Annals of human genetics

Familial Mediterranean Fever associated with MEFV mutations in a large cohort of Cypriot patients

Journal:	*Annals of Human Genetics*
Manuscript ID:	AHG-MS-14-0111
Manuscript Type:	Regular manuscript
Date Submitted by the Author:	16-Jun-2014
Complete List of Authors:	Neocleous, Vassos; The Cyprus Institute of Neurology and Genetics,, Molecular Genetics, Function and Therapy Costi, Constantina; The Cyprus Institute of Neurology and Genetics,, Molecular Genetics, Function and Therapy Kyriakou, Christina; The Cyprus Institute of Neurology and Genetics,, Molecular Genetics, Function and Therapy Kyriakides, Tassos; Yale University, Dept of Epidemiology & Public Health Shammas, Christos; The Cyprus Institute of Neurology and Genetics,, Molecular Genetics, Function and Therapy Skordis, Nicos; Hospital 'Archbishop Makarios III', Department of Pediatrics; St George's, University of London Medical School at the University of Nicosia, Department of Medicine Toumba, Meropi; Iasis Hospital, Department of Pediatrics Koliou, Maria; Hospital 'Archbishop Makarios III', Department of Pediatrics Kousparou, Marianna; Hospital 'Archbishop Makarios III', Department of Pediatrics Onoufriou, Margarita; Hospital 'Archbishop Makarios III', Department of Pediatrics Atamyan, Vick; Corner Limassol Av. & Armenias Str, Department of Internal Medicine Pierides, Alkis; Hippocrateon Hospital, Department of Nephrology Anastasiadou-Christophidou, Violetta; Hospital 'Archbishop Makarios III', Department of Pediatrics Tanteles, George; The Cyprus Institute of Neurology and Genetics, Department of Clinical Genetics Phylactou, Leonidas; The Cyprus Institute of Neurology and Genetics, Molecular Genetics, Function and Therapy
Keywords:	Cyprus, FMF, Hereditary Recurrent Fevers, MEFV

Familial Mediterranean Fever associated with *MEFV* mutations in a large cohort of Cypriot patients

VASSOS NEOCLEOUS[1], CONSTANTINA COSTI[1], CHRISTINA KYRIAKOU[1], TASSOS C KYRIAKIDES[2], CHRISTOS SHAMMAS[1], NICOS SKORDIS[3,4], MEROPI TOUMBA[5], SOPHIA KYRIAKOU[6], MARIA KOLIOU[3], MARIANNA KOUSPAROU[3], MARGARITA ONOUFRIOU[3], ADAMOS HADJIPANAYIS[7,8], MICHALIS IASONIDES[9], VICK N ATAMYAN[10], ALKIS PIERIDES[11], VIOLETTA ANASTASIADOU-CHRISTOPHIDOU[3,12], GEORGE A TANTELES[12] and LEONIDAS A PHYLACTOU[1]

[1]Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics, 1683 Nicosia, Cyprus

[2]Dept of Epidemiology & Public Health, Yale University, USA

[3]Department of Pediatrics, Hospital 'Archbishop Makarios *III*', 1474 Nicosia, Cyprus

[4]St George's, University of London Medical School at the University of Nicosia;

[5]Iasis Hospital, 8036 Paphos, Cyprus

[6]University of Cyprus, Department of Economics

[7]Department of Pediatrics, Larnaca General Hospital, Larnaca, Cyprus

[8]European University of Cyprus, The School of Medicine

[9]Iliaktida Peadiatric & Adolescent Medical Centre, 4001 Limassol, Cyprus

[10]Corner Limassol Av. & Armenias Str., Acropolis, Nicosia, Cyprus

[11]Department of Nephrology, Hippocrateon Hospital, Nicosia, Cyprus

[12]Department of Clinical Genetics, The Cyprus Institute of Neurology and Genetics, 1683 Nicosia, Cyprus

Correspondence: Leonidas A Phylactou, PhD

Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics, P.O. Box 23462, 1683 Nicosia, Cyprus. Tel.: +35722358600; Fax: +35722392817; E-mail: laphylac@cing.ac.cy

