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A Missing Correlation between the Number and Type of *MEFV* Mutations and Severity of Clinical Manifestations in Egyptian Pediatric FMF Patients

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Authors' contributions

Author AIA designed and supervised the study and the writing of the manuscript. Authors HMET and IOF contributed equally in writing the manuscript. Authors MMEW and FHEB managed the literature searches. Author SS diagnosed and assessed all patients and managed all clinical aspects of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Previous researches identified the gene for Familial Mediterranean Fever (FMF) and found several different gene mutations that cause this inherited rheumatic disease. The aim of this work is to investigate the correlation between severity of symptoms of FMF and the number and type of *MEFV* variants, as well as to shed light on the correlation between the genotype and phenotype of Egyptian pediatric FMF patients.
Study Design: Retrospective study.
Place and Duration of Study: Department of Pediatrics, Kasr El Aini Hospital, Cairo University Medical School, Cairo, Egypt, between January 2012 and February 2013.
Methodology: This study involved 35 childhood cases of Egyptian ethnic origin suspected to suffer from FMF. They include 19 males and 16 females of age range between 1-17 years. *MEFV* mutations in each patient were determined by performing

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DNA isolation and purification, in vitro amplification (PCR), and reverse hybridization using an FMF StripAssay.
Results: Our results revealed 14% homozygous, 34% single heterozygous, 35% compound heterozygous-bi, and 17% compound heterozygous-tri patients. Twelve *MEFV* mutations were covered where all mutations were concentrated on exons 10 and 2. Severity of clinical manifestations and severity score did not linearly correlate with the number of variants, nor with the type of variant.
Conclusion: Our results question the strength of genotype-phenotype correlation in FMF and indicate that *MEFV* genotypes express much more variable phenotypes than previously suggested. Our results also revealed no association between the number of mutations and severity of clinical manifestations.

Keywords: FMF; MEFV mutations; genotype-phenotype correlation; pediatrics.

1. INTRODUCTION

Familial Mediterranean fever (FMF) is a systemic autoinflammatory disorder which is inherited mainly in an autosomal recessive pattern and predominantly affects Eastern Mediterranean populations, especially of Jewish, Turkish, Armenian, and Arab descent [1,2]. The disease is characterized by periodically recurrent self-limited episodes of fever, accompanied by apparently unprovoked peritonitis, pleuritis, arthritis and/or skin rash [3]. A fatal complication experienced in some patients is development of AA amyloidosis which may lead to renal failure and death but this can be largely prevented by prophylactic administration of the neutrophil suppressor, colchicine, which is currently the most commonly utilized drug for treatment of FMF [4,5].

Before identification of the causative gene for FMF, diagnosis was based solely on clinical manifestations and exclusion of similarly presenting diseases, family history of FMF, patient ancestry and response to colchicine but difficulties were frequently encountered in diagnosis of atypical presentations of the disease. The discovery of the *MEFV* gene located on chromosome 16p13.3 and the development of a test for detecting mutations involved in FMF have added a novel tool to help make definitive diagnoses [6]. Most FMF patients carry mutations in the *MEFV* gene encoding the protein pyrin which is associated with inflammation [7,8]. To date, over 70 *MEFV* gene mutations – predominantly positioned in exon 10 – have been identified which are associated with FMF and can be accessed through the Infevers database http://fmf.igh.cnrs.fr/infevers/ [9]. The majority of FMF cases are caused by four mutations clustered on exon 10: p.M694V, p.V726A, p.M680I and p.M694I [7,8]. Some studies have suggested that homozygosity and compound heterozygosity of these founder mutations, particularly p.M694V, are associated with severe phenotype and higher frequency of renal amyloidosis [10-12]. On the other hand, few studies found no relationship between *MEFV* variations and phenotypic features of the disease [13-14]. Therefore in this study, we aimed to investigate the correlation between the genotype or number of *MEFV* variants and the presence of certain clinical manifestations and phenotypic disease severity in a population of Egyptian children diagnosed with FMF, in an attempt to re-evaluate the link between the mutations and the severity of clinical manifestations.

