The relation of *MEFV* gene variants to clinical phenotype and selected laboratory markers in Egyptian patients with familial Mediterranean fever

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Background/aim
Familial Mediterranean fever (FMF) is an autoinflammatory disease, with a high prevalence in the Mediterranean region. It is brought out by variants in the *MEFV* gene. The present goal is to describe the demographic, clinical features, and *MEFV* gene variants among Egyptian FMF patients and to explore the relation of *MEFV* variants with clinical features and selected laboratory markers.

Patients and methods
The present study enrolled 302 patients with FMF from both sexes with a mean age 18.01±8.73 years. Patients were recruited from the Clinical Genetic Clinic, Medical Research Centre of Excellence, National Research Centre, Cairo, Egypt, during the period from 2021 to 2023. All patients were subjected to complete history taking, clinical evaluation, and laboratory investigations. C-reactive protein, serum amyloid A (SAA) protein and vitamin D were measured using enzyme-linked immunosorbent assay technique, while erythrocyte sedimentation rate was measured by Westergren method. In addition, *MEFV* genetic variants were investigated using a real-time PCR genotyping assay and direct sequencing of exon 2 and exon 10 of the *MEFV* gene.

Results
The average age of FMF cases was 18.01±8.73 years (with a range between 2 and 34 years), and the female/male ratio was 1.07. The most prevalent symptoms were abdominal pain, fever, and arthritis. Genotyping of the *MEFV* gene demonstrated that 215 (71.2%) patients were heterozygotes, 26 (8.6%) patients were compound heterozygotes and 12 (4.0%) patients were homozygotes, while 49 (16.2%) patients had no detected mutation. p. Met 694Ile was the most common *MEFV* variant (36.7%), followed by p. Met680Ile (21.5%), p.Val726Ala (9.6%), p. Glu148Gln (8.94%), and p.Met694Val (7.94%). There was no significant variation in clinical manifestations between different *MEFV* gene variants. The level of SAA protein was higher in FMF patients carrying the Met694Val variant, while carriers of the p. Glu148Gln variant showed lower erythrocyte sedimentation rate, SAA, and higher serum vitamin D.

Conclusion
The most commonly encountered *MEFV* gene variants among our Egyptian FMF cases were p. Met694Ile followed by p. Met680Ile. No phenotype-genotype association was observed. The p. Met694Val variant could be a possible risk factor for developing amyloidosis. Investigating the whole *MEFV* gene is recommended to fully understand the molecular background of FMF cases and properly establish a good correlation with the variable phenotypes.

Keywords:
familial Mediterranean fever, genotype, MEFV mutations, phenotype

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Introduction

Familial Mediterranean fever (FMF) is an autoinflammatory disease with a high prevalence (around 1/500 inhabitants) among people in the Mediterranean basin, such as Turks, Armenians, and Arabs [1]. It is characterized by repeated attacks of fever and recurrent serositis affecting the peritoneum, pleura, and joints leading to amyloidosis, which can progress to renal failure [2]. Colchicine is the gold standard of treatment, reducing the attack frequency and amyloidosis risk [3]. Mediterranean fever is related

to variants within the familial Mediterranean fever (*MEFV*) gene encoding the pyrin (or marenostrin protein), a cytosolic protein of activated monocytes, neutrophils, and eosinophils [4]. Cytokines are essential players in the inflammation of the serous membranes. They stimulate the production of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum amyloid A (SAA) [5,6].

The *MEFV* gene is located in the 16p13.3 region containing 10 exons. Exons 2 and 10 turn out to be two hotspot regions for mutations, with exon 10 carrying the majority of them. Among the 370 identified *MEFV* variants, p.Met680Ile, p. Met694Val, p.Met694Ile, p.Val726Ala in exon 10 and the p.Glu148Gln in exon 2 are present with a frequency of 80% in FMF cases [7,8].

