Familial Mediterranean fever: Correlation between Gene Mutations and Clinical Findings at Royal Medical Services in pediatric patients

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ABSTRACT

Aim: Familial Mediterranean Fever (FMF) is an autosomal recessive inherited disorder that is caused by an inflammation due to pyrin protein deficiency, encoded by the *MEFV* gene. This retrospective study aims to correlate the genetic mutations with the clinical symptoms and complications in FMF paediatric patients.

Materials and methods: This retrospective study was analysed in the Immunology Department at Princess Iman Research and Laboratory Sciences Centre in the period between January 2019 and May 2021. The medical files including the demographic and clinical data of all patients diagnosed to have FMF were collected, then reviewed. Whole blood samples of patients under 14 years old with the clinical picture of FMF were collected at the Jordanian Royal Medical Services (JRMS) Hospitals and sent to the Immunology Department at Princess Iman Research and Laboratory Sciences Centre for analysis of MEFV gene mutations by polymerase chain reaction (PCR).

Results: Among the 250 paediatric patients tested for gene mutations, the mean age of onset was 7.6 ± 3.45 years. Abdominal pain was a presenting symptom in 84% of patients. The most frequently encountered allele was M694V at 29.8%, followed by E148Q (24.2%). Moreover, they were associated with more severe clinical courses and complications.

Conclusion: M694V and E148Q mutations have been found to be prevalent and may be used as biomarkers for disease severity.

Keywords: Familial Mediterranean Fever, *MEFV* gene, mutation, pyrin.

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Introduction

Familial Mediterranean fever (FMF) is an autoinflammatory disorder with an autosomal recessive pattern of inheritance, manifested by recurrent fever episodes and inflammation of the serosa, which can present as abdominal pain, arthralgia and chest pain [1, 2]. The disease is distributed among the populations of Mediterranean origin: Arabs, Jews, Armenians, and Turks [2]. The prevalence of FMF worldwide is 1/1000–1/500 [1]. Turks have the highest prevalence of FMF Globally, with an estimated rate of 1:150 to 1:1000 [3].

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Since the clinical symptoms of FMF present early, they could be confused with different illnesses occurring in paediatrics; for example, recurrent febrile tonsillitis might be a presenting complaint of FMF, particularly in young children [4]. Many patients present in childhood or in adolescence complaining of episodes that usually last for 3 days or less and recur every few weeks or months [5]. FMF can be differentiated from PFAPA (periodic fever, aphthous stomatitis, pharyngitis, adenitis syndrome) by the presence of family history and a differential diagnosis [2]. Although, patient age at the presenting time is highly related to the severity of the disease, patients who are at the onset of the disease aged more than 12 years had a decreased frequency of fever episodes than the younger patients. Moreover, patients aged less than 5 years at the onset had more severe episodes [6].

FMF is caused by a *MEFV* gene mutation that is found in the short arm of chromosome 16p13.3 and contains 10 exons parted by 9 introns; mutations were reported particularly in exons 2, 3, 5 and 10 of the *MEFV* gene [7]. The most common *MEFV* gene mutations are confined in the exons 2 and 10 and are randomly dispersed in multiple populations. Four gene mutations account more than 70% of the mutations in FMF patients of Mediterranean origin among different ethnic groups: M694V, M694I, M680I and V726A, all are present in the exon 10 [8]. This gene encodes a protein named pyrin that is found almost always in neutrophils and their precursors, which regulates the inflammatory, apoptotic processes, and/or cytokine secretion [9]. In fact, pyrin protein has an essential part in the activation of inflammasome and production of the potent pyrogenic cytokine interleukin (IL-1β); it is implicated in the stimulation of caspase-1 and produces active IL-1, as a mechanical part of the inflammasome complex, which has a pivotal role in the pathogenesis of FMF [10]. While in some studies a genotype-phenotype correlation was defined, it was not exactingly predictive and patients having similar phenotypes in one family can present with diverse symptoms [11]. Even though FMF is an autosomal recessive disorder, patients with heterozygous gene mutations are found to show typical disease-associated symptoms [12].