2. METHODOLOGY

2.1 Subjects

The current study consists of 35 childhood cases of Egyptian ethnic origin suspected to suffer from the FMF recruited at the Kasr El Aini Hospital of Cairo University Medical School in Cairo, Egypt. The symptoms noticed abided by those of FMF, ranging from fever and mild abdominal pain only to more discomforting symptoms including joint pain, leg pain and peritonitis. The subjects were selected upon clinical basis where all patients satisfied 2 major or one major and 2 minor criteria of the Tel Hashomer criteria for diagnosis of FMF as previously described by Livneh A [3]. Briefly the major criteria include recurrent febrile episodes accompanied by serositis, amyloidosis of AA-type without a predisposing disease, and favorable response to continuous colchicine treatment; the minor criteria include recurrent febrile episodes, erysipelas-like erythema, and FMF in a first-degree relative. Those who did not match 2 major or one major and 2 minor criteria were excluded from further study. The subjects include 19 males and 16 females with ages ranging from 1-17 years. According to the mutations expressed, cases were classified into four different genotypes; a homozygous mutation, a single heterozygous mutation, compound heterozygous for two mutations (bi) and compound heterozygous for three mutations (tri). The mean age at FMF onset was 6.6 ± 7.3, 5.2 ± 2.7, 6.0 ± 4.6, and 4.6 ±2.8 years, for each genotype category respectively. The mean duration of disease follow-up was 1.30 ± 0.45, 1.21 ± 0.45, 1.25 ± 0.58, and 1.42 ± 0.49 years, respectively. All parents or legal guardians provided their written informed consent. All experiments were performed in compliance with the guidelines of the Institutional Review Board of Kasr El Aini Medical School in Cairo University and in accordance to the ethical standards of the Declaration of Helsinki.

2.2 DNA Isolation and Purification

Fresh blood samples from the 35 patients were collected in the presence of an anticoagulant (EDTA). DNA isolation was carried out using Lysis Solution and GEN^{x}TRACT Resin provided in the ViennaLab StripAssays® FMF StripAssay kit (ViennaLab Diagnostics GmbH, Vienna, Austria), according to the manufacturer's protocol.

2.3 In Vitro Amplification (PCR)

A fresh working dilution (0.2 U/µl) of Taq DNA Polymerase (Qiagen, Germany) in Taq Dilution Buffer (ViennaLab Diagnostics GmbH, Vienna, Austria) was prepared. The amplified products were separated by electrophoresis on a 3% agarose gel followed by UV visualization of the ethidium bromide-stained gel for confirmation of the amplification process.

2.4 Reverse Hybridization

The ViennaLab StripAssays® FMF StripAssay was used where amplification products were selectively hybridized to the StripAssay teststrip, which contains oligonucleotide probes (wild type- and mutant-specific) immobilized as parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and colour substrates, according to manufacturer protocol.

3. RESULTS

3.1 Classification of Subjects

Amplification products were selectively hybridized to a test strip containing wild-type and mutant-specific oligonucleotide probes. The assay covers twelve *MEFV* mutations on the following exons: exon 2: p.E148Q; exon 3: p.P369S; exon 5: p.F479L; exon 10: p.M680I (G/C), p.M680I (G/A), p.I692del, p.M694V, p.M694I, p.K695R, p.V726A, p.A744S, and p.R761H. The 35 patients were genotypically divided into four categories according to their polymorphic position and number: homozygous (14%), single heterozygous (34%), compound heterozygous-bi (2 mutations) (35%), and compound heterozygous-tri (3 mutations) (17%), as shown in Fig. 1.

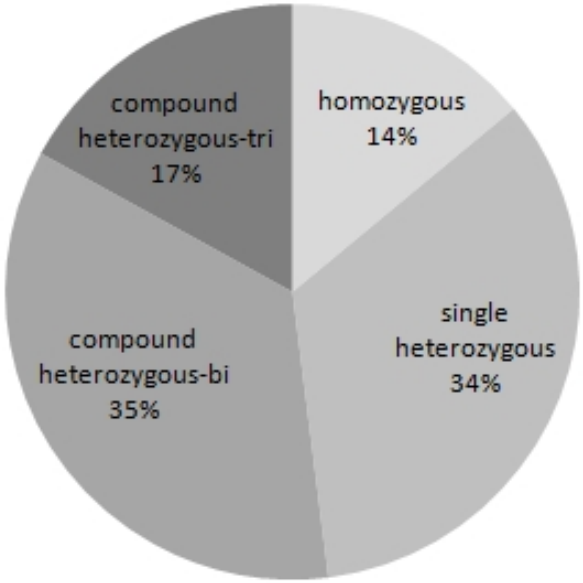

Fig. 1. Classification of the 35 FMF patients into four categories according to their genotype