Although some studies have shown an association between the p.Met694Val variant and a severe FMF phenotype [9], no evidence of genotype-phenotypic correlation has been confirmed, making more challenging the patient's management [10–12]. The analysis of the typical FMF cases showed an autosomal recessive model of inheritance. The disease can segregate either in a homozygous or a compound heterozygous modality. However, it has been noticed that a substantial number of cases with clinical phenotype of FMF are either heterozygous or carry no *MEFV* mutation [13].

Herein, the present study intends to describe the demographic, clinical features, and *MEFV* gene variants among Egyptian cases with FMF and to determine the relation of the identified *MEFV* variants to clinical features and laboratory findings.

Patients and methods

Patients

This cross–sectional study enrolled 302 patients with FMF who attended the Clinical Genetic Clinic at Medical Research Centre of Excellence of the National Research Centre (NRC), Egypt, during the period from 2021 to 2023.

Inclusion criteria

The study included FMF patients of both sexes with ages ranging from 2 to 34 years, clinically diagnosed according to Tel-Hashomer criteria [14]. The major criteria are: 1) recurrent febrile episodes accompanied by peritonitis, synovitis, or pleuritis; 2) AA amyloidosis without predisposing disease; and 3) favorable response to colchicine treatment. Minor criteria are: 1) recurrent febrile episodes; 2) erysipelas-like erythema; and 3) FMF in a first-degree relative. Two major criteria or one major plus two minor criteria indicated the definite diagnosis of FMF.

Exclusion criteria

Patients with a history of infection, and pre-existing medical conditions, including heart disorders, chronic lung diseases, hepatic injury, and diabetes, were excluded from the study.

Ethical approval

The present research was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki, 1975. This study has been approved by the local Ethics Committee of the National Research Centre, Cairo, Egypt with approval number 054190923. A written informed consent was provided by all participants and/or their parents prior to inclusion in this study.

Methods

All participants were subjected to complete history taking and thorough clinical examination. ESR, CRP, SAA proteins, and vitamin D were measured for all participants. Moreover, the genetic analysis of the *MEFV* gene was also performed.

Clinical investigation

The clinical evaluation of FMF individuals was established following detailed history taking, pedigree analysis, and thorough clinical examination. The demographic and clinical data included age, gender, consanguinity, and family history. Clinical characteristics, such as fever, abdominal manifestations, chest manifestations, arthritis, arthralgia, skin and renal manifestations, were also obtained from each participant. In addition, the frequency of attacks/month as well as the doses of colchicine were recorded.

Laboratory investigations:

Five ml of venous blood was withdrawn from each case, and divided into three tubes, 2 ml blood in Na-citrate tubes for ESR measurement, 2 ml for serum laboratory investigations comprising CRP, SAA, and vitamin D. The remaining blood was added to EDTA tube for genetic analysis of the *MEFV* gene.

Quantitative measurement of CRP, SAA protein, and vitamin D was done by enzyme-linked immunosorbent assay, using kits supplied by Elabscience Co. (USA), according to the manufacturer's protocols in

each kit. Vitamin D deficiency was defined as serum 25-hydroxyvitamin D levels less than 20 ng/ml [15]. Additionally, ESR was assessed manually by the Westergren method [16].

Genetic analysis of the *MEFV* gene

DNA was obtained from blood samples collected on EDTA using ZYMO DNA extraction miniprep (Willfort, USA) following the manufacturer's instructions. DNA concentration and purity were evaluated by a Thermo Scientific NanoDrop spectrophotometer (Wilmington, USA). Detection of *MEFV* gene variants was performed using two methods, as follows:

Real-Time PCR based SNP genotyping assay

The FMF real-time PCR Genotyping Kit, followed by melting curve analysis (DNA Technology, Russia), was used to identify the thirteen common gene variants of the *MEFV* gene: p.Glu148Gln (c.442 G/C) in exon 2, p.Pro369Ser (c.1105 C/T) and p.Arg408Gln (c.1223 G/A) in exon 3, p.Phe479Leu (c.1437C/G) in exon 5, p.Met680Ile (c.2040 G/A), p.Met680Ile (c.2040 G/C), p.Ile692del (c.2076_2078del), p.Met694Val (c.2080 A/G), p.Met694Ile (c.2082 G/A), p.Lys695Arg (c.2084 A/G), p.Val726Ala (c.2177 T/C), p.Ala744Ser (c.2230 G/T), and p.Arg761His (c.2282 G/A) exon 10.