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 with no mutations. Of the 268 identified alleles, p.Val726Ala (27.61%) was the most frequent followed by p.Met694Val (19.40%). The missense 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 Hardy-Weinberg equilibrium (*p-value* <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, 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 causing gene more than 15 years ago resulted into numerous investigations worldwide that examined the frequency and the genotypic variability of the disease (Pras *et al.*, 1992, 1997a, 1997b). Since the discovery of the *MEFV* gene, more than 250 sequence variants have been reported and recorded in Infevers database (http://fmf.igh.cnrs.fr/ISSAID/infevers/) (Sarrauste de Menthiere *et al.*, 2003, Touitou *et al.*, 2004, Milhavet *et al.*, 2008). The majority of these mutations are undoubtedly pathogenic and 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 the 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 lead to the idea that FMF might manifest also in heterozygotes (Booty *et al.*,

2009, Marek-Yagel *et al.*, 2009, Jeru *et al.*, 2013, Grandemange *et al.*, 2009, Medlej-Hashim *et al.*, 2010).

Previous research in the Cyprus population showed identified the *MEFV* allelic frequency in a smaller sample of Cypriot origin (Deltas *et al.*, 2002). In the present 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 Cyprus National Bioethics Committee and informed consent was obtained from all patients that 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 the 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 3program of the Whitehead Institute for Biomedical Research (*http://bioinfo.ut.ee/primer3-0.4.0/primer3/*). The conditions of the PCR amplification of the *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 is satisfied) and the same test was also 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}) i.e.

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

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

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 fifty one (8.60%) were compound heterozygous for various combinations of mutations. The remaining 395 individuals (66.61%) of the present 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.Glu148Qln (15.67%), p.Met680Ile (6.72%) and p.Met694Ile (6.34%). The missense p.Arg761His (3.73%) and p.Ala744Ser (2.24%) were identified as the rarest.

A comparison per gender of the allelic frequency for each one of the identified alleles was attempted. In males, the missense p.Val726Ala (31.01%) was the most frequent mutation followed by p.Met694Val (19.38%), p.Phe479Leu-p.Glu167Asp (19.38%), p.Glu148Qln (13.95%), p.Met680Ile (6.20%) and p.Met694Ile (5.43%). The least frequent mutations in the males were the missense 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 p.Val726Ala (24.46%) was also the most frequent and was followed by p.Met694Val (19.42%), p.Glu148Qln (17.27%) and p.Phe479Leu-p.Glu167Asp (17.27%). The least frequent mutations in the females were p.Arg761His and p.Ala744Ser and represented the 4.32% and 2.88%, respectively of the *MEFV* identified alleles.

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 sequel to our analysis with a χ^2 test 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 Hardy-Weinberg equilibrium (Table 3). The results of the above analysis revealed that the distribution of genotypes among Cypriot patients differs significantly from a binomial distribution (*p-value* <0.001) (Table 3). The distribution of p.Val726Ala and p.Glu148Qln does not differ from Hardy-Weinberg equilibrium at the 5% significance level. However, all five of the above tested mutations except p.Met694Val differ from Hardy-Weinberg equilibrium at the 1% significance level and their distribution is considered to comply with the Hardy-Weinberg equilibrium (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 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

The present 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 the *MEFV* gene analysis. Therefore, the testing strategy adopted by the present study 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 the *MEFV* exons 2, 3, 5 and 10 where most of the *MEFV* frequent mutations are located (Shinar *et al.*, 2012).

