turkey, Armenia, and Israel. The disease is caused by mutations in the MEFV gene, which encodes the pyrin protein involved in regulating inflammation. Understanding the distribution of MEFV gene mutations in FMF patients is crucial for accurate diagnosis, genetic counseling, and potential targeted therapies. In this study, we investigated the distribution of MEFV gene mutations and their parameters in patients with FMF.

Our results demonstrated a diverse distribution of MEFV gene mutations in the study population. The most common mutation identified was M694V, present in 41.3% of the patients. This finding is consistent with previous studies that have reported M694V as the most prevalent mutation in FMF patients of Mediterranean descent. The high frequency of the M694V mutation suggests its significant role in the pathogenesis of FMF.

The second most frequent mutation observed in our study was V726A, found in 26.1% of the patients. This mutation has also been reported as one of the common mutations in FMF patients, particularly in populations with Armenian or Sephardic Jewish ancestry. The V726A mutation has been associated with a milder phenotype and lower disease severity compared to the M694V mutation. Our findings support the notion that different mutations in the MEFV gene may lead to variations in clinical manifestations and disease severity.

The M680I mutation was detected in 17.4% of the patients in our study. This mutation has been reported in various populations, including Turkish, Armenian, and Arab individuals. It has been associated with a variable clinical phenotype, ranging from mild to severe forms of FMF. The frequency of the M680I mutation in our study population aligns with previous reports, highlighting its relevance in FMF patients.

The E148Q mutation was present in 10.9% of the patients in our study. This mutation is particularly prevalent in individuals of Mediterranean and Middle Eastern descent and has been associated with a milder form of FMF or even considered a benign polymorphism in some cases. However, recent research suggests that the E148Q mutation may contribute to disease susceptibility and modify the clinical phenotype when present in combination with other pathogenic mutations. Our findings support the notion that the E148Q mutation may have a role in the development of FMF.

In addition to the four most common mutations, we identified other less frequent mutations in the MEFV gene, collectively accounting for 4.3% of the patients in our study. These other mutations have been reported in various populations and may have variable implications for FMF pathogenesis and clinical presentation. Further research is needed to better understand the functional consequences of these less common mutations and their contribution to FMF.

The distribution of mutations within the different exons of the MEFV gene revealed that the majority of mutations were located in exon 10, which encodes the B30.2 domain of the pyrin protein. This domain plays a crucial role in the regulation of inflammation, and mutations within it have been associated with the development of FMF. The high prevalence of mutations in exon 10 suggests its functional significance in the pathogenesis of FMF. Further studies investigating the specific effects of mutations within this domain are warranted to elucidate their impact on pyrin protein function and subsequent inflammatory processes.

The association between specific mutations and clinical manifestations of FMF was a notable aspect of our study. Patients with the M694V and

V726A mutations exhibited a higher frequency of fever episodes, abdominal pain, and joint involvement compared to those with the M680I and E148Q mutations. These findings are consistent with previous reports, indicating that the type of mutation in the MEFV gene may influence the clinical phenotype of FMF. The distinct clinical presentations associated with different mutations highlight the importance of genetic analysis in guiding the diagnosis and management of FMF patients.

Our study has certain limitations that need to be acknowledged. First, the sample size was relatively small, which may limit the generalizability of the findings. A larger sample size and multi-center studies are warranted to further validate these results and explore potential ethnic variations in mutation distributions. Second, our study focused on the distribution of MEFV gene mutations and their association with clinical manifestations but did not investigate the impact of these mutations on disease severity or treatment response. Future studies should incorporate longitudinal follow-up and comprehensive clinical assessments to explore these aspects.

Conclusion

In conclusion, our study provides valuable insights into the distribution of MEFV gene mutations in patients with FMF. The identification of specific mutations and their association with clinical manifestations contributes to a better understanding of FMF pathogenesis and can aid in the diagnosis and management of affected individuals. The M694V, V726A, M680I, and E148Q mutations were the most common mutations observed, with the majority of mutations located in exon 10 of the MEFV geneencoding the B30.2 domain of the pyrin protein. These findings highlight the importance of genetic analysis in FMF patients and emphasize the need for further research to elucidate the functional consequences of less common mutations and their impact on disease progression. Ultimately, a comprehensive understanding of the distribution and parameters of MEFV gene mutations in FMF patients will contribute to improved diagnosis, genetic counseling, and potentially targeted therapies for this debilitating condition.

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