The homozygous category consists of 5 patients, the single heterozygous consists of 12 patients, the compound heterozygous-bi consists of 12 patients, and the compound heterozygous-tri consists of 6

3.2 Positions of the *MEFV* Gene Mutations

Of the 12 mutations covered by the FMF strip assay, all mutations expressed were found to take place in exon 2 and exon 10 on the *MEFV* gene, the majority of which were positioned on the latter, as indicated in Table 1. All mutations in the homozygote cases were restricted to exon 10 (Table 1).

3.3 Correlation of FMF Genotypes with *MEFV* Gene Mutations

Among all 35 patients, the most common mutations were p.M694I (28%) followed by p.M694V (22%), p.V726A (16%), p.M680I (16%) and p.E148Q (9%). Correlating the four different genotype categories of the FMF patients and mutations, each genotype showed

different mutations in different percentages. Among homozygous patients, p.M694V (40%) and p.M694I (40%) constituted the most frequently occurring mutations. In single heterozygotes, the highest percentage corresponded to the p.V726A (33%) mutation. In compound heterozygous patients carrying 2 mutations, p.M694I (37.5%) and p.M694V (25%) displayed highest frequencies within this category. Finally, among compound heterozygous patients carrying 3 mutations, p.V726A (22%) and p.M694V (17%) comprised the highest percentages. The p.P369S and p.F479L mutations were not detected in any patients (Table 1).

Table 1. Position of each detected mutation on the *MEFV* gene and correlation between the four genotype categories and the *MEFV* variants detected

Genotype of patient	Gender	*MEFV* variant(s)	Position of detected polymorphism(s)
Homozygous			
1	Male	p.M694I	Exon 10
2	Female	p.M694I	Exon 10
3	Female	p.M680I	Exon 10
4	Male	p.M694V	Exon 10
5	Male	p.M694V	Exon 10
Single Heterozygous			
1	Male	p.E148Q	Exon 2
2	Male	p.M694I	Exon 10
3	Male	p.V726A	Exon 10
4	Male	p.M694I	Exon 10
5	Female	p.V726A	Exon 10
6	Female	p.M680I	Exon 10
7	Female	p.M680I	Exon 10
8	Female	p.E148Q	Exon 2
9	Male	p.V726A	Exon 10
10	Female	p.A744S	Exon 10
11	Male	p.M694I	Exon 10
12	Male	p.V726A	Exon 10
Compound Homozygous-bi			
1	Male	p.E148Q, p.M694I	Exon 2 and Exon 10
2	Female	p.M680I, p.M694I	Exon 10
3	Female	p.M694I, p.K695R	Exon 10
4	Female	p.M694V, p.M680I	Exon 10
5	Male	p.M680I, p.V726A	Exon 10
6	Male	p.M680I, p.M694I	Exon 10
7	Male	p.E148Q, p.M694V	Exon 2 and Exon 10
8	Male	p.M694V, p.M694I	Exon 10
9	Male	p.M694I, p.V726A	Exon 10
10	Female	p.M694V, p.M694I	Exon 10
11	Male	p.M694V, p.M694I	Exon 10
12	Male	p.M694V, p.M694I	Exon 10
Compound Heterozygous-tri			
1	Female	p.E148Q, p.I692del, p.V726A	Exon 2 and Exon 10

Table 1 continues……..

