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Familial Mediterranean Fever Associated with *MEFV* Mutations in a Large Cohort of Cypriot Patients

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Summary

Familial Mediterranean fever (FMF) is caused by mutations in the *MEFV* gene and the spectrum of mutations among Greek–Cypriots with FMF-related symptoms was examined. Sequence analysis for exons 2, 3, 5, and 10 of the *MEFV* gene was performed in a cohort of 593 patients. A total of 70 patients carried mutations in the homozygote or compound heterozygote state, 128 were identified with one *MEFV* mutation and 395 had no mutations. Of the 268 identified alleles, p.Val726Ala (27.61%) was the most frequent followed by p.Met694Val (19.40%). The missense mutations p.Arg761His (3.73%) and p.Ala744Ser (2.24%) were identified as the rarest. An interesting finding is the high frequency (18.28%) of the complex p.Phe479Leu–p.Glu167Asp that was identified in 49 of the mutated alleles. The *MEFV* genotypes did not follow a binomial distribution and proved not to satisfy the HWE ($P < 0.001$). The high percentage (66.61%) of patients with unidentified mutations could be due to mutations in the rest of the coding or noncoding *MEFV* gene or due to mutations in other genes that are also causing Hereditary Recurrent Fevers. Results from this work indicate the high incidence of FMF in Cyprus and describe the spectrum of the mutations which occur in the country.

Keywords: Cyprus, FMF, hereditary recurrent fevers, *MEFV*

Introduction

Familial Mediterranean fever (FMF) belongs to the family of the Hereditary Recurrent Fevers (HRFs) and is one of the most frequent autosomal recessive disorders, commonly found among individuals of Mediterranean origin and particularly the non-Ashkenazi Jews, Armenians, North Africans, Arabs and Turks (La Regina et al., 2003; Lidar & Livneh,

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2007). The diagnosis is made after clinical suspicion based on the Tel–Hashomer criteria (Pras, 1998) and is characterized by recurrent self-limiting episodes of fever and serositis, that appear every few weeks to months or years (Livneh et al., 1997). The most severe complication of FMF is secondary amyloidosis, commonly influencing the kidneys and sometimes other vital organs such as the adrenals, intestine, spleen, lung, and testis (Livneh et al., 1997; Touitou, 2001).

The identification of *MEFV* as the causative gene more than 15 years ago resulted in numerous investigations worldwide that examined the frequency and the genotypic variability of the disease (Pras et al., 1992; French FMF Consortium, 1997; The International FMF Consortium, 1997). Since the discovery of the *MEFV* gene, more than 250 sequence variants have been reported and recorded in the Infevers database (http://fmf.igh.cnrs.fr/ISSAID/infevers/) (Sarrauste de Menthiere et al., 2003; Touitou et al., 2004; Milhavet et al., 2008). Several of these mutations are apparently pathogenic but most are unconfirmed or seemingly nonpathogenic variants (Shinar et al., 2012). Five of the most commonly observed mutations are responsible for 65%–95% of observed mutations in different ethnic groups. These five mutations include: p.M680I (c.2040G > C), p.M694V (c.2080A > G), p.M694I (c.2082G > A), and p.V726A (c.2177T > C), and p.E148Q (c.442G > C) (Touitou, 2001). A substantial number of Mediterranean ancestry patients clinically diagnosed with recessive FMF have been found to carry only one mutation in the *MEFV* gene despite extensive investigation for a second pathogenic mutation in the coding and regulatory region of the gene. Such heterozygote patients usually respond well to colchicine treatment, which led to the idea that FMF might also manifest in heterozygotes (Booty et al., 2009; Grandemange et al., 2009; Marek-Yagel et al., 2009; Medlej-Hashim et al., 2010; Jeru et al., 2013;).

Previous research in the Cyprus population identified the *MEFV* allelic frequency in a smaller sample of Cypriot origin (Deltas et al., 2002). In this study, we report the results of a large cohort of patients with HRFs who underwent genetic analysis for the *MEFV* gene. Since studies in neighboring countries in the Mediterranean region have reported FMF as one of the most prevalent inherited disorders, we aimed to further analyze the spectrum of mutations in the Greek–Cypriot patients.

Materials and Methods

Ethics Statement

The study has been approved by the Cyprus National Bioethics Committee and informed consent was obtained from all patients who participated in the study.

