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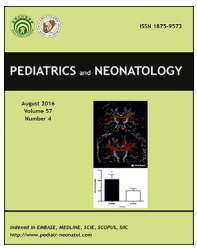

Original Article

# Genotype-phenotype correlation in Jordanian children with genetically-proven familial Mediterranean fever: The effect of R202Q mutation

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**Key Words**
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*Background:* Familial Mediterranean fever (FMF) is a hereditary periodic fever syndrome inherited as an autosomal recessive pattern; nonetheless, patients with symptomatic heterozygous variants exist. This study aimed to review children with genetically-proven FMF, to describe their mutation maps and clinical characteristics, and to explore the genotype—phenotype correlation.

*Methods:* Medical charts of pediatric FMF patients who were diagnosed by both genetic mutation and clinical criteria and followed up at our hospital were reviewed. Demographic and clinical data, results of *MEFV* genetic testing, procedures, concomitant medical conditions, disease severity, and treatment response were recorded and analyzed.

*Results:* A total of 132 patients (71 females [54%]) were included in the final analysis. The average ages at presentation and diagnosis were 6.2 ± 3.1 and 7.6 ± 4.4 years, respectively. The most common clinical features were abdominal pain (n = 120, 91%), fever (n = 97, 73.5%), and arthritis (n = 75, 56.2%). Gastrointestinal endoscopy was the most frequently reported procedure (n = 27, 20.45%). The most common mutation was R202Q (n = 71, 53.8%), followed by E148Q (n = 36, 27.3%), M694V (n = 30, 22.7%), and V726A (n = 22, 16.7%). Two rare variants with potential pathogenicity were identified—namely, c.-15 and c.-330. A novel *MEFV* mutation (p. Lys629 Met) was noted. Abdominal pain, arthritis, arthralgia, and skin rashes were more common with the R202Q mutation. Patients with compound heterozygous

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mutations showed a higher rate of abdominal pain (94.1%) and exhibited the best response to colchicine (67.6%). Patients with complex alleles had the highest rate of fever (80%) and arthritis/arthralgia (70%).
*Conclusion:* FMF is endemic in Jordan. Genetic testing is important in FMF evaluation; however, the genotype–phenotype correlation needs further study. The R202Q mutation is possibly pathogenic and is associated with the manifestation of the full spectrum of FMF features; hence, it needs to be considered in the diagnosis of FMF patients in Jordan.


## 1. Introduction

Familial Mediterranean fever (FMF) is a common hereditary periodic fever syndrome. FMF is a monogenic autoinflammatory disease secondary to *MEFV* gene mutations on chromosome 16p13, which encodes the pyrin protein. Pyrin is a regulator of inflammation and cytokine production (e.g., IL-1β).[1]

FMF has an autosomal recessive pattern of inheritance; nonetheless, symptomatic patients with heterozygous mutations have been reported.[2,3] For these reasons, diagnostic criteria have been developed, including the Tel Hashomer Hospital criteria, Turkish pediatric FMF criteria, and PRINTO clinical classification criteria for autoinflammatory periodic fevers.[4]

Populations originating from the Mediterranean basin such as Sephardic Jews, Armenians, Turks, and Arabs have the highest incidence of FMF. However, FMF is being increasingly recognized worldwide.[3]

Clinically, FMF is characterized by recurrent self-limited attacks of fever, aseptic serositis, joint pain, aphthous changes in the lips and/or oral mucosa, and erythema. Attacks are also associated with increased erythrocyte sedimentation rate (ESR) and elevated levels of C-reactive protein (CRP), serum amyloid A, leukocytes, and neutrophils. Moreover, these attacks may have different severities in the same patient, with symptom-free intervals of different lengths. Symptoms frequently start prior to 20 years of age. The clinical phenotype is usually more severe in patients with precocious attack onsets. FMF attacks can be triggered by infections, stress, menses, cold exposure, fat-rich foods, and some drugs.[2,3,5]

Atypical presentations seem to play an essential role in misdiagnosis and delayed diagnosis.[2] The most important complication of FMF is renal amyloidosis, leading to renal failure.[6]

Colchicine is the standard FMF treatment; however, anti-IL-1β agents are used if colchicine resistance or intolerance occurs.[2]