2	Female	p.M680I, p.M694V, p.V726A	Exon 10
3	Female	p.E148Q, p.I692del, p.V726A	Exon 2 and Exon 10
4	Female	p.M694V, p.M694I, p.R761H	Exon 10
5	Female	p.M694V, p.M694I, p.A744S	Exon 10
6	Male	p.M694V, p.M680I, p.V726A	Exon 10

3.4 Correlation of FMF Genotypes with Clinical Picture

Diagnostic criteria for FMF disease were classified into major, minor and supportive criteria upon which FMF was diagnosed. Within the major criteria, the symptoms were manifested variably among the four genotype categories. The most common manifestation within all categories was fever (Table 2). Of the major criteria, most homozygotes complained just of fever and only 20% of peritonitis however none complained of monarthritis and pleuritis. Single heterozygotes displayed a range of these manifestations however the latter three were uncommon. The majority of compound heterozygous-bi patients reported fever and 50% reported monoarthritis, while almost all compound heterozygous-tri patients manifested both. Regarding the minor criteria, single heterozygous patients experienced the most manifestations compared to other genotypes categories, where mild abdominal pain was the most frequent complaint followed by joint and leg pain. Compound heterozygous-bi patients also displayed a range of these manifestations however less frequently than the former genotype category. Some homozygous patients experienced leg and mild abdominal pain and several compound heterozygous-tri patients complained of only mild abdominal pain. From the supportive criteria, of note, favorable response to colchicine was reported by the majority of patients, regardless of the genotype category, where the dose of colchicine used in all patients was 0.05 mg/kg/day with a maximum dose of 2 mg/day (Table 2). When correlating between the clinical manifestations and the number of mutations, there was very little difference between the frequency of manifestations experienced in patients carrying one mutation compared to those carrying two mutations (homozygotes and compound-bi patients). Similarly, few noticeable differences were observed when comparing patients carrying three mutations with the exception that compound heterozygous-tri patients had higher frequency of monoarthritis but lower frequency of leg pain compared to patients carrying two variants.

Table 2. Correlation between the genotype categories and the clinical manifestations of FMF pediatric patients (n=35)

Clinical Picture	Homozygous (n=5)	Single Heterozygous (n=12)	Compound Heterozygous-bi (n=12)	Compound Heterozygous-tri (n=6)
Major Criteria				
Fever	100% (n=5/5)	93% (n=11/12)	93% (n=11/12)	100% (n=6/6)
Peritonitis	20% (n=1/5)	8% (n=1/12)	8% (n=1/12)	0% (n=0/6)
Pleuritis	0% (n=0/5)	8% (n=1/12)	0% (n=0/12)	17% (n=1/6)
Monoarthritis	0% (n=0/5)	25% (n=3/12)	50% (n=6/12)	83% (n=5/6)
Minor Criteria				
Mild abdominal pain	40% (n=2/5)	66% (n=8/12)	42% (n=5/12)	66% (n=4/6)
Chest pain	20% (n=1/5)	17% (n=2/12)	17% (n=2/12)	17% (n=1/6)
Joint pain	0% (n=0/5)	42% (n=5/12)	25% (n=3/12)	0% (n=0/6)
Leg pain	60% (n=3/5)	42% (n=5/12)	33% (n= 4/12)	0% (n=0/6)
Supportive Criteria				
Favorable response to colchicine	100% (n=5/5)	83% (n=10/12)	75% (n=9/12)	83% (n=5/6)
Consanguinity	20% (n=1/5)	17% (n=2/12)	33% (n=4/12)	17% (n=1/6)
Family History	0% (n=0/5)	0% (n=0/12)	25% (n=3/12)	0% (n=0/6)
Appendectomy/ Laparotomy	0% (n=0/5)	17% (n=2/12)	0% (n=0/12)	17% (n=1/6)