Patients

A total of 593 unrelated patients (272 males, 321 females) with recurrent fevers were referred to the Cyprus Institute of Neurology and Genetics. All patients were clinically diagnosed with FMF, according to Tel–Hashomer criteria (described above) or demonstrated symptoms related to FMF and Hereditary Recurrent Fevers (HRFs).

Amplification and direct sequencing of MEFV exons 2, 3, 5, and 10

The sequence information of the *MEFV* gene was obtained from http://www.ensembl.org (ENSG00000103313) and exons 2, 3, 5, and 10 of all patients were analyzed using genomic DNA isolated from peripheral blood samples. The *MEFV* gene exons were amplified using the primers Exon 2F: 5'-CTC CTC TGC CCT GAA TCT TG-3' and Exon 2R: 5'-CTC AAA GTC TTG GCC TCC AG-3'; for Exon 3F: 5'-CCT GTT TGC TTC CTC ACT GG-3' and Exon 3R: 5'-TAA TGC ACC AAC AAC CCA GA-3'; Exon 5F: 5'-AGC CCA CCT CTT ATC CAC CT-3' and Exon 5R: 5'-GTG GGT CAC CAA GAC CAA GT-3'; Exon 10F: 5'-TAC CCT GTC CCT GTT TCC TG-3' and Exon 10R: 5'-GTC GGC ATT CCG TGA CTA TT-3'. The Primers were designed using the Primer 3 program of the Whitehead Institute for Biomedical Research (http://bioinfo.ut.ee/primer3--0.4.0/primer3/). The conditions of the PCR amplification of *MEFV* exons 2, 3, 5, and 10 are available upon request. PCR amplification was carried out using BigDye terminator v1.1, cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Amplification products were run on an automated Applied Biosystems 3130*xl* Genetic Analyzer.

Statistical Analyses

The statistical analysis was carried out in a sample of 593 patients (272 males, 321 females). The statistical program *IBM SPSS* Statistics 20.0 was used for the descriptive statistics summary of the cohort under investigation (homozygotes, compound heterozygotes, heterozygotes and patients with no identified mutation in the *MEFV* gene). The statistical analysis tested whether the distribution of genotypes among all patients follows a binomial distribution [i.e., Hardy–Weinberg equilibrium (HWE) is satisfied] and the same test was applied for each of the most common *MEFV* mutations separately.

Following Cazeneuve et al. (2003) the number of patients with FMF symptoms which are not related to the *MEFV* mutations (N_{OTHER}) was calculated by subtracting the number of patients whose disease phenotype was due to *MEFV* mutations (N_{MEFV}) that is, the patients who carry two mutated

alleles (*I/I* genotype), from the total number of patients, that is

$$N_{OTHER} = N_{TOTAL} - N_{MEFV},$$

where

$$N_{MEFV} = \frac{\left(n_{I/I} + \frac{n_{I/NI}}{2}\right)^2}{n_{I/I}}$$

In the N_{MEFV} equation it is also considered the assumption that some mutated alleles might have escaped the screening procedure of the present study in patients with FMF symptoms carrying the *I/NI* genotype.

Results

The spectrum and frequency of the *MEFV* gene defects in the cohort of 593 Cypriot HRF patients is depicted in Table 1. A total of 198/593 patients with FMF-related symptoms were identified with *MEFV* mutations in the heterozygote, homozygote or compound heterozygote state.

Seventy patients (11.80%) were verified with mutations in the *MEFV* gene in both alleles and 128 individuals (21.59%) in the heterozygote state. Nineteen patients (3.20%) were homozygous for the same mutation while 51 (8.60%) were compound heterozygous for various combinations of mutations. The remaining 395 individuals (66.61%) of this study with clinical suspicion of FMF were identified with no mutations in the *MEFV* gene (Table 1).

The overall allelic frequency of *MEFV* defects in the Cypriot cohort of 1186 unrelated alleles is illustrated in Table 2. The most frequent defect among the 268 Cypriot identified alleles was p.Val726Ala (27.61%) followed by p.Met694Val (19.40%), the complex allele p.Phe479Leu-p.Glu167Asp (18.28%), p.Glu148Gln (15.67%), p.Met680Ile (6.72%), and p.Met694Ile (6.34%). The missense mutations p.Arg761His (3.73%) and p.Ala744Ser (2.24%) were identified as the rarest.