Notably, FMF is associated with an increased risk of Behçet disease, ankylosing spondylitis, psoriasis, Crohn disease, and ulcerative colitis (UC), indicating that the tissue-specific dysregulation of innate immunity shared between FMF and spondyloarthritis spectrum disorders may influence adaptive immune MHC class I-associated conditions.[2,3]

The FMF genotype has been studied in the Arab population, with M694V and V726A having been identified as the most common mutations, followed by M694I, M680I, and E148Q.[7] The genotypes M694V/M694V and M694V/V726A have a severe clinical course in Arab FMF patients, whereas M694I/M694I are associated with mild disease.[8] The M694V mutation is associated with the development of amyloidosis and protracted febrile myalgia syndrome.[9]

The majority of the reported literature from our area examined mutations using customized mutation testing and described the clinical characteristics. This study aimed to review the phenotype of all children diagnosed with FMF between January 2015 and January 2020 in our facility using Sanger DNA sequencing of the 10 exons of the *MEFV* gene. Further, we attempted to explore the genotype–phenotype correlation in our study population.

## 2. Methods

### 2.1. Patient identification

This was a cross-sectional retrospective study. The laboratory database for all FMF genotype tests conducted at a tertiary hospital between January 2015 and January 2020 was reviewed. All patients with *MEFV* mutations were identified, and their electronic hospital and clinic records were reviewed. Patients who met the clinical criteria for diagnosis (Turkish FMF criteria/Tel Hashomer Hospital criteria) were included. The diagnostic criteria for FMF comprised two or more of the following: ≥3 attacks with 6–72-h duration of fever, abdominal pain, chest pain, oligoarthritis, and family history of FMF.[10] Disease severity was determined according to the criteria by Pras et al., including age of onset, frequency of attacks on admission, severity of arthritis, presence of erythema, and requirement for colchicine.[11]

### 2.2. Genetic testing

From each patient, 3 mL of peripheral venous blood was collected in anticoagulant test tubes. DNA extraction from blood samples was performed using the Gentra Puregene DNA extraction kit (Qiagen, Valencia, CA, USA). The 10 coding exons of the *MEFV* gene were amplified by polymerase chain reaction (PCR). Forward and reverse primers were designed using Primer3 (http://frodo.wi.mit.edu/primer3) and encompassed exon–intron junctions in accordance with NCBI reference sequences for the *MEFV*

gene (NG_007871.1) and *MEFV* cDNA (NM_000243.3). The PCR conditions for the 10 exons were as follows: initial denaturation at 95 °C for 10 min; additional 35 cycles at 95 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were visualized with 1% agarose gel electrophoresis. Thereafter, the PCR products were purified and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit on a 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were compared to reference sequences using ChromasPro 1.34 (Technelysium Pty. Ltd., Australia). Mutations and variants were designated according to the Human Genome Variation Society guidelines (https://www.hgvs.org/mutnomen).

### 2.3. Data collection

Demographic data, including age of onset, age of diagnosis, sex, family history, and presenting symptoms, were collected. The primary clinical data (e.g., fever, abdominal, joint, and chest pain, and erysipelas-like erythema) were also recorded. Results of the *MEFV* genetic testing, interventions or surgical procedures, concomitant medical conditions, and treatment response were recorded. The severity score was calculated according to the criteria by Pras et al. We excluded patients who did not meet the FMF diagnostic criteria or those with unavailable data (followed up outside our facility).

### 2.4. Statistical analysis

Data were collected on a pre-prepared spreadsheet. Data were analyzed using SPSS for Windows version 20.0 (IBM Corp., Armonk, NY, USA). The results were expressed as mean ± standard deviation for continuous variables and as frequency and percentages for categorical variables. Continuous and categorical data were analyzed using the t-test and chi-square test, respectively.

### 2.5. Ethics

This study was conducted in accordance with the principles embodied in the Declaration of Helsinki and was approved by the IRB committee of the Faculty of Medicine and Research Committee of the Jordan University of Science and Technology (approval no. 20190354). The requirement for the acquisition of informed consent from patients was waived owing to the retrospective nature of this study.

## 3. Results

A total of 234 patients were confirmed to have *MEFV* mutations by sequencing analysis. We excluded 39 outside referrals (no clinical data) and 63 patients with insufficient data or failure who did not meet the diagnostic criteria. Thus, 132 patients were finally included.