3.5 Correlation of Specific *MEFV* Mutations with Disease Severity

It has been previously suggested by La Regina and colleagues that within compound heterozygous patients, the presence of certain variants, such as p.M694V or p.M680I, can have an up-modulating effect on the other variant; conversely, the presence of p.V726A or p.E148Q mutations was suggested to have a down-regulating effect on the other variant [15]. To investigate this we correlated the presence of these mutations in compound heterozygous-bi and -tri patients to the major criteria manifestations reported by each patient (Table 3). The presence of p.M694V or p.M680I mutations does not appear to increase the severity of the clinical manifestations. In fact a compound heterozygous-bi patient possessing both of these supposedly up-modulating mutations showed fewer of these symptoms than other patients. Likewise, the presence of p.V726A or p.E148Q variants did not appear to lessen the symptoms; a compound heterozygous-tri patient carrying both these mutations displayed several severe symptoms including fever, pleuritis and monoarthritis (Table 3). As per the suggestion of a reviewer, we also attempted to correlate the genotype category with the severity score. Referring to the clinical data collected and based on the severity scoring system previously described by Pras *et al.* we calculated the severity score for each patient [16]. 5/5 (100%) of homozygous patients had intermediate disease. Among single heterozygotes 3/12 (25%) had mild disease, 5/12 (42%) had intermediate disease, and 4/12 (33%) had severe disease. For the compound heterozygous-bi patients 1/12 (8.3%) were with mild disease, 10/12 (83.3%) were with intermediate severity and 1/12 (8.3%) had severe disease. Finally for compound heterozygous-tri patients 2/6 (33%) had mild, 1/6 (17%) had intermediate, and 3/6 (50%) had severe disease. Furthermore, there was no correlation between the type of mutation and the severity score.

Table 3. Correlation between the presence of certain *MEFV* mutations in compound heterozygous patients and the clinical manifestations experienced

	Patient No.	*MEFV* Mutation	Clinical Manifestations (major criteria present)
Compound Heterozygous-bi	1	*p.E148Q**, p.M694I	Fever, peritonitis
	2	p.M680I**, p.M694I	Fever, monoarthritis
	4	p.M694V, p.M680I	Only fever
	5	p.M680I, *p.V726A*	Only monoarthritis
	6	p.M680I, p.M694I	Only fever
	7	*p.E148Q*, p.M694V	Only fever
	8	p.M694V, p.M694I	Fever, monoarthritis
	9	p.M694I, *p.V726A*	Fever, monoarthritis
	10	p.M694V, p.M694I	Fever, monoarthritis
	11	p.M694V, p.M694I	Fever, monoarthritis
	12	p.M694V, p.M694I	Only fever
Compound Heterozygous-tri	1	*p.E148Q, p.V726A*, p.I692del	Fever, pleuritis, monoarthritis
	2	p.M680I, p.M694V, *p.V726A*	Fever, monoarthritis
	3	*p.E148Q, p.V726A*, p.I692del	Fever, monoarthritis
	4	p.M694V, p.M694I, p.R761H	Only fever
	5	p.M694V, p.M694I, p.A744S	Fever, monoarthritis
	6	p.M680I, p.M694V, *p.V726A*	Fever, monoarthritis

**Mutations in bold are mutations which have been suggested to possess up-modulating effects on the other mutation in compound heterozygotes [15]*

*** Mutations in italics are mutations which have been suggested to possess down-modulating effects on the other mutation in compound heterozygotes [15]*

4. DISCUSSION

The correlation between the number and type of *MEFV* gene variants and FMF clinical manifestations has been questionable as many patients show variability in disease presentation, and the results of our study exemplify this. Our study which involved 35 pediatric FMF patients of Egyptian ethnic origin utilized a strip assay to test for twelve most frequently occurring *MEFV* gene variants; we were able to identify mutations in all 35 patients, in contrast to other studies using Egyptian cohorts where in some diagnosed FMF cases no mutations could be detected [17-18]. The patients were classified according to their mutations into 4 different genotype categories: homozygous, single heterozygous, compound heterozygous-bi with the mutated allele carrying 2 variants, and compound heterozygous-tri with the mutated allele carrying 3 variants (Fig. 1). All identified mutations were restricted only to exon 10 and exon 2; for homozygous patients all mutations were situated on exon 10 (Table 1). Among all 35 patients, the most common mutations were p.M694I (28%) followed by p.M694V (22%), p.V726A (16%), p.M680I (16%) and p.E148Q (9%). Similar findings are in previous studies of FMF patients of Egyptian origin where the same mutations represented the most frequently occurring variants in this ethnic group [10,17,19,20]. Homozygous patients had only p.M694I, p.M694V, or p.M680I mutations, a finding similar to a recent study by Talaat and colleagues which reported that these 3

mutations contributed to 65% of the pathogenic variants detected in homozygotes of Egyptian ethnicity [18].