For each variant, a total volume of 35 µl contains 20 µl of PCR mix, 10 µl of PCR buffer with Taq AT polymerase, and 5 µl of DNA (1 ng). Real-time PCR (Light Cycler 480, Roche, Germany) thermal conditions were as follows: the deactivation of Taq polymerase at 80°C for 2 min, denaturation at 94°C for 5 min, and 45 cycles (94°C for 30 s, 67°C for 15 s), the temperature was then reduced to 25°C and further increased gradually to 75°C, and the melting temperature (Tm) of Fam (wild allele) and Hex (mutant allele) was then estimated (Fig. 1).

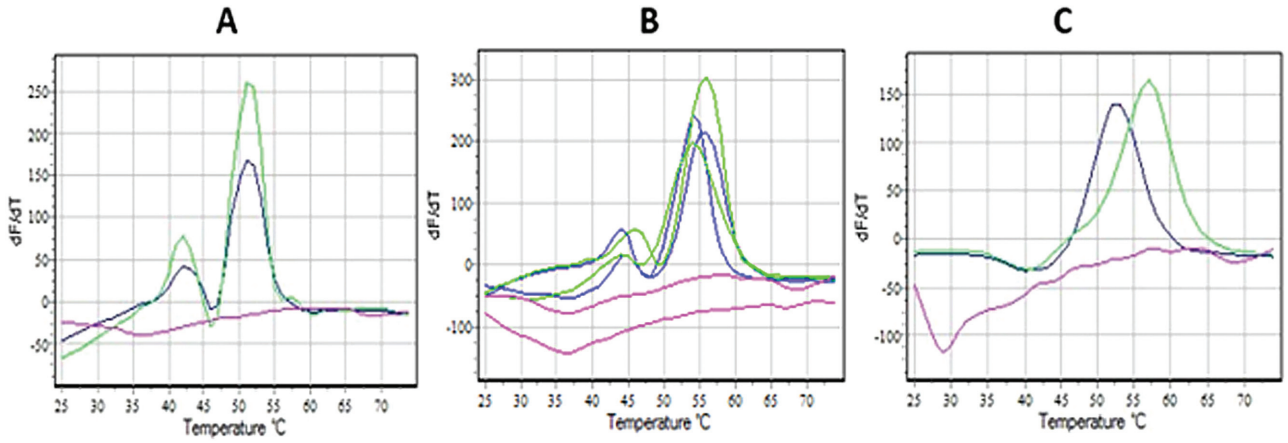
Sanger sequencing

PCR amplification was done using designed primers to amplify exon 2 and 10 under the following conditions: initial denaturation at 94°C, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and final extension at 72°C for 5 min. Primer sequences were as follows F: 5' CTA AAC GTG GGA CAG CTT CAT C 3' and R: 5' CTT CCT TCA GGT CCG CAG AT 3' for exon 2 and F: 5' CAG AAA CCG CTT ACC CCA AC 3' and R: 5'GCC AGA AGC AGG AAG AGA GAT G 3' for exon 10. Purification of the PCR products was done utilizing thermo sensitive Exonuclease I and Shrimp Alkaline Phosphatase (EXO-rSAP) (Neb, USA). Purified samples were then sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit on an ABI Prism 3500 XL Analyzer (Applied Biosystems, USA), following the manufacturer's protocols. The resultant sequences were then compared with the wild-type of gene sequence using a blasting procedure at the URL https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Statistical analysis

Data were analyzed using Microsoft Excel 2016 and the statistical software IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, New York, USA). Quantitative data were presented as mean±SD and ANOVA test was used to compare between more than two groups with parametric data, while qualitative variables were presented as number

Figure 1



Analysis of variants by real-time PCR and melting curve according to Tm of Fam (blue) and Hex (green). (a): Heterozygous in p.Val726Ala mutation. (b): compound heterozygous in p.Met694Ile and p.Met680Ile mutations. (c): Homozygous p.Met694Ile mutation.

and percent, and χ^2 test was used to compare the significant variance between more than two categorical variables. *P* value less than 0.05, was considered significant.