### 3.1. Demographics

Out of the 132 patients, 71 (54%) were female. The average ages at presentation and diagnosis were 6.2 ± 3.1 and 7.6 ± 4.4 years, respectively. Furthermore, 49 (37.1%) patients had a family history of FMF. The average time between symptom onset and diagnosis was 9.8 months (Table 1).

### 3.2. Clinical manifestations and laboratory findings

The most common clinical features were abdominal pain (n = 120, 91%), fever (n = 97, 73.5%), and arthritis (swollen painful joints with movement limitations) or arthralgia (painful joints in the absence of swelling) (n = 75, 56.2%). Forty (30.0%) patients reported chest pain. Classical erysipelas-like erythema occurred in 17 (12.9%) patients, whereas relapsing Henoch–Schönlein purpura was detected in two (1.5%) patients. Two patients had recurrent seronegative meningitis.

Elevated levels of inflammatory markers (ESR, CRP) were observed in 71 (53.8%) patients; specifically, ESR alone was elevated in 8 patients, CRP alone was increased in 21 patients, and both were elevated in 42 patients. Levels of autoimmune antibodies (antinuclear antibody and rheumatoid factor) were elevated in 15 (11.4%) patients (two patients with positive rheumatoid factor and 13 patients with positive antinuclear antibody). Proteinuria was detected in 16 (12.1%) patients, and two (1.5%) developed nephrotic-range proteinuria. Seven (5.3%) patients showed abnormal flow cytometry results, and five (3.78%) had elevated serum IgD levels (Table 2).

### 3.3. Procedures

Gastrointestinal endoscopy was the most common procedure in our patients. Of 27 patients (20.45%), 17 were diagnosed with gastrointestinal pathology; 12 (9%), with *Helicobacter pylori* gastritis; five (3.78%), with nonspecific gastritis; four (3%), with inflammatory bowel disease (IBD); and one (0.75%), with biopsy-proven celiac disease. Four patients underwent renal biopsy, and one was noted with renal amyloidosis. The most common surgery was appendicectomy (n = 5, 3.78%), followed by cholecystectomy

**Table 1** Demographic data of patients (n = 132).

| Characteristic | Number (n) | Percentage (%) |
|---|---|---|
| Male | 61 | 46 |
| Female | 71 | 54 |
| Average age at presentation of symptoms | 6.2 ± 3.1 years | |
| Average age at diagnosis | 7.6 ± 4.4 years | |
| Confirmed family history of FMF | 49/132 | 37.12 |

(n = 4, 3.8%), splenectomy (n = 2, 1.5%), and adenoidectomy/tonsillectomy (n = 3, 2.0%) (Table 2).

### 3.4. Colchicine use

Colchicine (0.5–5 mg daily) was started in 85 (64.4%) patients. Good-to-partial response was documented in 63 (74.1%), and 30 (35.3%) required dose escalation (Table 2).

### 3.5. Mutation genotypes

The *MEFV* gene was sequenced in all patients: 27 (20.4%) were homozygous, 51 (38.6%) were heterozygous, and 34 (25.8%) were compound heterozygous. Complex alleles were found in 20 (15.2%) patients. The most common variants were R202Q (n = 71, 53.8%), which was homozygous in 17 (12.9%) patients, heterozygous in 21 (15.9%), and in combination with other mutations (compound heterozygous or complex allele) in 33 (25.0%). The second most common mutation was E148Q, which was identified in 36 (27.3%) patients, followed by M694V (n = 30, 22.7%) and V726A (n = 22, 16.7%) (Supplementary file A). Two rare variants with potential pathogenicity were identified (c.-15 and c.-330). We also noted a novel *MEFV* mutation (p. Lys629 Met) (Supplementary file A). R202Q was the most frequent (n = 103, 42%), followed by M694V (n = 43, 17.6%), E148Q (n = 41, 16.7%), V726A (n = 24, 9.8%), and M680I (n = 8, 3.3%) (Table 3).

### 3.6. Genotype–phenotype correlation

Abdominal pain, arthritis, arthralgia, and skin rashes were more common with the R202Q variants. All patients with V726A mutations reported fever, which was more frequent, albeit not significantly, in these patients than in those with R202Q or E148Q mutations. Skin rashes were reported in 20% of patients with R202Q, E148Q, and V726A mutations, whereas more chest pain was reported in patients with E148Q mutations than in those with R202Q and V726A mutations. Only one patient in our cohort developed renal amyloidosis. This patient had double homozygous mutations (R202Q and M694V) (Table 4).