Among our patients, fever was the most common feature of the major criteria, present in almost 100% of the cases regardless of the genotype of the patient or the number of the mutations. This finding corroborates previous studies on Egyptian patients [18,20] but is in contrast to other studies where mild abdominal pain was the most common symptom followed by fever [19]. Heterozygotes – despite having only one mutated allele – presented more aggressive symptoms compared to homozygous patients, which is not in agreement with the finding of Talaat and colleagues on Egyptian pediatric patients as well as other studies involving different ethnicities [12,21-22]. Single heterozygous patients showed a range of other major criteria including peritonitis, pleuritis and monoarthritis; however these clinical manifestations were rare. In our study, for the first time, we further classified complex heterozygous polymorphisms in Egyptian patients into compound heterozygous-bi and -tri genotypes; previous studies have not made this differentiation and have only analyzed the data for compound heterozygotes as a whole [17]. The majority of patients with compound mutations experienced more than one of the major criteria. Over 80% of compound heterozygous-tri patients and 50% of compound heterozygous-bi patients complained of monoarthritis. Homozygotes, on the other hand, despite having pathogenic variants on both alleles, in fact had fewer major clinical manifestations; apart from fever which was present in all patients only 20% suffered of pleuritis (Table 2). These findings differ from several studies which suggested that homozygotes display more severe symptoms and have a more aggressive clinical presentation of the disease [11-12, 22-24]. Many of these studies however examined data of Turkish patients therefore differences in disease presentation between ethnicities can be expected; in fact it has been previously suggested that Egyptian patients may display milder forms of FMF compared to other populations [10]. These results further elucidate the phenotypic diversity of FMF patients and question the strength of the previously suggested correlation between genotype and disease severity. Taken together our results also question the previously proposed “dosage effect” causing symptoms in heterozygotes or a proportionality between the number of mutations and the severity of the disease [25].

As a possible cause for the large phenotypic diversity among FMF patients, a previous study by La Regina and colleagues suggested that in compound heterozygote cases, the presence of certain variants could modulate the effects of the other existing mutation [15]. The proposed up-modulating variants were p.M694V and p.M680I, and down-regulating variants were p.V726A and p.E148Q. Also, several previous studies have described a correlation between the presence of p.M694V variant and severe disease phenotype [10, 17,24,26-27]. However, analysis of variants and clinical manifestations in compound heterozygous-bi and -tri patients revealed no correlation between the presence of any of these mutations and the severity of the manifestations reported by the patients (Table 3) and furthermore there was no correlation between the severity score and the number and type of mutations. Nevertheless it is important to note that the study performed by La Regina and colleagues evaluated the effects of these variants in simple compound heterozygotes and not on the background of two variants as in the case of compound heterozygous-tri patients [15].

Similar discrepancies were observed regarding the minor criteria (Table 2). Single heterozygous and compound heterozygous-bi patients showed a range of minor symptoms, mainly mild abdominal pain and also joint pain, leg pain and less frequently chest pain. It is reasonable to assume that most severe symptoms would be associated with the more numerous mutations of compound heterozygous-tri genotype, but this is not always the case

as can be seen in Table 2. Strangely, compound heterozygous-tri patients did not suffer as many or as frequently of these symptoms. Sixty percent of homozygotes complained of leg pain but only 20% of chest pain and none suffered joint pain. Although in general in all genotypes categories the main complaint was mild abdominal pain, the homozygous genotype had the smallest percentage of patients complaining of this symptom. Moreover, homozygotes responded greatest to treatment compared to other genotypes where 100% experienced favorable response to colchicine (Table 2). This again differs from previous studies which concluded that homozygotes were most unresponsive to treatment [13,18]. Few patients reported consanguinity of parents and an even lesser number had family history of the disease as supportive criteria. It is worth mentioning that this work is not without its limitations, chief of which is the small number of subjects involved in our experiments which could generate considerable type II error. It would be of great value to pursue this study using a larger study cohort.