Results

Clinical and laboratory results

Demographic, clinical manifestations and laboratory data are presented in Table 1. A total of 302 FMF cases were studied. There were 156 (51.7%) females and 146 (48.3%) males (F/M= 1.07). Their ages ranged from 2 to 34 years (18.01±8.73). Abdominal pain was the most prevalent symptom (96.7%), followed by fever (87.1%), and the least common manifestation was erysipelas-like erythema (20.2%). Among the 302 patients, 253 (83.8%) carried one or two *MEFV* gene mutations while, 49 (16.2%) were negative for the screened variants. All participants were treated with colchicine (0.5–2 mg/day), with 20% of cases having colchicine resistance.

Genetic analysis of the *MEFV* gene

MEFV gene variants of the examined FMF participants are listed in Table 2. Sequencing results of commonly encountered mutations are illustrated in Fig. 2.

Association of *MEFV* gene variants with clinical and laboratory data

We assessed the genotype/phenotype association among patients carrying p.Met694Ile, p.Met680Ile, p.Val726Ala, p.Glu148Gln, and p.Met694Val mutant variants, as they were the most prevalent mutations among the studied patients. The results did not reveal any significant variance as regards the prevalence of clinical manifestations or frequency of inflammatory attacks between the five most common mutations (Table 3). As regards SAA and vitamin D, SAA was highest among patients with the p. Met694Val variant. However, patients carrying the p.Glu148Gln genotype showed the lowest ESR, median SAA, and the highest median serum vitamin D (Table 4).

Table 1 Demographic, clinical, and laboratory data of the examined Familial Mediterranean fever cases

	FMF cases (*n*=302)
Demographic	
Age (years) [$]	18.01±8.73
Sex: Males [#]	146 (48.3)
Females [#]	156 (51.7)
Consanguinity [#]	175 (57.9)
Family history of FMF [#]	139 (46.0)
Clinical Presentation [#]	
Abdominal pain	292 (96.7)
Fever	263(87.1)
Arthritis	224 (74.2)
Vomiting	190 (62.9)
Diarrhea	152 (50.3)
Constipation	61 (20.2)
Erysipelas-like erythema	57 (18.9)
Chest Pain	20 (6.6)
Laboratory data [$]	
CRP (mg/dl)	29.47±10.50
ESR (mm/1st hour)	32.04±9.46
Serum Amyloid A (U/L)	68.81±22.24
Vitamin D (ng/ml)	15.38±5.48

[#]:Data are represented as number (N) and percentage (%). [$]: Data are represented as Mean±SD.

Table 2 Genotyping of *MEFV* gene variants in Familial Mediterranean fever cases

	FMF cases (*n*=302)
MEFV gene variants	
p.Met694Ile	111 (36.7)
p.Met680Ile (c.2040 G>A or G>C)	65 (21.5)
p.Val726Ala	29 (9.6)
p.Glu148Gln	27 (8.94)
p.Met694Val	24 (7.94)
p.Ala744Ser	7 (2.3)
p.Ile692del	3 (1.0)
p.Pro369Ser	3 (1.0)
p.Arg408Gln	2 (0.7)
p.Lys695Arg	2 (0.7)
p.Phe479Leu	1 (0.3)
p.Arg761His	1 (0.3)
Mutational Alleles	
No detected mutation	49 (16.2)
Heterozygous	215 (71.2)
Compound heterozygous	26 (8.6)
Homozygous	12 (4.0)

All Data are represented as number (N) and percentage (%).