The current study established 8 *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. They are also usually associated with milder clinical features (Touitou, 2001, Solak *et al.*, 2008). Recently, it was reported that it is also predominant among Arabs as well, 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 p.Met694Val (c.2080A>G) mutation is comparable to the one observed in Jordanian patients (Medlej-Hashim *et al.*, 2000), and significantly lower to the one reported in Armenians, non-Ashkenazi and Turks, ranging from 37% to 71% (Touitou, 2001). In the Greek HRF patients the missense 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). Contradicting results on the severity of the FMF disorder have been generated for the carriers of p.Met694Val. An initial report suggested that carriers of p.Met694Val manifest more severe symptoms and are in greater risk for developing amyloidosis (Dewalle *et al.*, 1998). Several studies that followed have also shown that p.Met694Val is associated with a generally more severe form of the disease (Delibas *et al.*, 2005, Mattit *et al.*, 2006, Pasa *et al.*, 2008). However, recent studies demonstrated that individuals with FMF who were homozygous, heterozygous or compound heterozygous for the p.Met694Val mutation experienced a more severe clinical course but with lower rates of amyloidosis (Caglayan *et al.*, 2010, Inal *et al.*, 2009, Ureten *et al.*, 2010) .

The missense mutation p.Phe479Leu (c.1437C>G) was found as the third most frequent mutation, representing the 18.28% of the characterized alleles (Table 2). In a previous study that also investigated the genetic makeup of FMF in Cypriot patients, p.Phe479Leu was reported as the second most common mutation but the alleles under investigation were significantly less 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 of the present study. The possibility of p.F479L-p.E167D *in cis* combination to have originated as a *de novo* mutation in Cyprus is possible. In populations of other ethnic origins these variants are inherited separately, therefore the possibility of having a Cypriot ancestor carrier of p.Phe479Leu prior to this event cannot be excluded (Deltas *et al.*, 2002).

In the present study, the allelic frequency of the debated p.Glu148Qln (c.442G>C) was found to be 15.67% (Table 2). Various studies have established p.Glu148Qln 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.Glu148Qln as a disease causing mutation and considered it as a polymorphism (Ben-Chetrit *et al.*, 2000, Tchernitchko *et al.*, 2006). In general, p.Glu148Qln is characterized as a solely European mutation in populations where FMF is distinctly rare (Lidar & Livneh, 2007). However, it was recently reported that p.Glu148Qln 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 *et al.* (2002) described the clinical severity exhibited in compound heterozygous patients for p.Glu148Qln/p.Val726Ala 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 the present study identified as compound heterozygous for the p.Glu148Qln/p.Val726Ala also exhibited severe clinical manifestations.

The earlier targeted mutation analysis by Deltas *et al*. (2002) in a cohort of Cypriot patients for eight mutations of the *MEFV* gene failed to detect p.Met680Ile as this was not included in the panel under investigation. 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%. Similar allelic frequencies for the p.Met680Ile were also reported in Arab populations (Majeed *et al.*, 2005). Several other reports demonstrated p.Met680Ile as more frequent in Armenians and Turks (Yalcinkaya *et al.*, 2000). It is speculated that the severe causing phenotype of p.Met680Ile to be attributed for unknown reasons to codon 680 of the *MEFV* protein. In general mutants located within the 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 the present study the severe missense 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 (Majeed *et al.*, 2005, Belmahi *et al.*, 2006, Touitou, 2001).

The two least common mutations were found to be the missense 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 p.Ala744Ser is the second mutation Deltas *et al*. (2002) failed to detect in the examined Cypriot cohort. This was also probably due to the restrictions of their methodology used back then. The missense p.Arg761His mutation was also found as rare in Turks while it is more prevalent in Armenians (Solak *et al.*, 2008) (Yalcinkaya *et al.* 2000). On the other hand, p.Ala744Ser is more prevalent in

Arabs (Sharkia *et al.*, 2013). 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).

It should be noted that the five most common *MEFV* mutations of the present study represent the 75.75% of the identified alleles while in the classically affected populations this frequency is equal to 85%. The reason for observing lower frequencies in the Cypriot cohort of the present study could be attributed to the migration trends in the island, outlining the present-day gene pool of the Greek-Cypriots (Shammas *et al.*, 2012).