The response to colchicine in patients with the most common mutations ranged from 25% for E148Q to 47.4% for R202Q. There was no significant difference with respect to patients with R202Q, E148Q, and V726A mutations, which were described as severe diseases according to the Pras score.

Patients with compound heterozygous mutations showed a higher rate of abdominal pain (94.1%) and exhibited the best response to colchicine (67.6%). Patients with complex alleles had the highest rate of fever (80%) and arthritis/arthralgia (70%) (Table 5).

### 3.7. Exon distribution of the mutations

In our cohort, most mutations were in exon 2 (n = 108, 56%), followed by exon 10 (n = 73, 37.8%) and exon 3 (n = 8, 4.1%). One patient had an exon 1 mutation.

**Table 2** Clinical and laboratory characteristics of the study population (n = 132).

| Characteristic | Number of patients | Percentage (%) |
|---|---|---|
| Abdominal pain | 120 | 91 |
| Fever | 97 | 73.5 |
| Arthritis/arthralgia | **75** | **56.8** |
| Oligo | 5 | 3.8 |
| Poly | 14 | 10 |
| Undetermined | 56 | 34 |
| Cardiac manifestations | **5** | **3.8** |
| Pericardial disease | 2 | 1.5 |
| Myocardial disease | 1 | 0.75 |
| Undetermined | 2 | 1.5 |
| Skin manifestations | 22 | 16.6 |
| Erysipelas-like erythema | 17 | 12.9 |
| Papules and pustules | 1 | 0.75 |
| Skin peeling | 1 | 0.75 |
| Soles and palm petechiae | 1 | 0.75 |
| Relapsing HSP | 2 | 1.5 |
| Jaundice | 3 | 2.0 |
| Chest pain | 40 | 31.0 |
| Mouth ulcers | 8 | 6.0 |
| Lymphadenopathy | 15 | 11.3 |
| Headache | 17 | 12.9 |
| Scrotal swelling | 2 | 1.5 |
| Back pain | 5 | 3.0 |
| Eye manifestations | 6 | 4.5 |
| Meningitis (seronegative, recurrent) | 2 | 1.5 |
| Anemia | 3 | 2.3 |
| Neutropenia | 4 | 3.0 |
| Elevated levels of inflammatory markers: | | |
| ESR alone | 8 | 6.1 |
| CRP alone | 21 | 15.9 |
| Both ESR and CRP | 42 | 31.8 |
| Rheumatoid factor (positive) | 2 | 1.5 |
| Antinuclear antibody (positive) | 13 | 9.8 |
| Abnormal flow cytometry results[a] | 7 | 5.3 |
| Elevated IgD levels (>144 mg/dL) | 5 | 3.78 |
| Abnormal gastrointestinal endoscopy findings: | 17 | 13.0 |
| HP gastritis | 12 | |
| Non-specific gastritis | 5 | |
| IBD | 4 | |
| Celiac | 1 | |
| Genetic test results: | | |
| Heterozygous | 51 | 38.6 |

**Table 2** (*continued*)

| Characteristic | Number of patients | Percentage (%) |
|---|---|---|
| Compound heterozygous | 34 | 25.8 |
| Homozygous | 27 | 20.4 |
| Complex alleles | 20 | 15.2 |
| Associated diseases: | | |
| JIA | 4 | 3.0 |
| Hyper IgD | 1 | 0.7 |
| HSP | 4 | 3.0 |
| Rheumatic fever | 3 | 2.0 |
| Kidney disease (amyloidosis, 1; IgA nephropathy, 2; MCD, 1) | 4 | 3.0 |
| Surgical procedures: | 14 | 10.6 |
| Appendectomy | 5 | 3.8 |
| Cholecystectomy | 4 | 3.0 |
| Adenoidectomy/ tonsillectomy | 3 | 2.0 |
| Splenectomy | 2 | 1.5 |
| Response to colchicine | | |
| Good | 52 | 39.3 |
| Partial | 11 | 8.3 |
| Poor | 22 | 16.6 |
| Pras score severity | | |
| Mild | 51 | 39.0 |
| Intermediate | 74 | 56.0 |
| Severe | 7 | 5.3 |

HSP, Henoch–Schönlein purpura; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IBD, inflammatory bowel disease; HP gastritis, *Helicobacter pylori* gastritis; JIA, juvenile idiopathic arthritis; MCD, minimal change disease.