Association of the number of mutant alleles with clinical and laboratory findings

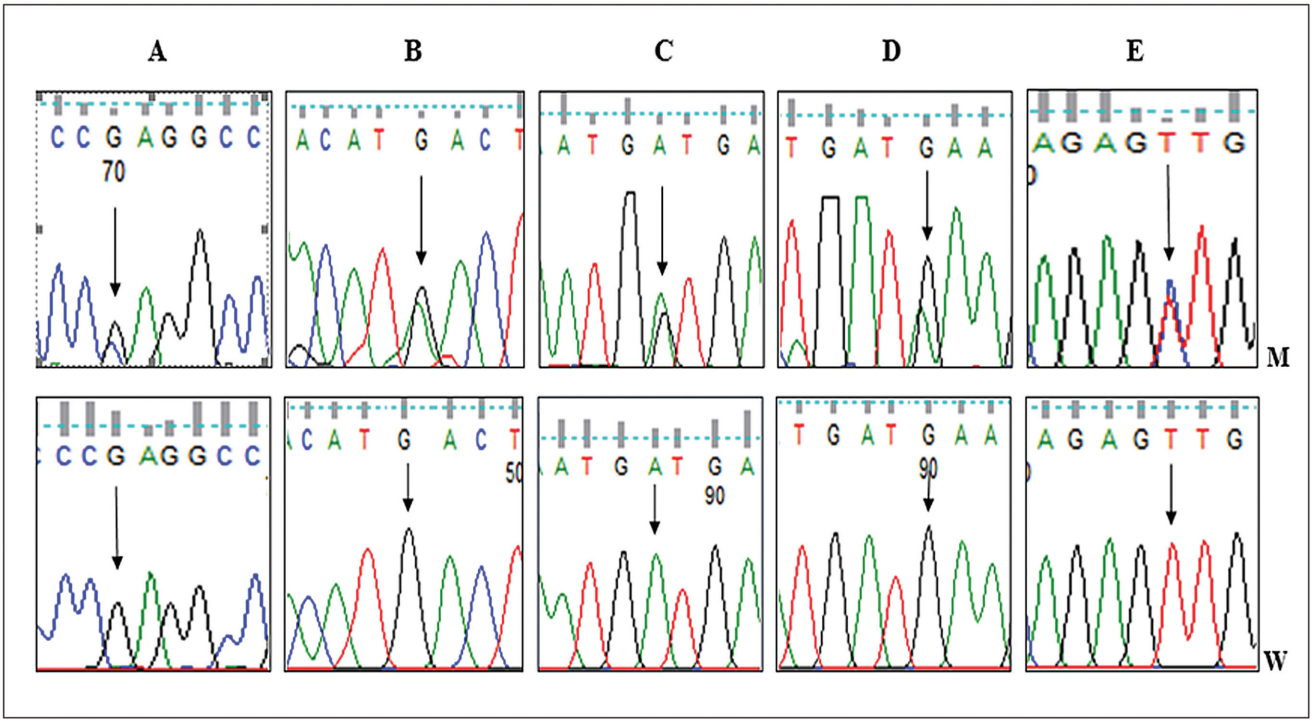
Regarding the number of mutant alleles among the 302 studied FMF patients, 215 (71.2%) patients were heterozygous, 12 (4%) patients were homozygous, 26 (8.6%) patients were compound heterozygous, and 49 (16.2%) patients were negative for the studied mutations. No significant association was found between the number of mutant alleles and clinical manifestations (Table 5).

In addition, we assessed the relationship between different laboratory findings with the number mutant alleles, and we found that CRP, SAA, and serum vitamin D were statistically higher in cases with compound heterozygosity (Table 6).

Discussion

FMF is a common auto-inflammatory condition affecting mainly the Eastern Mediterranean area,

Figure 2



Partial nucleotide sequence of the *MEFV* gene showing A: p.Glu148Gln (c.442 G>C) variant in exon 2. B: p.Met680Ile (c.2040 G>A) variant in exon 10. C: p. Met694Val (c.2080 A>G) variant in exon 10. D: Met694Ile (c.2082 G>A) variant in exon 10. E: p. Val726Ala (c.2177 T>C) variant in exon 10 (M for mutant and W for wild).

related to variants in the *MEFV* gene found on chromosome 16p13.3. According to Mediterranean studies, the most commonly encountered genetic *MEFV* alleles in FMF are p.Met680Ile, p. Met694Val, p.Met694Ile, p.Val726Ala, in exon 10 and the p.Glu148Gln in exon 2, which account for about 80% of FMF mutations [7,8].