The frequency of FMF patients carrying only one *MEFV* mutation was also evidenced in the present study and found to be consistent with the hypothesis that clinical symptoms of the disorder may be also 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 the present study did not follow a binomial distribution and the Hardy-Weinberg 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 the 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). No consanguinity or biased sampling was observed in the cohort of patients under

investigation of the present study. However, when testing the distribution for each of the most common *MEFV* mutations separately, these did not differ significantly from Hardy-Weinberg expectations, indicating that FMF patients were randomly selected and that the requirements for Hardy-Weinberg equilibrium were satisfied. The most predominant scenario in the present study could be the presence of an FMF-like phenotype which 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 with 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 and could be explained by the presence of mutations in other exons that have not been sequenced. It was reported that rare or private mutations are more frequent in populations that are not classically affected (Konstantopoulos *et al.*, 2003). Disease-causing mutations may also reside in the non-coding or regulatory regions affecting splicing or the messenger RNA expression (Booty *et al.*, 2009). Nevertheless, some investigators have failed to detect any mutations when complete sequencing of the gene was performed (Booty *et al.*, 2009). The presence of dominant negative

mutations or mutations with a high penetrance could also be another explanation (Booty *et al.*, 2009). Furthermore, several authors suggested that although most disease-associated mutations are missense nucleotide changes, genomic rearrangements (deletions, copy number variations) could be involved in the pathogenesis of the disease. However, a recent study with MLPA did not identify any *MEFV* copy number variations, suggesting that genomic rearrangements could not be considered as another disease mechanism (Booty *et al.*, 2009).

Moreover, the expulsion of the “Chuetas”, descendants of Jews, from Spain to Palma de Mallorca in the 11th century, make this hypothesis stronger. This community had a population of eighteen families, from which more than sixty members were diagnosed with FMF. Their symptoms and haplotypes were similar with those of North African Jews FMF patients, descendants of those expelled from Spain in the 16th century (Ben-Chetrit & Touitou, 2009). The presence of FMF in Armenia can be explained either by the neighboring interactions with Turkey or by the migration of Jews from the Middle East to the Khazars’ kingdom, through the Caspian Sea, in the 8th century (Ben-Chetrit & Touitou, 2009).

In a recent study by Yepiskoposyan et al. (2007) a map of the known *MEFV* mutations around the world was established and haplotype analysis dated p.Met694Val, p.Val726Ala and p.Glu148Qln in the Middle East more than 2,500 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 as the most frequent among the Ashkenazi Jewish, the Druze and the Armenian FMF patients (Yepiskoposyan & Harutyunyan, 2007).

It is speculated that the missense mutations p.Met694Val and p.Val726Ala migrated from the Middle East to Spain and North Africa either, in the early days, via Phoenicians

sailors who travelled across the Mediterranean Sea or in the 8th century, during the Muslim conquest of North Africa and Spain (Ben-Chetrit & Touitou, 2009).

In conclusion, the present 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 the present study and 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 towards the effective diagnosis, 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.

Acknowledgements

This work was supported by the A.G. Leventis Foundation.

References

(1997a) Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. The International FMF Consortium. *Cell,* 90**,** 797-807.

(1997b) A candidate gene for familial Mediterranean fever. *Nat Genet,* 17**,** 25-31.

Belmahi, L., Sefiani, A., Fouveau, C., Feingold, J., Delpech, M., Grateau, G. & Dode, C. (2006) Prevalence and distribution of MEFV mutations among Arabs from the Maghreb patients suffering from familial Mediterranean fever. *C R Biol,* 329**,** 71-4.

Ben-Chetrit, E., Lerer, I., Malamud, E., Domingo, C. & Abeliovich, D. (2000) The E148Q mutation in the MEFV gene: is it a disease-causing mutation or a sequence variant? *Hum Mutat,* 15**,** 385-6.

Ben-Chetrit, E. & Touitou, I. (2009) Familial mediterranean Fever in the world. *Arthritis Rheum,* 61**,** 1447-53.

Booty, M.G., Chae, J.J., Masters, S.L., Remmers, E.F., Barham, B., Le, J.M., Barron, K.S., Holland, S.M., Kastner, D.L. & Aksentijevich, I. (2009) Familial Mediterranean fever with a single MEFV mutation: where is the second hit? *Arthritis Rheum,* 60**,** 1851-61.