Amyloidosis is the most life-threatening complication of misdiagnosed, mistreated or neglected FMF, which is caused by the precipitation of insoluble material that is a product of serum amyloid-A destruction and is an acute-phase reactant formed by the liver. Renal amyloidosis complicated by renal failure, which happens in up to 60% of patients with untreated FMF, is the leading cause of mortality in FMF patients [8].

The diagnosis of FMF was mainly clinical, and it was later confirmed by the *MEFV* gene mutation analysis. Patients were tested for 14 variants: M694V, M694I, M680I, V726A, R761H, A744S, E167D, T267I and I692del are obviously pathogenic, while variants K695R, E148Q, P369S, F479L and I591T are of unknown importance [13]. This retrospective study focuses on the genetic analysis, clinical symptoms and the correlation between them in 250 FMF paediatric patients.

Patients and methods

Study design and patients

This retrospective study was analysed at the Immunology Department at Princess Iman Research and Laboratory Sciences Centre in the period between January 2019 and May 2021. During this period, there were 250 patients under 14 years old with FMF diagnosed using Turkish diagnostic criteria in childhood.

Criteria included; Fever (axillary temperature > 38 °C), abdominal pain, chest pain, oligoarthritis- with a duration of (6–72 h) and ≥3 attacks- and family history of FMF where the diagnosis requires the presence of 2 out of 5 criteria [11].

Patients were referred from paediatric outpatient clinics of JRMS Hospitals. Demographic and clinical data of the study group were collected from patients' charts in those hospitals, including patient sex and age, family history of FMF, presence of episodic fever, abdominal pain, joint pain, chest pain, other associations and renal complications. Patients were analysed for *MEFV* gene mutations by molecular diagnosis.

Sample collection and molecular analysis

First, 3–5 ml of blood were withdrawn from patients into ethylenediamine-tetraacetic acid-containing tubes. The procedure included three stages: DNA extraction using a commercial kit (Wizard@Genomic DNA Purification Kit/ Promega), followed by PCR amplification for exons 2, 3, 5, 10 of the MEFV gene by a Biorad T100 Thermal Cycler using biotinylated primers, then identification by reverse hybridisation of amplification products to a test strip containing allele-specific oligonucleotide probes immobilised as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and colour substrates containing nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphatase (BCIP) using a Vienna Lab Established Innovation in Diagnostics GmbH FMF Strip Assay. The assay tests 12 mutations in the MEFV gene: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S and R761H [14]. Gene mutation types and allele frequencies were also analysed.

Statistical analysis

The relationships between the presence of M694V and E148Q gene mutations with the severity of clinical course and the progression to complications in FMF patients were analysed using Chi-square. A P-value of < 0.05 was considered statistically significant.

Results

Patient characteristics

Among the 250 paediatric FMF patients tested for *MEFV* gene mutations, the mean age of onset was 7.6 ± 3.45 years. Females were more frequent, 131 (52.4%) were females and 119 (47.6%) were males, with a female-to-male ratio of 1.1:1 and a positive family history in 30.3%.

Clinical presentations

The most common presenting symptom was abdominal pain in (n = 210, 84%), followed by fever, which present in (n = 180, 72 %), and 36 patients who presented with chest pain, constituting 14.4%. The frequencies of presenting symptoms are listed in **Table I.**

Table I: The frequency of presenting symptoms

Presenting symptom	Number*	Percentage**
Abdominal pain	210	84%
Fever	180	72%
Chest pain	36	14.4%
Tonsillitis	30	12%
Vomiting	29	11.6%
Arthralgia	29	11.6%
Appendicitis	21	8.4%
Arthritis	14	5.6%
Skin rash	13	5.2%

* Number of patients with the presenting symptom
** Number of patients with the presenting symptom among all FMF patients (n=250)

Gene mutations

Of the *MEFV* gene mutations, heterozygous gene mutations were found in 184 patients (73.6%); in the group study, compound heterozygous gene mutations were in 20.8% (n = 52) of patients, and a homozygous gene mutation was in 5.6% (n = 14) of the study group. The frequency of *MEFV* gene mutations is shown in **chart (1).**