There is a discrepancy in the distribution of the *MEFV* mutations between countries and even within the same country. This discrepancy may be related to geographic distribution, racial differences, sample size, and ethnic group. In the current investigation, we studied the *MEFV* mutational profile and the diverse clinical presentations in a cohort of 302 Egyptian patients with FMF. We found that p.Met694Ile (36.7%) is the commonest *MEFV* genetic variant, followed by p. Met680Ile (21.5%), p.Val726Ala (9.6%), p. Glu148Gln (8.94%), and p.Met694Val (7.94%). A previous study from Egypt reported that the commonest genetic variant was p.Val726Ala, which represented 41.2%, followed by p.Met694Val (32.4%), p.Met680Ile (29.4%), p.Glu148Gln (25%), and p.Met694Ile (20.6%) [17], another study in upper and lower Egypt, stated that the commonest variant was p.Glu148Gln (38%), followed by p.Met694Ile (18.1%), p.Val726Ala, (15.8%), and p.Ala744Ser (9.3%) [18].

Table 3 Association of clinical investigations with different *MEFV* gene variants

	p.Met694Ile (*n*=111) *N* (%)	p.Met680Ile (*n*=65) *N* (%)	p.Val726Ala (*n*=29) *N* (%)	p.Glu148Gln (*n*=27) *N* (%)	p.Met694Val (*n*=24) *N* (%)	None (*n*=49) *N* (%)	*P* value
Abdominal pain	107 (96.4)	63 (96.9)	28 (96.6)	26 (96.3)	22 (91.7)	48 (98.0)	0.730^{+}
Diarrhea	50 (45)	41 (63.1)	15 (51.7)	17 (63)	11 (45.8)	22 (44.9)	0.106^{+}
Constipation	21 (18.9)	13 (20)	6 (20.7)	3 (11.1)	5 (20.8)	9 (18.4)	0.882^{+}
Vomiting	71 (64)	38 (58.5)	23 (79.3)	16 (59.3)	11 (45.8)	26 (53.1)	0.082^{+}
Arthritis	86 (77.5)	51 (78.5)	22 (75.9)	17 (63)	13 (54.2)	37 (75.5)	0.091^{+}
Fever	97 (87.4)	57 (87.7)	26 (89.7)	24 (88.9)	19 (79.2)	43 (87.8)	0.802^{+}
Erysipelas-like erythema	26 (23.4)	13 (20)	6 (20.7)	3 (11.1)	5 (20.8)	10 (21.4)	0.728^{+}
Frequency of attacks/ month: Mean±SD	5.9±2.6	4.2±2.5	3.8±1.7	5.7±2.3	4.9±1.8	3.7±1.5	0.293^{++}

$^{+}$: Data are represented as number (N) and percentage (%) and insignificantly difference at *P* value greater than 0.05 using Chi-Square test.
$^{++}$: Data are represented as mean±SD and insignificantly difference at *P* value greater than 0.05 using ANOVA test.

Table 4 Association between laboratory findings and different *MEFV* gene variants

Laboratory Parameters	p. Met694Ile (*n*=98)	p. Met680Ile (*n*=53)	P. Val726Ala (*n*=16)	p.Glu148Gln (*n*=21)	p. Met694Val (*n*=19)
Serum Amyloid A (U/L)	63.25±19.51[a]	70.11±28.82[a]	62.49± 22.55[a]	47.82±18.38[b]	102.32±32.58[c]
Vitamin D (ng/ml)	14.19±5.09[a]	14.51±5.32[a]	18.83±4.87[b]	19.36±3.23[b]	15.24±6.74[a]
CRP (mg/dl)	23.92±10.76[a]	29.41± 9.75[a]	26.18±12.26[a]	34.90±19.66[b]	40.51±11.23[c]
ESR (mm/h)	30.27±12.62[a]	38.9±10.71[a]	33.95± 11.74[a]	16.8±7.00[b]	19.3±6.60[b]

All Data are represented as mean±SD. Data with different superscript letter (a, b, c, d) within the same raw are significantly changed at *P* value less than 0.05, using ANOVA test.