Our results question the strength of genotype-phenotype correlation in FMF and indicate that *MEFV* genotypes express much more variable phenotypes than previously suggested which is exemplified in our observations that heterozygotes may suffer an aggressive disease whereas homozygotes a milder one in several cases. One possible hypothesis is the existence of genetic or environmental *MEFV* gene modifier factors. One of these modifier factors may be related to sex since both in our study and in previous studies using Egyptian cohorts males represent a larger fraction of the patient population (approximately 1.2:1) [10,18]. A potential explanation for this imbalance, as previously suggested by others, is the incomplete penetrance of the disease phenotype in females or increased embryonic death of female zygotes with two mutated *MEFV* alleles [19,28-29]. Moreover, the variability could arise from patient age; a novel study which involved pediatrics diagnosed with FMF with one mutated *MEFV* allele found that in several of these patients, upon reaching puberty, the clinical signs of FMF completely disappeared [21]. Another important disease-modifying factor is the environment. A recent paper by Ozen and colleagues has shed light on the possible contribution of the country of residence where patients living in eastern Mediterranean countries showed a higher frequency of disease manifestations, and interestingly eastern Mediterranean FMF patients present a milder disease phenotype once they migrate to European countries [30]. Furthermore among other propositions a recently published article by Federici and colleagues has suggested a dosage effect causing symptoms in heterozygotes rather than the concept of a pure autosomal recessive inheritance pattern accompanied by recurrence of mutations and loss of protein function [25].

5. CONCLUSION

In conclusion the lack of a linear correlation between severity of symptoms and number of mutations makes it evident that there is a multitude of modifier factors affecting the phenotype of *MEFV* mutations and this proposal is supported by a recent study by Ben-Zvi *et al.* [31] and is further validated by the existence of multiple phenotypes varying in severity for the same genotype.

CONSENT

All authors declare that written informed consent was obtained from the parents or legal guardians of the patients.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. Am J Med. 1967;43(2):227-53.
2. Gershoni-Baruch R, Shinawi M, Leah K, Badarnah K, Brik R. Familial Mediterranean fever: prevalence, penetrance and genetic drift. Eur J Hum Genet. 2001;9(8):634-7.
3. Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T, et al. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum. 1997;40(10):1879-85.
4. Stojanov S, Kastner DL. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. Curr Opin Rheumatol. 2005;17(5):586-99.
5. Rigante D, La Torraca I, Avallone L, Pugliese AL, Gaspari S, Stabile A. The pharmacologic basis of treatment with colchicine in children with familial Mediterranean fever. Eur Rev Med Pharmacol Sci. 2006;10(4):173-8.
6. Nir-Paz R, Ben-Chetrit E, Pikarsky E, Hassin D, Hasin Y, Chajek-Shaul T. Unusual presentation of familial Mediterranean fever: role of genetic diagnosis. Ann Rheum Dis. 2000;59(10):836-8.
7. The International FMF Consortium. Ancient missense mutations in new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell. 1997;90:797-807.
8. The French FMF Consortium. A candidate gene for familial Mediterranean fever. Nat Genet. 1997;17:25-31.
9. Touitou I, Lesage S, McDermott M, Cuisset L, Hoffman H, Dode C, et al. Infevers: an evolving mutation database for auto-inflammatory syndromes. Hum Mutat. 2004;24(3):194-8.
10. el-Garf A, Salah S, Iskander I, Salah H, Amin SN. MEFV mutations in Egyptian patients suffering from familial Mediterranean fever: analysis of 12 gene mutations. Rheumatol Int. 2010;30(10):1293-8.
11. Inal A, Yilmaz M, Kendirli SG, Altintas DU, Karakoc GB. The clinical and genetical features of 124 children with Familial Mediterranean fever: experience of a single tertiary center. Rheumatol Int. 2009;29(11):1279-85.
12. Sarkisian T, Ajrapetyan H, Shahsuvaryan G. Molecular study of FMF patients in Armenia. Curr Drug Targets Inflamm Allergy. 2005;4(1):113-6.
13. Soylemezoglu O, Arga M, Fidan K, Gonen S, Emeksiz HC, Hasanoglu E, et al. Unresponsiveness to colchicine therapy in patients with familial Mediterranean fever homozygous for the M694V mutation. J Rheumatol. 2010;37(1):182-9.