A comparison by gender of the allelic frequency for each one of the identified alleles was attempted. In males, the missense mutation p.Val726Ala (31.01%) was the most frequent mutation followed by p.Met694Val (19.38%), p.Phe479Leu-p.Glu167Asp (19.38%), p.Glu148Gln (13.95%), p.Met680Ile (6.20%), and p.Met694Ile (5.43%). The least frequent mutations in the males were the missense mutation p.Arg761His and p.Ala744Ser with a frequency of 3.10% and 1.55%, respectively.

In females the mutation frequencies were comparable to some extent to the ones observed in the males. The missense mutation p.Val726Ala (24.46%) was also the most frequent and was followed by p.Met694Val (19.42%), p.Glu148Gln (17.27%), and p.Phe479Leu–p.Glu167Asp (17.27%). The least frequent mutations in the females were p.Arg761His and p.Ala744Ser which represented 4.32% and 2.88%, respectively of the *MEFV* identified alleles. One limitation of this study is that the analyzed cohort is probably heterogeneous and contains patients which may not strictly fulfill the Tel–Hashomer criteria for the diagnosis of FMF (as described in the Materials and Methods' patients section). This is an inherent drawback as our organization, which is a National referral center, receives referrals from a great number

Table 1 Types and frequency of molecular *MEFV* defects in the cohort of 593 Cypriot HRF patients.

	# of HRF Patients with *MEFV* defects	%
Homozygotes		
p.Val726Ala/p.Val726Ala	1	5.3%
p.Met694Val/p.Met694Val	8	42.1%
p.Met694Ile/p.Met694Ile	1	5.3%
p.Met680Ile/p.Met680Ile	1	5.3%
p.Phe479Leu–p.Glu167Asp⁇/p.Phe479Leu–p.Glu167Asp⁇	8	42.1%
	19	100%
Compound heterozygotes		
p.Val726Ala/p.Arg761His	5	9.8%
p.Val726Ala/p.Met680Ile	3	5.9%
p.Val726Ala/p.Ala744Ser	1	1.95%
p.Val726Ala/p.Met694Val	9	17.7%
p.Val726Ala/p.Glu148Gln	2	3.9%
p.Val726Ala/p.Phe479Leu–p.Glu167Asp*	19	37.25%
p.Met694Val/p.Met680Ile	2	3.9%
p.Met694Ile/p.M680Ile	1	1.95%
p.Glu148Gln/p.Met694Val	7	13.7%
p.Glu148Gln/p.Met680Ile	1	1.95%
*p.Phe479Leu–p.Glu167Asp/p.Met694Val	1	1.95%
	51	100%
Heterozygotes		
p.Val726Ala/X	33	25.8%
p.Met694Val/X	17	13.3%
p.Met694Ile/X	14	10.9%
p.Arg761His/X	5	3.9%
p.Met680Ile/X	9	7.0%
p.Glu148Gln/X	32	25.0%
*p.Phe479Leu–p.Glu167Asp/X	13	10.15%
p.Ala744Ser/X	5	3.9%
	128	100%
X/X	395	66.6%
Total	593	100%

*p.Phe479Leu–Glu167Asp is known to be coinherited.

Table 2 The overall allelic *MEFV* frequency in the cohort of 593 Cypriot patients.

Mutation	# of alleles	% alleles in the Cypriot cohort of patients under investigation ($n = 1186$)	% identified *MEFV* alleles ($n = 268$)
p.Val726Ala	74	6.24%	27.61%
p.Met694Val	52	4.38%	19.40%
p.Met694Ile	17	1.43%	6.34%
p.Arg761His	10	0.84%	3.73%
p.Met680Ile	18	1.52%	6.72%
p.Glu148Qln	42	3.54%	15.67%
p.Phe479Leu–p.Glu167Asp	49	4.13%	18.28%
p.Ala744Ser	6	0.51%	2.24%
No mutations	918	77.40%	–
Total	1186	100%	100%

of physicians throughout the island, some of whom may refer if only certain and not all criteria are met. This in turn may lead to imprecise allele distributions which may represent an explanation as to why we identify a lower percentage of *MEFV* positive patients compared to those in the literature.