Six mutations were the ones most frequently detected in our study: M694V, E148Q, V726A, M680I (G/C), P369S and M694I. The most frequently encountered allele was M694V, found in 90 patients, followed by E148Q in 73 patients, V726A in 56 patients, accounting for 29.8%, 24.2%, 18.5% respectively. The most frequent gene mutation in heterozygotes was the E148Q gene mutation (n = 63, 34.2%), the most frequent compound heterozygous gene mutation was M694V-V726A (n = 21, 40.5%), and the most frequent homozygous gene mutations were M694V and V726A (n = 4, 28.6%) for each mutation. The frequency of the 12 alleles in the study group are shown in **chart (2).** The frequency of different genotypes in *MEFV* gene mutations are shown in **Table II.**

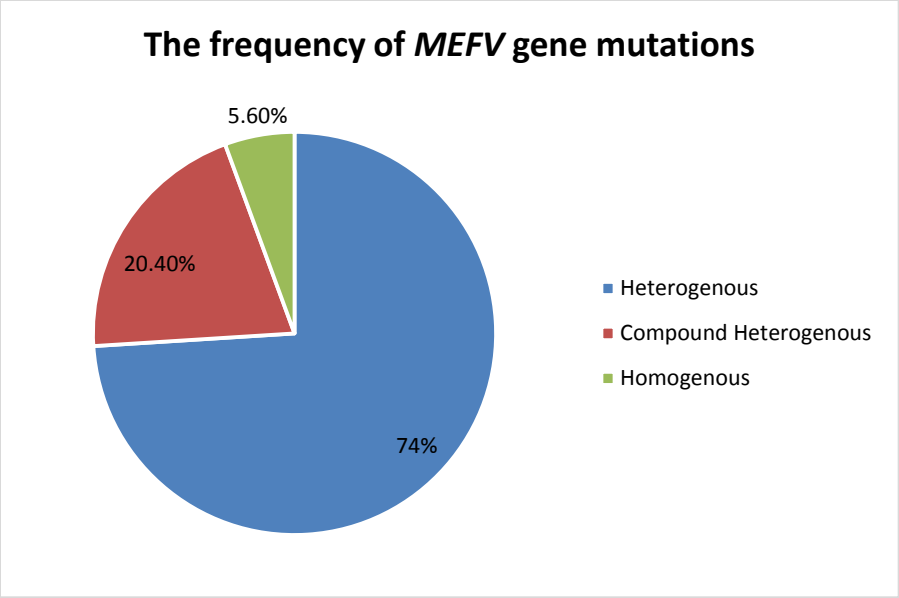

Chart 1: The frequency of *MEFV* gene mutations.

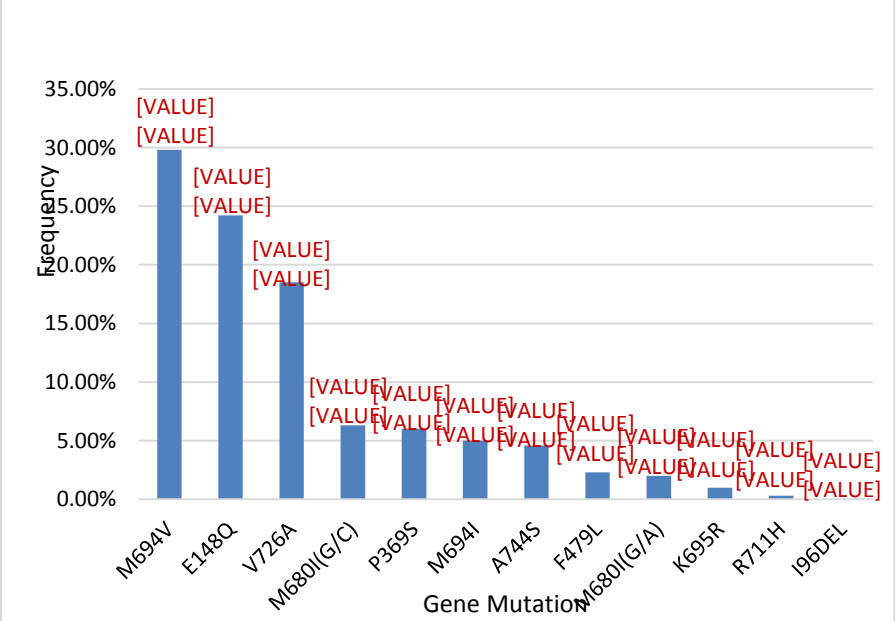

Chart 2: The frequency of the 12 alleles in all mutation types (Heterozygous, Compound Heterozygous, Homozygous) in the study group

