Original article

Genotypes and Phenotypes of Arab Patients with Familial Mediterranean Fever in North Jordan

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Abstract:

Objectives: Familial Mediterranean Fever (FMF) is an autosomal-recessive disorder caused by mutations in *MEFV* gene. Hundreds of mutations have been described in patients with FMF. The aim of this study was to analyze the common genotypes of Arab patients with FMF from Jordan and to establish genotype-phenotype correlation patterns. ***Methods:*** This cross-sectional retrospective study was performed in a tertiary hospital in the north of Jordan. A total of 123 patients with FMF were recruited from the rheumatology outpatient clinic at King Abdullah University Hospital. Patients were diagnosed according to Tel-Hashomer criteria and a carrier of at least one previously identified *MEFV* gene mutation. Demographics and clinical manifestations were recorded. ***Results:*** Mean age at diagnosis was 17.49 years and M:F ratio was 1.05:1. The following mutations were common among our patients: R202Q (41.4%), M694V (22.1%), E148Q (10.3%), V726A (11.3%), M680I (5.3%), and M694I (3.7%). Among them 26% were homozygous, 11% were compound homozygous, 26% heterozygous, 42% were compound heterozygous, and 18% were other complex genotype including homozygosity and heterozygosity of more than one mutation. As for genotype-phenotype correlation, 65% of the patients with skin rash had R202Q mutation, while M694V mutation was associated with abdominal and chest pain. Amyloidosis correlated most with the M694I mutation (66.7%). ***Conclusion:*** The results confirm the frequency of the previously and commonly identified mutations and highlight the association of R202Q polymorphism with skin rash phenotype among adult Jordanian FMF patients. Additionally, both M694V and M694I mutations were associated with different clinical presentations.

Keywords: Familial Mediterranean Fever; Jordan, MEFV gene mutation; Autoinflammatory disease.

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Introduction:

Familial Mediterranean Fever (FMF) is an autosomal-recessive autoinflammatory condition that presents with recurrent periodic, self-limiting episodes of fever and painful serositis [1]. The episodes start most often during childhood, with more than 80% of patients presenting at an age younger than 20 years and only a few after the age of 40 years [2]. These symptoms can be controlled by colchicine and Interleukin-1 (IL-1) inhibitors [3]. The disease mostly affects people in

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the Mediterranean region [4-6]. The prevalence varies from 1:100 to 1:2000. Male to female ratio is equal in Arabs, while male predominance is noted in other countries [6].

There are no specific markers to distinguish FMF from infectious diseases or appendicitis. However, during acute attacks elevation of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, and leukocytosis are observed [7]. The most severe complication of FMF is the development of secondary amyloidosis which mainly affects the kidneys, causing proteinuria and leading to kidney failure [8]. The diagnosis of FMF is a clinical diagnosis, which can be supported but not excluded by genetic testing.

The disease is caused by a mutation in the MEFV gene that has been mapped to chromosome 16p13.3. It is composed of 10 exons and spans approximately 14 Kb of genomic DNA. It encodes a 781 amino acid (~ 95 kDa) protein called Pyrin [9]. This protein is predominantly expressed in cells of the innate immune system [9]. Although it is still unclear what stimulates pyrin activity, it is now well established that it mediates inflammasome activation and caspase 1-dependent expression of IL-1, thus promoting a pro-inflammatory response [10].

The type and severity of FMF symptoms are correlated with the presence of different MEFV mutations [1]. More than 300 mutations have so far been identified [11]. The most common are M694V, M694I, M680I, V726A, and E148Q (12-14). The M694V mutation is the most frequent mutation observed in various ethnic groups, however its frequency varies from group to group. Among the Arab populations the distribution of mutations varies by country [7, 14-16]. In Jordan, some studies found that M694V was the most common mutation, followed by V726A or E148Q [17-20].

Although data on FMF genotype-phenotype correlations have been described in Jordanian children with FMF, data on adult patients is lacking [17-19]. The aim of this study was to analyze the genotypes of Arab patients with FMF from the northern region of Jordan and to establish the genotype-phenotype correlation patterns.

Material and methods:

1.1 Study subjects:

One hundred and twenty-three Jordanian patients diagnosed with FMF according to Tel-Hashomer criteria between January 2011 and February 2017 were enrolled in this retrospective study. Patients had regular follow-ups in the rheumatology outpatient clinic at King Abdullah University Hospital, Irbid, Jordan. All included patients' genomic DNA had been sequenced for the 10 exons of the *MEFV* gene and had at least one known MEFV gene mutation and /or polymorphism. The study was approved by the Institutional review board committees at Jordan University of Science and Technology and King Abdullah University Hospital and was conducted in accordance with the Declaration of Helsinki. Relevant patient demographic and clinical data were recorded.