[a] Variable abnormalities (decreased CD19 cells, reversed CD4/CD8 ratio, and increased CD19 cells).

**Table 3** MEFV mutation allele frequencies in our cohort.

| Mutation type | Number (n) | Percentage (%) of total alleles |
|---|---|---|
| Common mutations | | |
| R202Q | 103 | 42.0 |
| M694V | 43 | 17.6 |
| E148Q | 41 | 16.7 |
| V726A | 24 | 9.8 |
| M680I | 8 | 3.3 |
| A744T | 6 | 2.4 |
| P369S | 5 | 2.0 |
| R408Q | 4 | 1.6 |
| Rare mutations | | |
| F479L | 2 | 0.8 |
| R653H | 1 | 0.4 |
| R761C | 1 | 0.4 |
| R42W | 1 | 0.4 |
| E474K | 1 | 0.4 |
| E167D | 1 | 0.4 |
| K695R | 1 | 0.4 |
| c.-330 | 1 | 0.4 |
| c.-15 | 1 | 0.4 |
| K629M | 1 | 0.4 |
| TOTAL | 245 | 100 |

## 4. Discussion

FMF is the most common hereditary periodic fever syndrome in Jordan.[2] The *MEFV* gene, coding for pyrin, was identified in 1997. To date, 389 mutations have been identified (https://infevers.umai-montpellier.fr/web/search.php?n=1).

Fever and abdominal pain are common in children, and their occurrence is commonly associated with infectious causes. FMF is mostly diagnosed during childhood, with the first attack usually occurring before age 20.[3] The diagnosis requires a high index of suspicion. FMF notably mimics common pediatric disorders, which might result in delayed diagnosis. Data from neighboring countries such as Lebanon, Syria, and Turkey indicate an average age ranging from 1 to 13 years at presentation.[12–14]

However, delayed diagnosis up to 3 years has been reported.[13,14] In a study from Greece, diagnosis was delayed until adulthood.[15] A report from Jordan[16] reported the ages at presentation and diagnosis of 4.9 years and 6.6 years, respectively. Our numbers are consistent with these previous reports.[13,14] Timely diagnosis is essential as the most serious complication of FMF is renal amyloidosis, which can be prevented by early identification and treatment. We believe that improving physician awareness of the diverse presentations of FMF will shorten the duration to definitive diagnosis.

FMF is an autoinflammatory genetic disorder characterized by recurrent fevers and serosal inflammation of the abdomen, lungs, and joints, leading to severe pain.[3] Sudden-onset severe abdominal pain, classically affecting the whole abdomen and associated with board-like rigidity and rebound tenderness, affects approximately 90% of patients.[17]

Abdominal pain is the most common manifestation of FMF in reports from Jordan and surrounding regions.[12–14,16,18] Recurrent fever is typically high (>38 °C) and may reach 40 °C. The usual cycle is spontaneous rapidly rising fever followed by plateau and rapid decrease over 1–3 days. Fever was present in up to 100% of patients in some reports.[19] Arthritis affects 50–75% of FMF patients. FMF arthritis characteristically affects the large joints and is associated with high-grade fever and gradual resolution.[19]

In our cohort, abdominal pain was the most common manifestation (91%), followed by fever (73.5%) and arthritis (56.8%). Our results are consistent with the findings of a new report from Jordan as well as older reports from neighboring countries (Lebanon, Syria, and Turkey).[12–14,16,18] The relatively low prevalence of fever and arthritis might reflect underreporting rather than a real clinical difference.

The co-occurrence of abdominal pain and fever is a serious problem, particularly when associated with peritonism. Several FMF patients undergo unnecessary surgical