Table II: The frequency of different genotypes in *MEFV* gene mutations

Mutation	Allele	Number* and Percentage**	
Heterozygous	M694V	N=56	(30.4%)
	E148Q	N=63	(34.2%)
	V726A	N=21	(11.5%)
	A744S	N=11	(6%)
	P369S	N=10	(5.5%)
	M680(G/C)	N=8	(4.3%)
	M694I	N=7	(3.8%)
	F479L	N=3	(1.6%)
	K695R	N=3	(1.6%)
	M680(G/A)	N=2	(1.1%)
	Total	N=184	(73.6%)
Compound Heterozygous	M694V-V726A	N=21	(40.4%)
	E148Q-P369S	N=5	(9.6%)
	V726A-F479L	N=3	(5.8%)
	M694V-M680(G/C)	N=3	(5.8%)
	M694V-M694I	N=2	(3.9%)
	M694V-P369S	N=2	(3.9%)
	E148Q-V726A	N=2	(3.9%)
	M680(G/C)-V726A	N=2	(3.9%)
	M649V-A744S	N=1	(1.9%)
	M694I-R761H	N=1	(1.9%)
	E148Q-M680(G/C)	N=1	(1.9%)
	M680(G/C)-A744S	N=1	(1.9%)
	E148Q-F479L	N=1	(1.9%)
	M680(G/A)-M694I	N=1	(1.9%)
	M694I-V726A	N=1	(1.9%)
	M680(G/A)-V726A	N=1	(1.9%)
	V726A-P369S	N=1	(1.9%)
	M680(G/A)-M694V	N=1	(1.9%)

	E148Q-A744S	N=1	(1.9%)
	M680(G/C)-M694I	N=1	(1.9%)
	Total	N=52	(20.8%)
Homozygous	M694V	N=4	(28.6%)
	V726A	N=4	(28.6%)
	M680(G/C)	N=3	(21.4%)
	M694I	N=2	(14.3%)
	M680(G/A)	N=1	(7.1%)
Total		N=14	(5.6%)

* Number of patients with the corresponding allele
**Number of patients with the corresponding allele in relation to the mutation type (Heterozygous, Compound Heterozygous, Homozygous)

Association of gene mutations with clinical presentations

According to the presenting symptoms, patients with abdominal pain had M694V mutation as the most frequent (35.2%), followed by E148Q with (27%). Patients presented with fever were mostly found to have alleles M694V, V726A and E148Q in; 39%, 21.6% and 21.6%, respectively. Also patients who presented with chest pain, had the M694V gene mutation as the most common mutation (44.4%). The frequency of *MEFV* gene mutation in patients with a certain presenting symptom is listed in **Table III**.

Presenting symptom / Gene mutation	**Abdominal pain**	**Fever**	**Chest pain**	**Tonsillitis**	**Vomiting**	**Arthralgia**	**Appendicitis**	**Arthritis**	**Skin rash**
Number* and percentage **									
M694V	N=74 (35.2%)	N=71 (39%)	N=16 (44.4 %)	N=10 (33.3%)	N=6 (20.6%)	N=18 (62%)	N=9 (42.8%)	N=8 (57%)	N=5 (38.5%)
E148Q	N=57 (27%)	N=39 (21.6%)	N=4 (11%)	N=12 (40%)	N=14 (48%)	N=10 (34%)	N=6 928.6%)	N=3 (214%)	N=4 (30.8%)
V726A	N=41	N=39 (21.6%	N=4	N=6	N=4	N=5	N=5	N=2	N=2