1.2 Sequencing Analysis:

The 10 coding exons of *MEFV* were amplified from genomic DNA by polymerase chain reaction (PCR). Forward and reverse primers were designed in Primer3 (http://frodo.wi.mit.edu/primer3). Primer sequences and PCR parameters are available upon request. PCR products were purified and sequenced in both directions using Big-Dye Terminator v3.1 Cycle Sequencing Kit on a 3130xl Genetic Analyzer (Applied Biosystems). Sequencing data was compared to reference sequences using ChromasPro 1.34 (Technelysium Pty. Ltd., Australia). Mutations and variants were designated according to guidelines of the Human Genome Variation Society (http://www. hgvs.org/mutnomen), utilizing NCBI reference sequences for the MEFV gene (NG_007871.1) and cDNA (NM_000243.3).

Statistical analysis

Data was analyzed using SPSS statistical software, version 21.0 for Microsoft Windows. Descriptive analysis (mean and standard deviation), t-test, and Chi-square ($\chi 2$) test were applied to detect the differences between study variables. Exact test was used instead when the expected frequency was less than 5. A p-value of less than 0.05 was considered statistically significant.

Ethical Clearance:Approval and Consent to Participate

IRB approval was sought prior to initiation of this study. Patient consent obtained

Results:

Among the 123 patients with FMF, 63 patients (51.2%) were male and 60 patients (48.8%) were female. The mean age of patients at diagnosis was 17.49

years (ranging from 1 year to 63 years). Abdominal pain was the most common presenting feature in patients (79.7%), followed by fever (64.2%), arthritis (55.3%), chest pain (29.3%), headache (27.6%), and skin rash (16.3%). Family history of FMF in a first-degree member was reported in 40 patients (32.5%). Proteinuria was detected in 6 (4.9%) patients, and kidney biopsy performed in these patients revealed amyloidosis in 3 (0.8%) cases.

We identified 46 different genotypes in the MEFV gene. The most commonly observed mutations were R202Q (41.4%), M694V (22.1%), V726A (10.3%), E148Q (11.9%), M680I (5.3%), and M694I (3.7%) (Table 1) **(Figure 1 The distribution of homozygous and heterozygous alleles among the most common genotypes in MEFV gene).** Patients were then classified according to mutations and allele genotype status into 5 groups **(Table 2).** Group 1 (homozygous group) included 26 patients (21.1%); 16 were males and 10 were females. Group 2 (compound homozygous group) included 11 patients (8.9%); 6 were females and 5 were males. Group 3 (heterozygous group) included 26 patients (21.1%); 13 were males and 13 were females. Group 4 (compound heterozygous group) included 42 patients (43.2%); 24 were females and 18 were males. Group 5 (other complex genotypes group) included 18 patients (14.6%); 11 were males and 7 were females. The most common mutations in the heterozygous group were R202Q (10.6%), E148Q (4.1%), and V726A (4.1%). Homozygosity for R202Q, E148Q, and M694V was found in 14.6%, 3.3%, and 1.6% of patients respectively. R202Q/M694V was the most common genotype in compound homozygous and other complex genotype groups (8.13%).

Table 1 : The allele frequency of the *MEFV* genotypes among the study subjects

GENOTYPE	ALLELE (N)	FREQUENCY (%)
R202Q	101	41.39%
M694V	54	22.13%
V726A	25	10.25%
E148Q	29	11.89%
M680I	13	5.33%
M694I	9	3.69%
P369S	3	1.23%
A744S	2	0.82%
R653H	2	0.82%
E148V	1	0.41%
R408Q	1	0.41%
E167D	1	0.41%
A744S	1	0.41%
I692del	1	0.41%
K695R	1	0.41%
c.1588-69 G>A	1	0.41%