14. Tchernitchko D, Moutereau S, Legendre M, Delahaye A, Cazeneuve C, Lacombe C, et al. MEFV analysis is of particularly weak diagnostic value for recurrent fevers in Western European Caucasian patients. Arthritis Rheum. 2005;52(11):3603-5.
15. La Regina M, Nucera G, Diaco M, Procopio A, Gasbarrini G, Notarnicola C, et al. Familial Mediterranean fever is no longer a rare disease in Italy. Eur J Hum Genet. 2003;11(1):50-6.
16. Pras E, Livneh A, Balow JE, Jr., Kastner DL, Pras M, Langevitz P. Clinical differences between North African and Iraqi Jews with familial Mediterranean fever. Am J Med Genet. 1998;75(2):216-9.
17. El Gezery DA, Abou-Zeid AA, Hashad DI, El-Sayegh HK. MEFV gene mutations in Egyptian patients with familial Mediterranean fever. Genet Test Mol Biomarkers. 2010;14(2):263-8.
18. Talaat HS, Mohamed MF, El Rifai NM, and Gomaa MA. The expanded clinical profile and the efficacy of colchicine therapy in Egyptian children suffering from familial mediterranean fever: a descriptive study. Ital J Pediatr. 2012;38(1):66.
19. Settin A, El-Baz R, Abd Rasool M, El-Khalegy H, El-Sayed O, El-Bendary M, et al. Clinical and molecular diagnosis of Familial Mediterranean Fever in Egyptian children. J Gastrointestin Liver Dis. 2007;16(2):141-5.
20. Zekri AR, el-Bassuoni MA, Hammad OM, Sakr MA, Ibrahim AA. Application of refractory fragment amplification system for detection of Egyptian variant of Familial Mediterranean Fever. Egypt J Immunol. 2004;11(1):103-10.
21. Hentgen V, Grateau G, Stankovic-Stojanovic K, Amselem S, Jeru I. Familial Mediterranean fever in heterozygotes: are we able to accurately diagnose the disease in very young children? Arthritis Rheum. 2013;65(6):1654-62.
22. Ozturk C, Halicioglu O, Coker I, Gulez N, Sutcuoglu S, Karaca N, et al. Association of clinical and genetical features in FMF with focus on MEFV strip assay sensitivity in 452 children from western Anatolia, Turkey. Clin Rheumatol. 2011;31(3):493-501.
23. Yilmaz R, Ozer S, Ozyurt H, Erkorkmaz U, Sahin S. Familial Mediterranean fever gene mutations in the inner northern region of Turkey and genotype-phenotype correlation in children. J Paediatr Child Health. 2009;45(11):641-5.
24. Makay B and Unsal E. Does breast-feeding affect severity of familial Mediterranean fever? Clin Rheumatol. 2009;28(12):1389-93.
25. Federici S, Calcagno G, Finetti M, Gallizzi R, Meini A, Vitale A, et al. Clinical impact of MEFV mutations in children with periodic fever in a prevalent western European Caucasian population. Ann Rheum Dis. 2012;71(12):1961-5.
26. Fragouli E, Eliopoulos E, Petraki E, Sidiropoulos P, Aksentijevich I, Galanakis E, et al. Familial Mediterranean Fever in Crete: a genetic and structural biological approach in a population of 'intermediate risk'. Clin Genet. 2008;73(2):152-9.
27. Jarjour RA. Familial Mediterranean fever in Syrian patients: MEFV gene mutations and genotype-phenotype correlation. Mol Biol Rep. 2010;37(1):1-5.
28. Gershoni-Baruch R, Brik R, Lidar M, Shinawi M, Livneh A. Male sex coupled with articular manifestations cause a 4-fold increase in susceptibility to amyloidosis in patients with familial Mediterranean fever homozygous for the M694V-MEFV mutation. J Rheumatol. 2003;30(2):308-12.
29. Cazeneuve C, Sarkisian T, Pecheux C, Dervichian M, Nedelec B, Reinert P, et al. MEFV-Gene analysis in armenian patients with Familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype-genetic and therapeutic implications. Am J Hum Genet. 1999;65(1):88-97.
30. Ozen S, Demirkaya E, Amaryan G, Kone-Paut I, Polat A, Woo P, et al. Results from a multicentre international registry of familial Mediterranean fever: impact of environment on the expression of a monogenic disease in children. Ann Rheum Dis; 2013.

31. Ben-Zvi I, Brandt B, Berkun Y, Lidar M, Livneh A. The relative contribution of environmental and genetic factors to phenotypic variation in familial Mediterranean fever (FMF). Gene. 2012;491(2):260-3.

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