	(19.5%)	)	(11%)	(20%)	(13.8%)	(17%)	(23.8%)	(1%)4	(15%)
M680(G/C)	N=17 (8.1%)	N=11 (6.1%)	0	N=2 (6.6%)	N=3 (10.3%)	N=3 (10.4%)	N=3 (14.3%)	N=2 (14%)	N=2 (15%)
M680(G/A)	N=6 (2.8%)	N=3 (1.6%)	0	0	N=1 (3.450	0	N=3 (14.3%)	0	0
M694I	N=7 (3.3%)	N=9 (5%)	N=12 (33.6 %)	N=3 (10%)	0	N=2 (6.9%)	0	0	0
P369S	N=13 (6%)	N=7 (3.9%)	0	N=2 (6.6%)	N=3 (10.3%)	N=4 (13.7%)	N=2 (9.5%)	0	0
F479L	N=5 (2.3%)	N=4 (2.2%)	0	0	N=2 (6.9%)	0	0	0	0
A744S	N=8 (3.8%)	N=3 (1.6%)	0	N=2 (6.6%)	N=2 (6.9%)	N=1 (3.4%)	N=2 (9.5%)	N=1 (7%)	N=1 (7.7%)
K695R	N=3 (1.4%)	0	0	0	0	0	N=1 (4.7%)	0	0

Table III: The frequency of *MEFV* gene mutation in patients with a certain presenting symptom

* Number of patients with the gene mutation who had a certain presenting symptom
** Number of patients with the gene mutation who had a certain presenting symptom in relation to the total number of patients with the presenting symptom

Relation between gene mutations and FMF associations and complications

We noticed that 28 patients (11%) had associated inflammatory and autoimmune diseases: juvenile idiopathic arthritis (JIA) (n = 8, 3.2%), Henoch-Schonlein purpura (HSP) (n = 4, 1.6%), thyroiditis (n = 3, 1.2%), autoimmune hepatitis (n = 2, 0.8%), non-HSP vasculitis (n = 2, 0.8%). The frequency of associated inflammatory and autoimmune diseases in FMF patients is listed in **Table IV**.

Table IV: The frequency of associated inflammatory and autoimmune diseases in FMF patients

Associated autoimmune disease	Number[1]	Percentage[2]
JIA*	8	3.2%
HSP**	4	1.6%
Hashimoto's Thyroiditis	3	1.2%
Autoimmune Hepatitis	2	0.8%
Non- HSP Vasculitis (Leukocytocalstic Vasculitis and PAN*)**	2	0.8%
Aphthous Stomatitis	2	0.8%
Eosiniphilic Colitis	2	0.8%
Behcet's Disease	1	0.4%
Steven Jhonson syndrome	1	0.4%
Crohn's Disease	1	0.4%

*Juvenile Idiopathic Arthritis
**Henoch-Schonlein Purpura
***Polyarteritis Nodosa
[1] Number of FMF patients who had associated autoimmune disease
[2]Number of FMF patients who had associated autoimmune disease in relation to the total number of patients (n=250)

We declareded that our study group had complications related to FMF. Firstly, renal involvement manifested as proteinuria, haematuria and IgA nephropathy. Proteinuria was detected in 38 patients (15.2 %), and haematuria was found in 24 patients (9.6%). In our study, the M694V heterozygous genotype mutation was identified to be the most common gene mutation that carries a higher risk to develop proteinuria (n = 12, 31.5%) and haematuria (n = 9, 37.5%), followed by E148Q (n = 10, 26.3%; n = 5, 21%) in patients with proteinuria and haematuria, respectively. IgA nephropathy was seen in one patient who had the M694V heterozygous mutation. Secondly, pericardial and pleural effusions were detected in two patients who had the M694V heterozygous genotype mutation. Patients who have associated HSP and other vasculitis had M694V (n = 3, 50%) as the most common mutation, followed by the E148Q gene mutation, with 33.3%. The frequency of *MEFV* gene mutation in patients with FMF complications and other associations is shown in **Table V**.