Caglayan, A.O., Demiryilmaz, F., Ozyazgan, I. & Gumus, H. (2010) MEFV gene compound heterozygous mutations in familial Mediterranean fever phenotype: a retrospective clinical and molecular study. *Nephrol Dial Transplant,* 25**,** 2520-3.

Cazeneuve, C., Hovannesyan, Z., Genevieve, D., Hayrapetyan, H., Papin, S., Girodon-Boulandet, E., Boissier, B., Feingold, J., Atayan, K., Sarkisian, T. & Amselem, S. (2003) Familial Mediterranean fever among patients from Karabakh and the diagnostic value of MEFV gene analysis in all classically affected populations. *Arthritis Rheum,* 48**,** 2324-31.

Delibas, A., Oner, A., Balci, B., Demircin, G., Bulbul, M., Bek, K., Erdogan, O., Baysun, S. & Yilmaz, E. (2005) Genetic risk factors of amyloidogenesis in familial Mediterranean fever. *Am J Nephrol,* 25**,** 434-40.

Deltas, C.C., Mean, R., Rossou, E., Costi, C., Koupepidou, P., Hadjiyanni, I., Hadjiroussos, V., Petrou, P., Pierides, A., Lamnisou, K. & Koptides, M. (2002) Familial Mediterranean fever (FMF) mutations occur frequently in the Greek-Cypriot population of Cyprus. *Genet Test,* 6**,** 15-21.

Dewalle, M., Domingo, C., Rozenbaum, M., Ben-Chetrit, E., Cattan, D., Bernot, A., Dross, C., Dupont, M., Notarnicola, C., Levy, M., Rosner, I., Demaille, J. & Touitou, I. (1998) Phenotype-genotype correlation in Jewish patients suffering from familial Mediterranean fever (FMF). *Eur J Hum Genet,* 6**,** 95-7.

Gershoni-Baruch, R., Shinawi, M., Shamaly, H., Katsinetz, L. & Brik, R. (2002) Familial Mediterranean fever: the segregation of four different mutations in 13 individuals from one inbred family: genotype-phenotype correlation and intrafamilial variability. *Am J Med Genet,* 109**,** 198-201.

Grandemange, S., Soler, S. & Touitou, I. (2009) Expression of the familial Mediterranean fever gene is regulated by nonsense-mediated decay. *Hum Mol Genet,* 18**,** 4746-55.

Inal, A., Yilmaz, M., Kendirli, S.G., Altintas, D.U. & Karakoc, G.B. (2009) The clinical and genetical features of 124 children with Familial Mediterranean fever: experience of a single tertiary center. *Rheumatol Int,* 29**,** 1279-85.

Jeru, I., Hentgen, V., Cochet, E., Duquesnoy, P., Le Borgne, G., Grimprel, E., Stojanovic, K.S., Karabina, S., Grateau, G. & Amselem, S. (2013) The risk of familial Mediterranean fever in MEFV heterozygotes: a statistical approach. *PLoS One,* 8**,** e68431.

Konstantopoulos, K., Kanta, A., Deltas, C., Atamian, V., Mavrogianni, D., Tzioufas, A.G., Kollainis, I., Ritis, K. & Moutsopoulos, H.M. (2003) Familial Mediterranean fever associated pyrin mutations in Greece. *Ann Rheum Dis,* 62**,** 479-81.

Konstantopoulos, K., Kanta, A., Lilakos, K., Papanikolaou, G. & Meletis, I. (2005) Familial Mediterranean fever and E148Q pyrin gene mutation in Greece. *Int J Hematol,* 81**,** 26-8.

La Regina, M., Nucera, G., Diaco, M., Procopio, A., Gasbarrini, G., Notarnicola, C., Kone-Paut, I., Touitou, I. & Manna, R. (2003) Familial Mediterranean fever is no longer a rare disease in Italy. *Eur J Hum Genet,* 11**,** 50-6.

Lidar, M. & Livneh, A. (2007) Familial Mediterranean fever: clinical, molecular and management advancements. *Neth J Med,* 65**,** 318-24.