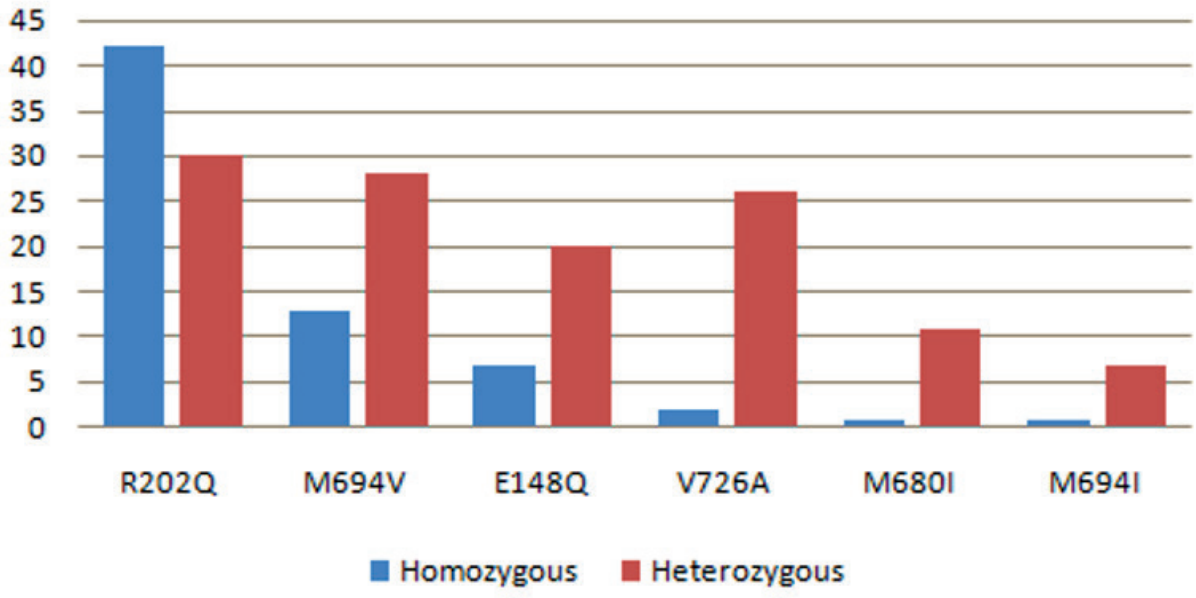

Figure 1: The distribution of homozygous and heterozygous alleles among the most common genotypes in *MEFV* gene.

Table 2: The Frequency of the *MEFV* genotypes among the different allelic groups.

ALLELE STATUS	GENOTYPE	NUMBER (N)	FREQUENCY (%)
HOMOZYGOUS	R202Q/R202Q	18	14.63%
HOMOZYGOUS	F148Q/F148Q	4	3.25%
HOMOZYGOUS	M694V/M694V	2	1.63%
HOMOZYGOUS	M694I/M694I	1	0.81%
HOMOZYGOUS	V726A/V726A	1	0.81%
COMPOUND HOMOZYGOUS	R202Q/R202Q/M694V/M694V	10	8.13%
COMPOUND HOMOZYGOUS	E148Q/ E148Q/V726A/V726A	1	0.81%
HETEROZYGOUS	R202Q/wt	13	10.57%
HETEROZYGOUS	E148Q/wt	5	4.07%
HETEROZYGOUS	V726A/wt	5	4.07%
HETEROZYGOUS	A744S/wt	1	0.81%
HETEROZYGOUS	F148V/wt	1	0.81%
HETEROZYGOUS	c.1588-69 G>A/wt	1	0.81%
COMPOUND HETEROZYGOUS	E148Q/V726A	6	4.88%
COMPOUND HETEROZYGOUS	R202Q/E148Q	4	3.25%
COMPOUND HETEROZYGOUS	V726A/M600I	4	3.25%
COMPOUND HETEROZYGOUS	M694I/V726A	3	2.44%
COMPOUND HETEROZYGOUS	R202Q/M694V	3	2.44%
COMPOUND HETEROZYGOUS	M680I/V72kA	1	0.81%
COMPOUND HETEROZYGOUS	E148Q/M694I	1	0.81%
COMPOUND HETEROZYGOUS	E148Q/M694V	1	0.81%
COMPOUND HETEROZYGOUS	E148Q/A744S	1	0.81%
COMPOUND HETEROZYGOUS	E148Q/I692del	1	0.81%
COMPOUND HETEROZYGOUS	M694I/M694V	1	0.81%
COMPOUND HETEROZYGOUS	M694I/M680I	1	0.81%
COMPOUND HETEROZYGOUS	R202Q/V726A	1	0.81%
COMPOUND HETEROZYGOUS	R202Q/A744S	1	0.81%
COMPOUND HETEROZYGOUS	V726A/M694V	1	0.81%
COMPOUND HETEROZYGOUS	V726A/R653H	1	0.81%
COMPOUND HETEROZYGOUS	V726A/E167D	1	0.81%
COMPOUND HETEROZYGOUS	M694V/M680I	1	0.81%
COMPOUND HETEROZYGOUS	P369S/R408Q	1	0.81%
COMPOUND HETEROZYGOUS	F148V/K595R	1	0.81%
COMPLEX HETEROZYGOUS	M694I/wt/R202Q/wt/M694V/wt	1	0.81%
COMPLEX HETEROZYGOUS	V726A/wt/R202Q/wt/M694V/wt	1	0.81%
COMPLEX HETEROZYGOUS	E148Q/wt/R202Q/wt/M694V/wt	1	0.81%
COMPLEX HETEROZYGOUS	M680I/wt/R202Q/wt/M694V/wt	1	0.81%