Table V: The frequency of *MEFV* gene mutation in patients with FMF complications and other associations

Complication / ***MEFV* Mutation**	**Proteinuria**	**Haematuria**	**IgA nephropathy**	**HSP & other Vasculitis**	**Pericardial & pleural effusions**

Number* and percentage **					
M694V	12 (31.5%)	9 (37.5%)	1 (100%)	3 (50%)	2 (100%)
E148Q	10 (26.3%)	5 (21%)	0	2 (33.3%)	0
V726A	6 (15.8%)	4 (16.5%)	0	1 (16.7%)	0
M680(G/C)	3 (7.9%)	1 (4.1%)	0	0	0
M680(G/A)	2 (5.2%)	1 (4.1%)	0	0	0
M694I	2 (5.2%)	1 (4.1%)	0	0	0
P369S	2 (5.2%)	3 (12.5%)	0	0	0
F479L	1 (2.6%)	0	0	0	0
A744S	1 (2.6%)	0	0	0	0
K695R	0	0	0	0	0

*Number of patients with the gene mutation who had a certain complication
** Number of patients with the gene mutation who had a certain complication in relation to the total number of patients who had the complication

In our study group, 36 FMF patients had an echocardiogram, and three of them had abnormal findings. One patient had pericardial effusion, which is considered an FMF complication; the others had accidental findings such as mitral valve regurgitation and mitral valve prolapse. Moreover, we noticed 25 (10%) patients who underwent enodoscopy and colonoscopy, 10 (4%) patients who underwent tonsillectomy and 8 (3.3%) patients who had appendectomy. Those who underwent appendectomy were diagnosed to have appendicitis before surgery**.**

Regarding the study group, 112 patients had a severe clinical course and developed complications (group 1), of which 78 cases (69.6%) had M694V and E148Q gene mutations, while 34 (30.3%) did not have these two mutations. On the other hand, 138 patients had mild clinical courses without complications (group 2), of which 42 (30.4%) had M694V and E148Q gene mutations and 96 (69.6%) did not have these two mutations.

The chi-square test analysis was 38.077 with a P-value of ≤ 0.001, which indicates a clinically significant result and means a significant correlation between the presence of M694V and E148Q gene mutations and the development of complications in FMF patients, as shown in **Table VI**.

Table VI: Outcome of FMF patients in relation to M694V and E18Q gene mutations

	Group1 With Complications N (%)	**Group 2 Without Complications N (%)**	**X^2**	**df**	**P value**	**Phi Effect size**
Presence of M694V or E148Q	78 (69.6)	42 (30.4)	38.077	1	≤0.001	0.39
Absence of M694V or E148Q	34 (30.4)	96 (69.6)				

Discussion

In this study we aimed to analyse the relationship between genotypes and clinical presentations and complications of paediatric FMF patients. Consequently, we studied the 12 most common mutations and related clinical and laboratory results.

Many studies of paediatric FMF have reported female predominance [15, 16, 17]. On the other hand, a study conducted in Egypt by Mansour et al. reported male predominance [5]. However, as our study reported, paediatric FMF studies of different ethnic groups showed that FMF affects both genders similarly, with no statistical significance of gender predominance [18, 19].

Positive family history was found in 30.3% of our study group. A different study in Jordan showed a similar finding with a family history positive for FMF in 29.1% of patients [20]. However, the family history for FMF was found to reach 40.8% in paediatric Lebanese patients. The higher rate of family history was explained by the authors to be caused by the higher rate of consanguinity among their population [18].

The most frequently presenting symptom was found to be abdominal pain followed by fever, which is similar to another study conducted in Turkey by Cecina et al. who declared that abdominal pain (76%) and fever (58%) were the two most frequently presenting symptoms [21]. Another study done in Lebanon showed that abdominal pain was the most frequently presenting symptom (84.7%) followed by fever (78.2%) [18]. Similarly, in a study reported by Arpacı et al. in Turkish patients, the most common clinical symptoms were abdominal pain (97.89%) and fever (92.46%) [6]. In contrast, fever was the most common symptom (97.3 %) in a study conducted by Kilic et al. in Turkey [19].

In our study group, patients with chest pain had the M694V mutation as the most frequently encountered genotype. This was similar to the findings that were reported by Mneimneh et al. and by Kilic et al. [18, 19].

In our study group, eight patients underwent appendectomy (3.3%). This is similar to a Lebanese study, in which 2.5% of their patients experienced appendectomy, and this was due to the severe abdominal pain that resembled appendicitis [18].