The relatively small proportion of patients found in the sample of 593 Cypriot patients with only one identified mutated allele, as well as the high proportion of patients with no identified mutations, provided a sequel to our analysis with a χ^2test for testing if the distribution of *I/I* (two identified *MEFV* mutations), *I/NI* (one identified *MEFV* mutation), and *NI/NI* (no identified *MEFV* mutations) genotypes complied with HWE (Table 3). The results of the above analysis revealed that the distribution of genotypes among Cypriot patients differs significantly from a binomial distribution ($P < 0.001$) (Table 3). The distribution of p.Val726Ala and p.Glu148Gln does not differ from HWE at the 5% significance level. However, four of the five tested mutations (p.Val726Ala, p.Met680Ile, p.Met694Ile, and p.Glu148Qln) do not differ from HWE equilibrium at the 1% significance level and their distribution is considered to comply with HWE (Table 4).

The proportion of patients in the cohort of 593 whose HRF phenotype did not result from mutations in the *MEFV* gene, $\frac{N_{OTHER}}{N_{TOTAL}}$, was calculated to be equal to 57%. This proportion ranges between 7% and 21% in the classically affected populations (Table 5).

The proportion of the 395 HRF patients with no identified mutations in the *MEFV* gene, $\frac{N_{OTHER}}{n_{NI/NI}}$, is 85% and was compared with the proportions observed in other classically affected populations (Table 5).

Moreover, the proportion of patients with HRF phenotype which is suspected to result from unidentified mutations in the cohort of 593, $\frac{n_{NI/NI}-N_{OTHER}}{N_{TOTAL}}$, was found to be equal to 10% while the same proportion varies from 0.1% to 2.3% for the classically affected populations (Table 5). The observed large proportion of the HRF patients whose phenotype did not result from mutations in the *MEFV* gene for all three of the above statistical combinations could be attributed to the presence of mutations in other exons that have not been sequenced.

Discussion

This study identified the *MEFV* spectrum of mutations in a total of 593 unrelated individuals of Cypriot origin with recurrent fevers and mean age of 25 years. The only objective tool that confirms FMF is *MEFV* sequencing. Therefore, the testing strategy adopted here is similar to the one suggested by the FMF genetic diagnosis guidelines that were prepared in a consensus document disseminated through the European Molecular Genetics Quality Network, and involves direct sequencing of *MEFV* exons 2, 3, 5, and 10 where most of the *MEFV* frequent mutations are located (Shinar et al., 2012).

The current study established eight *MEFV* mutations as the ones most commonly encountered in the Cypriot population with p.Val726Ala (c.2177T > C) being the most common. The allelic frequency of p.Val726Ala being 27.6% of the total *MEFV* alleles in the Cypriot patients conforms well to the known allelic frequencies observed in Israelis (29%) and Ashkenazi Jews (38%) (Table 2) (Touitou, 2001). In general, p.Val726Ala is more prevalent in non-classically affected populations, and affected individuals develop symptoms at an earlier age. Such individuals are also usually associated with milder clinical features (Touitou, 2001; Solak et al., 2008). Recently, it was reported that p.Val726Ala is also predominant among Arabs, with an average frequency of 33% (Sharkia et al., 2013).

In the present study, the allelic frequency of 19.4% for the second most prevalent mutation, p.Met694Val (c.2080A > G), is comparable to that observed in Jordanian patients

Table 3 The observed distribution of *I/I* (two *MEFV* mutations), *I/NI* (one *MEFV* mutation), and *NI/NI* (no *MEFV* mutation) *MEFV* genotypes with the theoretical proportion, expected from Hardy–Weinberg equilibrium, in the Cypriot cohort of 593 patients.

Genotypes	Observed (O)	Expected (E)	$O-E$	$(O-E)^2$	$\frac{(O-E)^2}{E}$
I/I	70	30.28	39.72	1577.68	52.10
I/NI	128	207.44	−75.52	5703.27	30.42
NI/NI	395	355.28	34.22	1170.85	4.44
					$X^2 = 86.96,\ P < 0.00001$

Table 4 The distribution of the five most common *MEFV* mutations in the Cypriot cohort of 593 patients tested with the X^2 test.

Mutations	X^2	*P* value
p.Met694Val	45.23	<0.001
p.Val726Ala	0.88	0.3482
p.Met680Ile	5.61	0.0179
p.Met694Ile	6.49	0.0108
p.Glu148Qln	0.82	0.3643

Table 5 Proportion of patients with genotype NI/NI whose phenotype results from or does not result from mutations in the *MEFV gene*.