Livneh, A., Langevitz, P., Zemer, D., Zaks, N., Kees, S., Lidar, T., Migdal, A., Padeh, S. & Pras, M. (1997) Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum,* 40**,** 1879-85.

Majeed, H.A., El-Khateeb, M., El-Shanti, H., Rabaiha, Z.A., Tayeh, M. & Najib, D. (2005) The spectrum of familial Mediterranean fever gene mutations in Arabs: report of a large series. *Semin Arthritis Rheum,* 34**,** 813-8.

Marek-Yagel, D., Berkun, Y., Padeh, S., Abu, A., Reznik-Wolf, H., Livneh, A., Pras, M. & Pras, E. (2009) Clinical disease among patients heterozygous for familial Mediterranean fever. *Arthritis Rheum,* 60**,** 1862-6.

Mattit, H., Joma, M., Al-Cheikh, S., El-Khateeb, M., Medlej-Hashim, M., Salem, N., Delague, V. & Megarbane, A. (2006) Familial Mediterranean fever in the Syrian population: gene mutation frequencies, carrier rates and phenotype-genotype correlation. *Eur J Med Genet,* 49**,** 481-6.

Medlej-Hashim, M., Nehme, N., Chouery, E., Jalkh, N. & Megarbane, A. (2010) 1Novel MEFV transcripts in Familial Mediterranean fever patients and controls. *BMC Med Genet,* 11**,** 87.

Medlej-Hashim, M., Rawashdeh, M., Chouery, E., Mansour, I., Delague, V., Lefranc, G., Naman, R., Loiselet, J. & Megarbane, A. (2000) Genetic screening of fourteen mutations in Jordanian familial Mediterranean fever patients. *Hum Mutat,* 15**,** 384.

Milhavet, F., Cuisset, L., Hoffman, H.M., Slim, R., El-Shanti, H., Aksentijevich, I., Lesage, S., Waterham, H., Wise, C., Sarrauste De Menthiere, C. & Touitou, I. (2008) The infevers autoinflammatory mutation online registry: update with new genes and functions. *Hum Mutat,* 29**,** 803-8.

Pasa, S., Altintas, A., Devecioglu, B., Cil, T., Danis, R., Isi, H., Bayan, K., Tuzun, Y., Ecer, S., Batun, S. & Ayyildiz, O. (2008) Familial Mediterranean fever gene mutations in the Southeastern region of Turkey and their phenotypical features. *Amyloid,* 15**,** 49-53.

Pras, E., Aksentijevich, I., Gruberg, L., Balow, J.E., Jr., Prosen, L., Dean, M., Steinberg, A.D., Pras, M. & Kastner, D.L. (1992) Mapping of a gene causing familial Mediterranean fever to the short arm of chromosome 16. *N Engl J Med,* 326**,** 1509-13.

Pras, M. (1998) Familial Mediterranean fever: from the clinical syndrome to the cloning of the pyrin gene. *Scand J Rheumatol,* 27**,** 92-7.

Sarrauste De Menthiere, C., Terriere, S., Pugnere, D., Ruiz, M., Demaille, J. & Touitou, I. (2003) INFEVERS: the Registry for FMF and hereditary inflammatory disorders mutations. *Nucleic Acids Res,* 31**,** 282-5.

Shammas, C., Neocleous, V., Toumba, M., Costi, C., Phedonos, A.A., Efstathiou, E., Kyriakou, A., Phylactou, L.A. & Skordis, N. (2012) Overview of genetic defects in

endocrinopathies in the island of Cyprus; evidence of a founder effect. *Genet Test Mol Biomarkers,* 16**,** 1073-9.

Sharkia, R., Mahajnah, M., Zalan, A., Athamna, M., Azem, A., Badarneh, K. & Faris, F. (2013) Comparative screening of FMF mutations in various communities of the Israeli society. *Eur J Med Genet,* 56**,** 351-5.