We have reported in our study 38 cases with proteinuria, 24 cases with haematuria and one case with IgA nephropathy identified by kidney biopsy, which indicates renal involvement. Regarding related FMF renal complications, Hüzmeli and colleagues reported that 950 FMF patients in the nephrology clinic were shown to have chronic proteinuria. They had nine patients with non-amyloid, IgA nephropathy, membranous glomerulonephritis, mesangioproliferative glomerulonephritis and immune complex glomerulonephritis [22]. In our study, the most frequently encountered gene mutation in FMF patients diagnosed with proteinuria and haematuria was M694V, followed by E148Q, which is similar to a study conducted among Turkish paediatric patients revealing that patients with proteinuria were mostly positive for the M694V genotype mutation and to a lesser extent with the E148Q genotype mutation [19]. This is in contrast to a study by Oskuz et al. in the Southern Marmara region, who demonstrated that there was no important correlation between genotype mutation and the development of chronic renal complications and presenting symptoms like fever, arthritis, arthralgia, abdominal pain and erysipelas-like erythema [9].

Since we did not observe any case of amyloidosis, we could not find any relation between the M694V genotype mutation and the progression into amyloidosis. Similarly a study conducted by Mneimneh et al.

among Lebanese patients demonstrated that no patient progressed into amyloidosis and no relation was found with the M694V gene mutation [18]. Moreover, amyloidosis seems to be rare among Arabs, Ashkenazi Jews and Iraqis of ethnic groups other than Arabs [8].

There were 11.2% patients diagnosed with associated inflammatory and autoimmune diseases, including JIA, HSP vasculitis, thyroiditis and autoimmune hepatitis. Fifty percent of them had the M694V mutation and another 33.3% had the E148Q genotype. In a similar study in Jordan by Alzyoud et al., associated autoimmune diseases constituted about 10.2% of patients, with JIA in 3.1% of patients, HSP in 3.1% of patients, recurrent aphthous stomatitis in 2.6% of patients, Crohn's disease in 1.0% of patients and systemic leukocytoclastic vasculitis in 0.5% of patients [20]. In a Turkish study, some patients were found to have FMF and HSP with either homozygous M694V or E148Q mutations [19]. Another study done by Salah et al. reported that the heterozygous E148Q and V726A mutations were the ones most frequently found in paediatric patients diagnosed with HSP [23]. Like some reports that revealed that HSP and other vasculitis are more frequent in FMF patients in comparison with the general population [22], a similar study done by Abbara et al. among Arabic, Turkish and Jewish origins, found that FMF could be linked to numerous vasculitis disorders, mainly HSP and polyarteritis nodosa (PAN) and the M694V/M694V was genotype as the most frequently encountered mutation, which indicated that the diagnosis of vasculitis disorders can both precede and follow the diagnosis of FMF [24]. In a previous study, some vasculitis disorders, such as HSP, polyarteritis nodosa and Behçet's disease were related to FMF at frequencies of 2.6–5, 0.8–1 and 0.5%, respectively [8]. FMF could be linked to various inflammatory diseases including HSP, polyarteritis nodosa, Behcet's disease and inflammatory bowel diseases [25].

This study affirmed that M694V gene mutations were highly associated with intense clinical presentation and outcome. Similar to other reports done among FMF paediatric patients in Turkey, the first one by Kilic et al. reported that the most frequent gene mutation accompanying the presenting symptoms was the M694V allele [19]. The other study conducted by Barut et al. demonstrated that homozygous M694V gene mutations are the most common mutations that are linked with the most serious symptoms and the worst complications in paediatric Turkish patients [16]. In addition, a study by Bilge et al. reported that the M694V gene mutation was the most frequent pathogenic allele in FMF patients [26]. The second most frequent allele linked to FMF severe presentations and complications is the E148Q gene mutation. A similar result found in Lebanon was that the M694V genotype mutation, accompanied by E148Q genotype to a lesser frequency, had more severe disease, compared to other genotypes [18]. In contrast to Arpacı et al., who reported in their study that the second most frequent gene mutation associated with a mild clinical picture was E148Q, they also declared that the occurrence of surgical procedures because of serious abdominal pain and renal failure was reasonably less in patients carrying the E148Q gene mutation. Consequently, this led to fewer FMF clinic visits in patients carrying the E148Q gene mutation [7].