Table 5 Clinical manifestations in regarding to the number of *MEFV* gene mutant alleles

No. of mutations	Abdominal pain *N* (%)	Diarrhea *N* (%)	Constipation *N* (%)	Vomiting *N* (%)	Arthritis *N* (%)	Fever *N* (%)	Erysipelas-like erythema *N* (%)	Chest pain *N* (%)
No mutation (*n*=49)	48 (98)	22 (44.9)	9 (18.4)	26 (53.1)	37(75.5)	43 (87.8)	10 (20.4)	25 (51.0)
One mutant allele (*n*=215)	207 (96.3)	110 (50.2)	42 (19.5)	141 (65.6)	163 (75.8)	189 (87.9)	44 (20.5)	119 (55.3)
Two Mutant alleles (*n*=38)	37 (97.4)	20 (52.6)	6 (15.8)	23 (60.5)	24 (63.2)	31 (81.57)	7 (18.4)	16 (42.1)
**P* value	0.536	0.343	0.891	0.250	0.791	0.836	0.951	0.394

All data are represented as number (N) and percentage (%). *: Insignificant difference at *P* value greater than 0.*05*, using Chi square test.

Table 6 Association of serum amyloid A protein, vitamin D, and erythrocyte sedimentation rate levels with the number of mutant *MEFV* gene alleles

MEFV gene alleles	Serum Amyloid A (U/L)	Vitamin D (ng/ml)	CRP (mg/dl)	ESR (mm/h)
No mutation (*n*=49)	39.94±17.91[a]	13.18±3.458[a]	25.52±10.85[a]	26.8±11.18[a]
Heterozygous (*n*=215)	52.62±20.52[b]	15.3±5.50[a]	28.88±13.89[a]	29.01±15.50[a]
Homozygous (*n*=12)	44.37±14.33[a]	13.2±5.2 0[a]	29.16±6.08[a]	28±0.50±6.36[a]
Compound heterozygous (*n*=26)	107.71±30.13[c]	24.42±5.62[b]	55.28±19.98[b]	15.46±6.38[b]

All Data are represented as mean±SD. Data with different superscript letter (a, b, c, d) within the same column are significantly changed at *P* value less than 0.05, using ANOVA test.

In accordance with our study, Belmahi and colleagues [19] reported that p.Met694Ile was the commonest pathogenic variant in Algerian Arabs, as it accounted for 80% of *MEFV* mutations, while in Moroccans and Tunisians; p.Met694Val was the commonest mutation, accounting for 49% and 50%, respectively [19]. In Jordan, the commonest variant was p.Met694Val, followed by p.Val726Ala and p.Met694Ile [20]. Additionally, a study in Jordan and Lebanon revealed that p.Met694Val and p.Met694Ile were the most common pathogenic variants [21]. In the Easter region of Turkey, Iran, and Amsterdam, p.Met694Val was the most frequently reported variant [22,23]. However, in the Mediterranean area of Turkey, p. Arg202Gln was the commonest variant (21.35%) [24].

The overall female/male ratio of our studied FMF participants was 1.07. Some researchers mentioned a higher incidence of FMF in males, while others reported a similar female/male ratio [25,26]. No significant difference was observed between males and females as regards gene variants or clinical manifestations which were explained by the autosomal recessive pattern of inheritance.

Abdominal pain and fever are the commonest clinical manifestations of FMF in Middle Eastern regions [17,27]. However, in the Japanese population, abdominal pain was less common (54.5%) [28]. In our research, abdominal pain was recorded in 96.7% of FMF patients followed by fever in 87.1%, and arthritis in 74.2%. On the other hand, a previous Egyptian survey noted that chest pain was the third most frequently occurring manifestation [29]. In Lebanon, the frequency of arthritis was 20%, which is lower than in our cohort [8].