**Table 4** Comparison of clinical features of the most common mutations.

| Clinical characteristics | R202Q N (%) | E148Q N (%) | V726A N (%) | p-value |
|---|---|---|---|---|
| Abdominal pain | 36 (94.7) | 15 (75.0) | 9 (90.0) | 0.03[a] |
| Fever | 28 (73.7) | 14 (70.0) | 10 (100.0) | NS |
| Arthritis/arthralgia | 23 (60.5) | 12 (60.0) | 4 (40.0) | NS |
| Skin rashes | 8 (21.1) | 4 (20.0) | 2 (20.0) | NS |
| Chest pain | 11 (28.9) | 9 (45.0) | 2 (20.0) | NS |
| Headache | 7 (18.4) | 3 (15.0) | 1 (10.0) | NS |
| Cardiac manifestations | 3 (7.9) | 2 (10.0) | 0 (0.0) | NS |
| Good-to-partial response to colchicine | 18 (47.4) | 5 (25.0) | 3 (30.0) | NS |
| Pras severity score: | | | | |
| Mild | | 13 (65.0) | 4 (40.0) | 0.026[a] |
| Intermediate | 13 (34.2) | 5 (25.0) | 5 (50.0) | 0.018[a] |
| Severe | 22 (57.9) | 2 (10.0) | 1 (10.0) | NS |

[a] Between R202Q and E148Q.

interventions prior to FMF diagnosis, such as appendicectomy/cholecystectomy and diagnostic laparotomy. Contrarily, patients with an established diagnosis of FMF are at risk for a delayed diagnosis of acute abdominal emergency, as physicians tend to relate their attacks to FMF.

A Turkish study reported an appendectomy rate of 12.8% in FMF patients,[20] with a rate of 16.1% in another study.[14] In an Italian study, the appendicectomy rate reached 20%.[18] Although we could not obtain national appendicectomy rates for comparison, our rate (3.7%) is similar to that reported by a previous study from Jordan in FMF patients (3.5%),[21] lying between the rates reported for Lebanon (2.5%)[12] and Egypt (6.94%), and it is much lower than that reported by a Turkish study.[14]

The discrepancy between our rates and reports from Turkey might be related to older FMF patients with a prolonged time between presentation and diagnosis. Tunca et al. reported delayed FMF diagnosis in the Turkish cohort for up to 6.9 years.[22]

*MEFV* mutation leads to failed pyrin synthesis, which leads to very high levels of inflammatory cytokines on the gastrointestinal mucosal surface and possible mucosal erosions.[23] Intestinal amyloidosis can occur secondary to longstanding untreated FMF. Both mechanisms lead to FMF patients presenting at the pediatric gastrointestinal clinic with mucosal ulceration-related symptoms or abdominal pain, dysmotility, diarrhea, pseudo-obstruction, perforation, and malabsorption.[24]

Colchicine, the treatment of choice for FMF, is associated with the development of chronic diarrhea, abdominal pain, and colitis, also leading to gastroenterology clinic visits and endoscopic interventions.[25]

Data on endoscopic findings in FMF are scarce. Sağ et al. reported the endoscopic and histopathological findings of FMF patients. In their cohort, 39 (23.8%) patients underwent endoscopic intervention. The most common indication was chronic abdominal pain followed by chronic diarrhea and dyspepsia. The most common finding was nonspecific gastritis for upper endoscopy and lymphonodular hyperplasia for colonoscopy. IBD was diagnosed more commonly than in the control group. Endoscopic procedure was involved in determining the etiology of additional symptoms in 12/26 (46.2%) patients.[25] In our cohort, 27 patients (20.45%) underwent endoscopy. Abdominal pain that was unresponsive to treatment was the most common indication as in a previous report.[25]

The relatively high incidence of *H. pylori* gastritis in our cohort reflects the endemicity of *H. pylori* in our community. Four patients had Crohn disease, which is the same number of cases in the cohort of Sağ et al.[25] However, we did not have any local numbers for comparisons. Seventeen (63%) of the endoscopic procedures contributed to the patients' management (proton pump inhibitors, triple therapy, immunosuppressants, and gluten-free diet).

In our cohort, the most common coexistent noninfectious diseases were juvenile idiopathic arthritis and vasculitis, followed by gastrointestinal disorders (Crohn