Shinar, Y., Obici, L., Aksentijevich, I., Bennetts, B., Austrup, F., Ceccherini, I., Costa, J.M., De Leener, A., Gattorno, M., Kania, U., Kone-Paut, I., Lezer, S., Livneh, A., Moix, I., Nishikomori, R., Ozen, S., Phylactou, L., Risom, L., Rowczenio, D., Sarkisian, T., Van Gijn, M.E., Witsch-Baumgartner, M., Morris, M., Hoffman, H.M. & Touitou, I. (2012) Guidelines for the genetic diagnosis of hereditary recurrent fevers. *Ann Rheum Dis,* 71**,** 1599-605.

Solak, M., Yildiz, H., Koken, R., Erdogan, M., Eser, B., Sen, T., Evirgen, N., Erdem, S. & Arikan, E. (2008) Analysis of familial Mediterranean fever gene mutations in 202 patients with familial Mediterranean fever. *Genet Test,* 12**,** 341-4.

Stoffman, N., Magal, N., Shohat, T., Lotan, R., Koman, S., Oron, A., Danon, Y., Halpern, G.J., Lifshitz, Y. & Shohat, M. (2000) Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. *Eur J Hum Genet,* 8**,** 307-10.

Tchernitchko, D.O., Gerard-Blanluet, M., Legendre, M., Cazeneuve, C., Grateau, G. & Amselem, S. (2006) Intrafamilial segregation analysis of the p.E148Q MEFV allele in familial Mediterranean fever. *Ann Rheum Dis,* 65**,** 1154-7.

Tomiyama, N., Higashiuesato, Y., Oda, T., Baba, E., Harada, M., Azuma, M., Yamashita, T., Uehara, K., Miyazato, A., Hatta, K., Ohya, Y., Iseki, K., Jinno, Y. & Takishita, S. (2008) MEFV mutation analysis of familial Mediterranean fever in Japan. *Clin Exp Rheumatol,* 26**,** 13-7.

Touitou, I. (2001) The spectrum of Familial Mediterranean Fever (FMF) mutations. *Eur J Hum Genet,* 9**,** 473-83.

Touitou, I., Lesage, S., Mcdermott, M., Cuisset, L., Hoffman, H., Dode, C., Shoham, N., Aganna, E., Hugot, J.P., Wise, C., Waterham, H., Pugnere, D., Demaille, J. & Sarrauste De Menthiere, C. (2004) Infevers: an evolving mutation database for auto-inflammatory syndromes. *Hum Mutat,* 24**,** 194-8.

Ureten, K., Gonulalan, G., Akbal, E., Gunes, F., Akyurek, O., Ozbek, M. & Ozturk, M.A. (2010) Demographic, clinical and mutational characteristics of Turkish familial Mediterranean fever patients: results of a single center in Central Anatolia. *Rheumatol Int,* 30**,** 911-5.

Yalcinkaya, F., Tekin, M., Cakar, N., Akar, E., Akar, N. & Tumer, N. (2000) Familial Mediterranean fever and systemic amyloidosis in untreated Turkish patients. *QJM,* 93**,** 681-4.

Yepiskoposyan, L. & Harutyunyan, A. (2007) Population genetics of familial Mediterranean fever: a review. *Eur J Hum Genet,* 15**,** 911-6.

	# 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.Glu148Qln	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.Glu148Qln/p.Met694Val	7	13.7 %
p.Glu148Qln/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.Glu148Qln/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 %**

Table 1. Types and frequency of molecular MEFV defects in the cohort of 593 Cypriot HRF patients. p.Phe479Leu-Glu167Asp is known to be co-inherited.*

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%**

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

Genotypes	Observed	Expected	$\mathbf{O}-\mathbf{E}$	$(\mathbf{O}-\mathbf{E})^{\mathbf{2}}$	$\frac{(\mathbf{O}-\mathbf{E})^{\mathbf{2}}}{\mathbf{E}}$
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
					$\chi^2 =$ **86.96**

Table 3. The observed distribution of I/I (two MEFV mutations), I/N (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.

Mutations	χ^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 4. The distribution of the five most common MEFV mutations using in the Cypriot cohort of 593 patients a χ^2 test.

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

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