The genotype/phenotype association in FMF was studied by several investigators; however, no clear consensus has been reached. In our cases, no significant genotype-phenotype association was found. In addition, no significant relation was identified between clinical phenotypes and the number of mutant alleles. In contrast, a previous Egyptian study involving 500 pediatric FMF cases observed that the phenotypes were influenced by the different genotypes [30]. The varied age groups examined in the two studies may be the cause of the disparity from our results.

Our results also suggested that nongenomic factors could affect the clinical phenotype of the disease such as epigenetic and environmental factors [31–33]. Among the epigenetic changes, miRNAs have been examined in many recent studies [34,35] and they have emerged as crucial players in several

processes implicated in FMF pathogenesis as apoptosis, inflammation and autophagy [36]. Additionally, the environmental circumstances as the microbial agents may impact the final disease expression, as stated by Ozen *et al.* [32]. They also hypothesized that other factors, including the feeding pattern or living conditions, could be considered.

Amyloidosis is the major consequence of FMF and SAA may have a pathogenic function in modulating the NALP3 inflammasome activity along with predicting the clinical course of AA amyloidosis [37]. In the present research, the median level of SAA was greater among cases with the p.Met694Val variant and the cases with compound heterozygosity. Most of the studies mentioned that p.Met694Val is the most commonly reported variant associated with amyloidosis [38–40]. Additionally, Kaşifoğlu and colleagues [41] reported in their study a six-fold increase in the risk of amyloidosis among FMF subjects carrying the p.Met694Val variant.

Comparing vitamin D between different genotypes revealed a higher median vitamin D among patients with the p.Glu148Gln genotype. In Onur and colleagues' study, patients with FMF showed lower vitamin D levels than healthy controls, with no significant variation between different *MEFV* mutations [42]. Vitamin D lack could worsen the clinical symptoms of FMF and colchicine response as mentioned by Kazem *et al.* [43]. Vitamin D has been considered an immunomodulatory as most immune cells carry vitamin D receptors and it decreases the expression of Toll-like receptors in human monocytes. Moreover, vitamin D is essential for the proliferation, differentiation, and maturation of cells in the immune system [42,44]. To date, it is not clarified whether the alteration of vitamin D is a cause or effect of FMF.

We observed in our cohort that cases with the p.Glu148Gln variant, known to be associated with mild FMF phenotype [45], having lower ESR, SAA levels, and higher vitamin D levels. Although several studies considered the p.Glu148Gln unlikely to cause the disease [46,47], we instead suggested either incomplete variant penetrance or even a modifying effect of other variants in the *MEFV* gene or other unexplored genes.

However, in our investigation, most of our patients with the p.Glu148Gln variants had mild clinical symptoms and responded to colchicine therapy, 81% of them were heterozygous. A recently published study indicated that Armenian patients with single or double p.Glu148Gln mutant alleles manifested clinically with FMF symptoms [48].

Conclusion

The most prevalent *MEFV* gene variants among the examined Egyptian FMF patients were p.Met694Ile (36.7%), followed by p.Met680Ile (21.5%), Val726Ala (9.6%), p.Glu148Gln (8.94%), and p.Met694Val (7.94%). There was no phenotype-genotype association in our cases. SAA was highest in patients with the p.Met694Val variant displaying its possible role as a genetic risk factor for developing amyloidosis, it was also noticed that the carriers of the p.Glu148Gln variant had the highest serum vitamin D and the lowest ESR and SAA implying its benign course. Further studies are recommended to complete the genetic profile of FMF patients by sequencing of the whole *MEFV* gene, followed by whole exome sequencing for negative cases, to properly establish a genotype/phenotype correlation and patient's management.

Author contributions: H.T.E: Study design, reviewing, and final manuscript proofing. G.N.E: Performing the molecular methodology. M.F.S: Performing the molecular methodology and manuscript preparation. M.B.T and M.M.S-A: Patient clinical examination and blood samples' collection. I.I.S: statistical analysis and writing the initial draft of the manuscript. H.M.R: Study design, reviewing, and final manuscript proofing. All authors revised and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

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