**Table 5** Common symptoms and response to colchicine according to genotype.

| Genotype | Abdominal pain N (%) | Fever N (%) | Chest pain N (%) | Arthritis/ Arthralgia N (%) | Heart involvement N (%) | Headache N (%) | Skin rashes N (%) | Good response to Colchicine N (%) |
|---|---|---|---|---|---|---|---|---|
| Homozygous (27) | 23 (85.2) | 21 (77.8) | 9 (33.3) | 12 (44.4) | 2 (7.4) | 2 (7.4) | 6 (22.2) | 17 (63.0) |
| Heterozygous (51) | 45 (88.2) | 39 (76.5) | 16 (31.4) | 31 (60.8) | 3 (5.9) | 8 (15.7) | 10 (19.6) | 13 (25.5) |
| Compound Heterozygous (34) | 32 (94.1) | 21 (61.8) | 11 (32.4) | 17 (50.0) | 0 | 3 (8.8) | 3 (8.8) | 23 (67.6) |
| Complex Alleles (20) | 18 (90.0) | 16 (80.0) | 5 (25.0) | 14 (70.0) | 1 (5.0) | 3 (15.0) | 3 (15.0) | 9 (45.0) |

disease and gallstones) and renal diseases (nephrotic disease, IgA nephropathy, and amyloidosis). A notable combination found in one of our patients, who was a 3-year-old boy with recurrent fever, was recurrent infections and abdominal pain. His gastrointestinal endoscopy revealed *H. pylori* gastritis, peptic duodenitis, and terminal ileitis with granulomas. He underwent whole-exome sequencing to investigate his very-early-onset Crohn disease vs. chronic granulomatous disease, which showed a heterozygous mutation (M694I) in addition to a hemizygous CYBB (c.-125C > G) mutation, confirming the coexistence of FMF and chronic granulomatous disease. In an Iranian cohort of 400 FMF patients, 2 cases of IBD (UC with E148Q-V726A mutation and Crohn disease with heterozygous M680I mutation) were identified.[26] Sari et al. reported positive *MEFV* mutations in 3/7 patients diagnosed with infantile UC that was unresponsive to medical therapy.[27] Giaglis et al. reported that 28% of unselected, consecutive patients with UC had *MEFV* mutations (2 patients with M680I/0, 3 with M694V/0, 1 with E148Q/E148Q, and 1 with A744S/0).[28]

All four patients with IBD identified in our cohort had Crohn disease. The genotype distribution of our patients was as follows: heterozygous R202Q (n = 2), homozygous R202Q (n = 1), and heterozygous R42W (n = 1). The discordance between our results and the findings of previous reports originated from the difference in IBD type and the method of mutation identification. Our results support the theory that *MEFV* is a modifying gene in pediatric IBD.

Classically, the most frequent mutations in FMF patients are M694V, V726A, M680I, E148Q, and M694I. However, in a considerable number of clinically diagnosed FMF patients, no mutations are detected in their genetic testing, especially in western patients.[29] M694V was reported to be the most common *MEFV* mutation in Palestine,[29] whereas E148Q was described as the most common mutation in Syria.[13] In a Lebanese cohort, M694V was the most common.[13,30] Hayder reported that E148Q was the most common mutation in Iraqi Kurds with FMF.[31] Egyptian studies showed different mutation distributions, with E148Q and M694I in different studies. M694V is the most common mutation in European countries (Greece[15] and Italy[18]), whereas Ashkenazi Jews had V726A mutations.[32]

The distribution of *MEFV* mutations is changing as new mutations are being described. In many centers, the methodology of detecting mutations has changed from the FMF strip assay, which is based on reverse hybridization of biotinylated PCR products on immobilized oligonucleotides for mutations and controls, to gene sequencing. Turkey provides an example of such an epidemiological shift. Balta et al. reported heterozygous M694V/R202Q as the most common mutation, followed by homozygous M694V.[7] On the other hand, a recent study conducted by Arpacı et al. revealed that R202Q was the most common mutation, followed by E148Q and M694V.[33]

Mansour et al. searched for the 10 most common mutations using the FMF PCR strip assay and reported E148Q as the most common mutation in an Egyptian cohort (26.2%); however, in 793 (57.2%) patients, no mutation was identified.[34] This high rate of unidentified mutations suggests that a more extensive analysis of the *MEFV* gene might result in a modified genotypic map.

Several reports from Jordan described M694Vas the most common mutation, and they all used PCR to identify the most common mutations.[7,9,16,21] Majeed et al. reported that M694V, V726A, M694I, M680I, and E148Q accounted for the most common mutations in 239 Jordanian FMF patients.[35] Homozygous M694V/M694V, which was the most common variant, was shown to be associated with the most severe course. In their cohort, two patients developed chronic renal failure, both of whom were untreated (i.e., did not receive colchicine) and were homozygous M694V/M694V patients.[4,35] Another report from Jordan[11] identified M694V as the most common mutation in their cohort. In the study conducted by Alzyoud et al., M694V was the most common mutation in their cohort, followed by V726A and E148Q. However, only one patient out of 196 developed amyloidosis. The methodology described in all of these studies[7–9,16,21] was looking for pre-identified mutations. None of them included R202Q in their strip, or complete sequencing of *MEFV* exons.