Population	$n_{NI/NI}$	N_{OTHER}	N_{TOTAL}	$\frac{N_{OTHER}}{N_{TOTAL}}$	$\frac{N_{OTHER}}{n_{NI/NI}}$	$\frac{n_{NI/NI}-N_{OTHER}}{N_{TOTAL}}$
Greek–Cypriots (Present study)	395	336.50	593	0.567	0.851	0.100
Arabs (40)	14	13.80	65	0.212	0.986	0.003
Armenians (40)	10	9.80	147	0.067	0.700	0.001
Turks (40)	36	30.60	230	0.133	0.850	0.023
non-Ashkenazi Jews (40)	14	12.20	178	0.069	0.871	0.010

(Medlej-Hashim et al., 2000), and is significantly lower than the frequencies reported in Armenians, non-Ashkenazi Jews and Turks, which range from 37% to 71% (Touitou, 2001). Moreover, in this study, the allelic frequencies of 42.1% and 13.3% were identified for the missense p.Met694Val in the homozygote and heterozygote state, respectively (Table 1). In the Greek HRF patients the missense mutation p.Met694Val was also reported as being the most frequent mutation and accounts for almost half of the identified alleles (48%) (Konstantopoulos et al., 2003).

The missense mutation p.Phe479Leu (c.1437C > G) was found to be the third most frequent mutation, representing 18.28% of the characterized alleles (Table 2). In a previous study that also investigated the genetic make-up of FMF in Cypriot patients, p.Phe479Leu was reported as the second most common mutation but the alleles under investigation were significantly less frequent compared to the ones examined in the present study (Deltas et al., 2002). Noticeably, p.Phe479Leu is rare in Armenians (<1%) and Jordanians (<1%) or nonexistent in other populations. An interesting finding is the *in cis* coinheritance of p.Phe479Leu with p.Glu167Asp (c.501G > C) observed in 18.28% of Cypriot FMF alleles in the present study. It is possible that the p.F479L-p.E167D *in cis* combination may have originated as a *de novo* mutation in Cyprus.

The allelic frequency of p.Glu148Gln (c.442G > C) which is a variant of uncertain clinical significance was found to be 15.67% (Table 2). Various studies have established p.Glu148Gln as a pathologic variant associated with a milder form of FMF (Stoffman et al., 2000; Konstantopoulos et al., 2005; Solak et al., 2008; Tomiyama et al., 2008). On the contrary, other studies have not ascertained p.Glu148Gln as a disease causing mutation and considered it as a polymorphism (Ben-Chetrit et al., 2000; Tchernitchko et al., 2006). In general, p.Glu148Gln is characterized as a solely European mutation in populations where FMF is distinctly rare (Lidar & Livneh, 2007). However, it was recently reported that p.Glu148Gln is the second most frequent variant in Turks (18.3%) (Solak et al., 2008), Arabs (21%), and Jews (16%) (Sharkia et al., 2013). A report by Gershoni-Baruch et al. (2002) described the clinical severity exhibited in compound heterozygous patients for p.Glu148Qln/p.Val726Ala to be

as severe as the one observed in homozygous patients for p.Met694Val (Gershoni-Baruch et al., 2002). In a similar fashion, the two patients of this study identified as compound heterozygous for p.Glu148Gln/p.Val726Ala also exhibited severe clinical manifestations.

The more detailed genetic analysis of the *MEFV* gene employed in the present study led to the identification of p.Met680Ile (c.2040G > C) with an allelic frequency of 6.72%. It is speculated that the severe phenotype resulting from p.Met680Ile may be attributed, for unknown reasons, to codon 680 of the *MEFV* protein. In general, mutants located within the regions characterized as mutational "hot-spots" (codons 680 and 694) of the *MEFV* gene have been known to be associated with the severe FMF format of the disorder (Touitou, 2001).

In this study the severe missense mutation p.Met694Ile (c.2082G > A) was identified with an allelic frequency of 6.34%. This mutation is fairly frequent among Arab populations and has been reported as the third most frequent mutation, representing 14% of the identified alleles (Touitou, 2001; Majeed et al., 2005; Belmahi et al., 2006).

The two least common mutations were found to be the missense mutation p.Arg761His (c.2282 G > A) and p.Ala744Ser (c.2230 G > T), with a frequency of 3.73% and 2.24%, respectively (Table 3). The missense mutation p.Ala744Ser is the second mutation which Deltas et al. (2002) failed to detect in the examined Cypriot cohort. This was probably due to restrictions of the methodology used at the time. These mutations are rarely found in other populations and their presence in a homogeneous population like the one in Cyprus could be attributed to the founder effect phenomenon (Shammas et al., 2012).