ALLELE STATUS	GENOTYPE	NUMBER (N)	FREQUENCY (%)
COMPLEX HETEROZYGOUS	M680I/wt/R202Q/wt/M694V/wt	3	2.44%
OTHER COMPLEX GENOTYPE	R202Q/R202Q/V726A/wt/ M694V/wt	1	0.81%
OTHER COMPLEX GENOTYPE	E148Q/E148Q/M694V/wt	1	0.81%
OTHER COMPLEX GENOTYPE	E148Q/E148Q/P369S/wt	1	0.81%
OTHER COMPLEX GENOTYPE	M694V/M694V/R202Q/wt	1	0.81%
OTHER COMPLEX GENOTYPE	R202Q/R202Q/A744S/wt	1	0.81%
OTHER COMPLEX GENOTYPE	M680I/M680I/P369S/wt	1	0.81%
OTHER COMPLEX GENOTYPE	R202Q/R202Q/R653H/wt/ M694V/wt	1	0.81%
OTHER COMPLEX GENOTYPE	R202Q/R202Q/M694V /wt	11	8.94%

Table 3 shows the distribution of the clinical characteristics among the 5 allelic groups of FMF patients. The presence of fever and headache was significantly higher in homozygous (80.77% and 50%, respectively), (p=0.007) and compound homozygous groups (81.82% and 45.45%, respectively), (p=0.02). While skin rash was more predominant in homozygous (30.77%), compound homozygous (27.27%), and other complex genotypes (27.78%) groups (p=0.005). Moreover, a family history of FMF was most prevalent in compound homozygous (63.64%) (p<0.001).

Table 3: Comparison of patient characteristics among the *MEFV* allelic groups.

Characteristic	Homozygous (n=26)	Compound Homozygous (n=11)	Heterozygous (n=26)	Compound and Complex heterozygous (n=42)	Other complex genotype (n=18)	P value
Age at diagnosis (mean±SD)	20.15±16.12	19.82±10.43	15.77±11.30	20.00±14.11	21.44±16.15	0.687
Male: female	16:10	5:6	13:13	18:24	11:7	0.533
Family history	3 (11.54%)	7 (63.64%)	1 (3.85%)	19 (45.24%)	10 (55.56%)	0.000018
Abdominal pain	18 (69.23%)	10 (90.91%)	18 (69.23%)	38 (90.48%)	14 (77.78%)	0.115
Fever	21 (80.77%)	9 (81.81%)	11 (42.31%)	30 (71.43%)	8 (44.44%)	0.007
Arthritis	14 (53.85%)	7 (63.64%)	13 (50%)	26 (61.90%)	8 (44.44%)	0.694
Chest pain	10 (38.46%)	6 (54.55%)	3 (11.54%)	13 (30.55%)	4 (22.22%)	0.064
Headache	13 (50%)	5 (45.45%)	4 (15.38%)	9 (21.43%)	3 (16.67%)	0.02
Skin rash	8 (30.77%)	3 (27.27%)	3 (11.54%)	1 (2.38%)	5 (27.78%)	0.005
Amyloidosis	0 (0%)	1 (9.09%)	0 (0%)	2 (4.76%)	0 (0%)	0.456

Regarding genotype-phenotype correlation, M694V heterozygous mutation was significantly higher in males than females; male to female ratio in this group was 2.11:1 (P=0.045). A total of 26 out of 28 patients (92.9%) within this group had abdominal pain (P=0.049), and 18 out of 28 (64%) patients significantly reported a family history of FMF (P=0.001). While M694V homozygous mutation was significantly associated with chest pain; 6 out of 13 patients (46%) had chest pain (P= 0.039). Amyloidosis was significantly associated with M694I heterozygous mutation; 2 out of 3 patients with amyloidosis (66.7%) had M694I heterozygous mutation (P=0.004).