We could argue that our results are consistent with those of previous reports; if we omit the results for R202Q, M694V would become the most frequent mutation [M694V homozygous (n = 9), heterozygous (n = 12), combined heterozygous (n = 5)]. Our study revealed that R202Q was the most common mutation in homozygous (n = 17, 63%) and heterozygous (n = 21, 41.1%) forms. The frequency of R202Q mutation in the Jordanian population is unknown; nevertheless, FMF patients with R202Q mutation exhibit a full-blown picture of FMF, suggesting that the mutation is truly disease-causing rather than a polymorphism with a disease-modifying effect. Our patients were already diagnosed with FMF based on the clinical criteria; hence, the mutations discovered had no effect on diagnosis establishment. New reports from multiple geographical areas in Turkey using genomic sequencing of the *MEFV* gene support our clinical findings that R202Q is a mutation with disease-causing properties.[36,37] Our findings suggest the need to add R202Q to the mutation test strip. We believe that adopting this approach universally might provide a proof of concept regarding the importance of R202Q as a disease-causing mutation.

Moreover, our cohort showed very rare mutations that could be pathogenic (c.-15C > G) or disease-causing (c.-330G > A), as well as a novel mutation (p. Lys629 Met [K629M]). This suggests that sequencing of the *MEFV* gene might have other rare or novel mutations.

Since the identification of the *MEFV* gene, the effects of genotype on the clinical presentation of FMF have been extensively studied with no clear genotype–phenotype correlation. Our results are a continuum of this debatable subject, in which some presentations were consistent with some studies while they were contradictory of others.

In our cohort, patients with R202Q mutation manifested the complete FMF spectrum in accordance with previous reports.[38] However, our findings contradict those of Yigit et al.[39] who reported no signs or symptoms with R202Q mutation, especially in the heterozygous status.

Patients with R202Q mutation in our cohort experienced abdominal pain, arthritis, arthralgia, and skin rashes, as compared with those with other mutations. The majority of our patients with R202Q mutation showed mild-to-moderate disease (92%), which is relatively consistent with the findings of Comak et al.[24] In contrast, Giaglis et al.[15] reported severe disease with this mutation. Another example is the E148Q mutation; while Yilmaz et al.[40] reported this mutation as protective against joint involvement, arthritis and arthralgia were most commonly observed with this mutation in our cohort.

M694V has been reported to be associated with severe disease,[41] poor response to colchicine,[42] and increased risk of amyloidosis.[43] The only patient who developed amyloidosis in our cohort had a complex allele (M694V homozygous/R202Q homozygous). Although this finding is consistent with previous reports from Jordan[16,35] this might not reflect a benign behavior (no development of amyloidosis complications) in our population, but a shorter follow-up duration, considering that many of our patients would be transferred to adult rheumatology service, where complications might subsequently develop.

In a systematic review, Gangemi et al. addressed the non-linearity of the genotype–phenotype correlation; nevertheless, none of the proposed theories could explain this discordance.[44] Altogether, the presence of *MEFV* mutation is insufficient to determine the disease onset, progression, or flare-up. However, the absence of *MEFV* mutation should not prevent physicians from diagnosing FMF.

### 4.1. Limitations

Our study presents new data on the clinical and genetic manifestations of FMF in Jordanian children. Nonetheless, this study has some limitations, including its retrospective design, with the inherit bias of retrospective data. Moreover, the cohort was relatively small, and we excluded FMF patients with no genetic testing or negative genetic test results. This might affect the comparability of our results to the findings of other reports.

## 5. Conclusion

FMF is endemic in Jordan. Genetic testing is important in FMF evaluation; however, the genotype–phenotype correlation needs further study. The R202Q mutation is possibly pathogenic and is associated with the manifestation of the full spectrum of FMF features. R202Q was the most common mutation and needs to be added to the customized FMF strip test in Jordan to increase the mutation detection rate.

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## Declaration of competing interest

The authors declare no conflict of interest related to this project.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2022.06.014.