In our study, we have found that the heterozygous gene mutations were found in 73.6% of patients, compound heterozygous gene mutations were present in 20.8% of patients, and the homozygous gene mutations were found in 5.6% of patients. In a similar study done by Cekin et al., 60% of patients were found to have heterozygous gene mutations, 24.7% of patients were found to have compound heterozygous mutations, 14% of patients were homozygous, and 1.3% of patients had a complex genotype [21].

The most frequently encountered gene mutations in heterozygous, compound heterozygous and homozygous patients were M694V (29.8%), E148Q (24.2%), V726A (18.5%), M680I (G/C) (6.3%), P369S (6%) and M694I (5%). In a similar study done in Jordan, the M694V genotype mutation was the most frequently encountered mutation (30%), followed by 21.5% E148Q, 20% V726A, 9% M6801 G/C, 8.3% M694I, 3.7 % P369S and 3.1% A744S [27]. This is similar to other studies reported on other Arab

populations that also stated that M694V was the most common allele, as in a study conducted in Lebanon by Mneimneh et al. where the frequency of M694V mutation was (37.2%) [18]. In other reports, including Turks, the frequencies of gene mutations were 48% M694V,18% E148Q, 15% M680I, 12.5% V726A, 3.3% P369S, 0.9% R761H, 0.9% K695R, 0.9% E148V and 0.5% A744S [21]. However, in Egyptian patients, the E148Q gene mutation was the most common in the studied group, with a rate of 38.6%, followed by the M694I, V726A and A744S mutations with rates of 18.1, 15.8 and 9.3%, respectively [5].

We have demonstrated that the most frequent allele in heterozygous gene mutation was E148Q (n = 63, 34.2%), and in compound heterozygous gene mutation was M694V-V726A (n = 21, 41%), while in homozygous gene mutation, the most frequent alleles were M694V and V726A, with a percentage of 28.6% for each mutation, and the least common alleles overall were K695R (1%) and R711H (0.3%). This is similar to one study done in Iran by Salehzadeh et al. who demonstrated that the most commonly encountered homozygous gene mutation was M694V (7.1%), the most frequent compound heterozygous gene mutation was M694V-V726A (10.46%), and the most frequent heterozygous gene mutation was E148Q (8.7%), in addition they reported that K695R and F479L were rare mutations with a frequency of 0.2 % [25].

In our study group, we found that 112 FMF patients had severe clinical courses and developed complications (group 1) of which 78 cases (69.6%) had M694V and E148Q gene mutations and 34 (30.4%) did not have these two mutations. Meanwhile, 138 FMF patients had mild clinical courses without complications (group 2), of which 42 (30.4%) had M694V and E148Q gene mutations and 96 (69.6%) did not have these two mutations.

By comparing these two groups, we found a significant correlation between the presence of M694V and E148Q gene mutations and the prediction of progression to severe complications. Chi square of independence showed a statistical significant association between gene mutations and sever complications. $X2(1)=38.077$, $p\leq0.001$, indicating the proportion of having complications in presence gene M694V or E148Q is significantly higher than proportion of having complications in absence of gene M694V or E148Q (69.6% vs. 30.4%) respectively. In addition, the phi coefficient for effect size was calculated and reveled there is a moderate association between two variables (Φ =0.39.) Moreover, by knowing independent variable the proportionate reduction error (PRE) to predict dependent variable is reduced by 39%. Therefore, M694V and E148Q gene mutations may be associated with severe clinical courses and can be considered indicators of more serious complications. In agreement with our study, many international studies have shown the M694V and E148Q genotypes were linked with higher disease severity scores (moderate and severe), which indicates a significant correlation between M694V and E148Q gene mutations in FMF patients and the severe clinical course and the predisposition to serious complications [18].

Conclusion

Our study analysed the correlation between the phenotype and genotype among FMF paediatric patients. M694V and E148Q mutations have been found to be prevalent and may be used as biomarkers for disease severity. These patients are high-risk patients that need treatment that is much more necessary to avoid further complications. Still, the phenotypic-genotypic association of FMF is intricate and multifactorial, we hope that in the future more studies will be done to evaluate the variable factors affecting this correlation in FMF and to focus on the prognosis of the disease in our society.

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