The frequency of FMF patients carrying only one *MEFV* mutation was also evidenced in this study and was found to be consistent with the hypothesis that clinical symptoms of the disorder may also be present in carriers. Another interesting explanation could be the digenic or oligogenic models of inheritance that until recently, were characterized as monogenic (Booty et al., 2009).

The *MEFV* genotypes of this study did not follow a binomial distribution and the HWE equilibrium is not satisfied (Table 3). This finding results from the relatively significant difference between the observed and expected frequencies of the patients with only one identified mutation and the patients with no identified mutations. The observed number of patients with only one identified *MEFV* allele was always smaller than expected, while the observed number of patients with no identified *MEFV* alleles always exceeded the expected frequencies (Cazeneuve et al., 2003).

According to Cazeneuve et al. (2003), three scenarios could explain this observation, such as consanguinity, biased sampling and the presence of an FMF-like phenotype which does not result from *MEFV* mutations but from alterations in other gene(s). As mentioned in the results section, one limitation of our study is that the analyzed cohort may be heterogeneous therefore a degree of sample bias could have been introduced. Consanguinity is very rare in Cyprus mainly due to religious constraints and is therefore unlikely to have contributed significantly to our results. However, when testing the distribution for each of the most common *MEFV* mutations separately, these did not differ significantly from HWE expectations, indicating that FMF patients were randomly selected and that the requirements for HWE were satisfied. The most likely scenario in the present study may be that the presence of an FMF-like phenotype does not result from *MEFV* mutations but from alterations in other gene(s) (Cazeneuve et al., 2003).

The proportion of the 395 patients with no identified mutations whose phenotype could not be explained by mutations in the *MEFV* gene is estimated to be 85% (Table 5). Similar proportions calculated among other classically affected populations were observed in the Turkish (85%), Armenian (98%), Arab (99%), and non-Ashkenazi Jewish (87%) populations (Cazeneuve et al., 2003). This further supports the presence of an FMF-like phenotype which is not related to mutations in the *MEFV* gene.

The proportion of patients in the cohort of 593 whose phenotype did not result from mutations in the *MEFV* gene was found to be equal to 57% (Table 5). The proportion of patients, whose phenotype is suspected to result from unidentified mutations in the *MEFV* gene in the same cohort, was calculated to be 10% (Table 5). These proportions are significantly larger than the relative proportions among the classically affected populations. The most likely explanation for this observation is cohort heterogeneity as some of the tested patients may not strictly fulfill the Tel–Hashomer diagnostic criteria. Another potential explanation may include the presence of mutations in other exons that have not been sequenced (Konstantopoulos et al., 2003). Disease-causing mutations may also reside in the noncoding or regulatory regions affecting splicing or the messenger RNA expression (Booty et al., 2009). The presence of dominant negative mutations or mutations with a high frequency could also be another explanation (Booty et al., 2009).

In a recent study by Yepiskoposyan & Harutyunyan (2007) a map of the known *MEFV* mutations around the world was established and haplotype analysis dated p.Met694Val, p.Val726Ala, and p.Glu148Gln in the Middle East to more than 2500 years ago. In this same study, the missense p.Met694Val mutation was shown to be present in about 80% of the North African Jewish population and p.Val726Ala was found to be most frequent among the Ashkenazi Jewish, the Druze, and the Armenian FMF patients (Yepiskoposyan & Harutyunyan, 2007).

In conclusion, this study identified the spectrum of *MEFV* mutations in a large cohort of Cypriot patients who presented FMF-like symptoms and which mirror the allelic heterogeneity which characterizes FMF in the island. The presence of an FMF-like phenotype which does not result from alterations in the *MEFV* gene and results from mutations in other gene(s) is very likely. The frequency of FMF patients carrying only one *MEFV* mutation was also evidenced in this study and was found to be consistent with the hypothesis that clinical symptoms of the disorder may also be present in carriers. Therefore, such studies that identify the genetic basis of detrimental disorders like FMF are extremely useful since they can be used in effective diagnosis, can assist in genetic counseling and can be used for the improvement of better therapeutic approaches.

Conflict of Interest

The authors declare no conflict of interest.

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