Discussion:

This cross-sectional study was performed in a tertiary hospital in north Jordan, our results showed that male: female ratio was 1.05:1, in contrast to data from other countries where male predominance was noted [21, 22]. However, it is in agreement with data on FMF among Arabs, where male: female ratio was reported to be almost equal [3].

The presenting symptoms among our patients were comparable to studies from Turkey [5, 13, 21, 23]. Abdominal pain was the most common presenting feature in 79.7% of cases as seen in a previous observation in Jordan [24]. While other studies have shown that fever was the most common presenting feature [12, 22]. Arthritis was documented in 55.3% of patients which is higher than that reported among other ethnic groups, while the frequency of chest pain (29.3%) and skin manifestations (16.3%) was similar to that reported in other studies [13, 22, 23].

M694V was reported to be the most common *MEFV* gene mutation in most studies [4, 5, 8, 12, 13, 18, 21, 23, 25-27]. However, our results showed that R202Q was the most common variant, which was identified in 41.39% of patients. Similar to our findings, R202Q was reported as the most common variant in a study that was conducted on 296 FMF patients in Turkey using next generation sequencing [11], as well as in another Turkish study of 514 FMF patients [28]. On the other hand, a different study involving 374 children with FMF showed that the R202Q/M694V compound mutation was the most common compound heterozygous genotype [29]. In our study, R202Q/M694V was the most common genotype in compound homozygous and other complex genotype groups. These differences could be due to variations in the spectrums of *MEFV* gene screening and the ethnic diversity of patients involved in the studies.

Among symptomatic patients who had only R202Q mutation; 14.63% were homozygous, and 10.57% were heterozygous, which contradicts the assumption of a Turkish study that the R202Q mutation represents a polymorphism [30]. Yet it supports the proposal of a Greek study that the presence of R202Q homozygosity might be considered as disease related [25]. In fact, R202Q was found to be the second most common homozygous variant in a study that was conducted on 182 FMF patients and screened 17 genes [31]. A similar finding was reported in a study of FMF children in the black sea region of Turkey [32]. In addition, a Turkish study that involved 122 FMF patients and 128 healthy subjects in 2017 reported R202Q as a novel FMF mutation, which was the second most common among patients [33].

The controversy on whether R202Q is a polymorphism or a pathogenic mutation is because most studies that reported this variant as disease linked have been conducted on FMF patients in the absence of healthy control groups. Nevertheless, a study that included 191 FMF patients and 150 healthy controls examined the genotype and allele frequency and clinical significance of R202Q and found that it was significantly higher in the FMF group [34].

FMF clinical manifestations vary among the different *MEFV* genotypes and allelic combinations. In our study, abdominal pain and arthritis were equally present among the 5 allelic groups, while fever and headache were more predominant in compound homozygous and homozygous groups, respectively.

Many studies have demonstrated a specific association between amyloidosis risk and the type of *MEFV* gene mutation. M694V homozygous genotype was found to be associated with a higher prevalence of renal amyloidosis in Armenians and Arabs [4,18]. However, our results showed that M694I heterozygous mutation was significantly associated with amyloidosis; 66.7% of patients who developed amyloidosis had M694I heterozygous mutation. This might be due to other genetic or epigenetic factors.

Conclusion:

Our data showed that the most common genotype in FMF patients from north of Jordan in our study was R202Q. However, it remains unclear whether it is a pathogenic one. Therefore, future studies are required to screen for this variant and determine its clinical significance in a larger cohort of FMF patients and compare it to a matching healthy control

group. Further knowledge about genotype-phenotype relations can help assess the severity of the disease and tailor patient treatment like that being studied in other diseases [35,36].

Author Contributions: Study design was developed by KA, TK, DZ, DA. Data generation was performed by KA, TK, DZ. Data analysis and interpretation were performed by KA, SJ, MO, TK, DZ, DA, and NS. Manuscript writing and editing were performed by KA, SJ, MO, TK, DZ, DA, NS. All authors read and approved final manuscript.

Human and Animal rights

Approval from Institutional review board (IRB) was granted prior to initiation of study. Studies involving human participants were according to Declaration of Helsinki.

Disclosures and Declarations There is no conflict of interest or any financial contributions/funding to any of the authors of this study.

Availability of data and material

The datasets used during the current study are available from the corresponding author on request.

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