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The Population Genetics of Familial Mediterranean Fever: A Meta-Analysis Study

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Summary

Our aim was to construct a Familial Mediterranean Fever (FMF) cumulative database and to propose a *MEFV* based phylogenetic tree. Data were collected from published studies. A meta-analysis based on 16,756 chromosomes from FMF patients and normal individuals from 14 affected populations was performed. Arlequin 2.0 and Phylip 3.2 software were used for population genetics analysis and phylogenetic tree construction.

We have shown that *MEFV* mutations are distributed non-uniformly along the Mediterranean Sea area. The most frequent mutations detected in FMF patients are M694V (39.6%), V726A (13.9%), M680I (11.4%), E148Q (3.4%), and M694I (2.9%), while 28.8% of chromosomes carry unidentified or no mutations, especially in Western Europeans. The mean overall carrier rate is 0.186 with peak values in Arabs, Armenians, Jews, and Turks. Only V726A obeys the Hardy-Weinberg law in FMF patients implying that this mutation is the most ancient. Jews present the most intense genetic isolation and drift; thus they might have nested *de novo* mutations and accelerated evolution. Besides Jews, three population groups might follow distinct evolutionary lines (Asia Minor, Eastern European, and Western European).

In conclusion, the *MEFV* mutation pattern is non-uniform regarding distribution, phenotypic expression, neutrality and population genetics characteristics. Jews are the candidate population for founder effects in *MEFV*.

Keywords: Familial Mediterranean Fever, autoinflammation, pyrin, *MEFV*, meta-analysis.

Introduction

Familial Mediterranean Fever (FMF) is a disease characterized by recurrent bouts of fever and serositis (Sohar et al., 1967) attributed to altered pyrin, the product of the *MEFV* gene (MIM:249100) (The international FMF consortium, 1997; The French FMF consortium, 1997; Bernot et al., 1998). More than 165 mutations and polymorphisms, primarily clustered in exon 10, have been identified in affected individuals (Infevers, 2008). Though *MEFV* mutations have been reported at lower but significant frequencies in all countries around the Mediterranean Sea, FMF mainly affects non-Ashkenazi Jews, Armenians, Arabs and Turks (Touitou, 2001).

Several studies have documented an increased frequency of healthy carriers of some *MEFV* mutations, at least in some populations (Aksentijevich et al., 1999; Yilmaz et al., 2001). This observation, in combination with the large number of known *MEFV* mutations (Touitou et al., 2004), was proposed to reflect some yet unidentified selective advantage for the heterozygous state of these mutations (Aksentijevich et al., 1999; Tunca et al., 1999; Stoffman et al., 2000; Schaner et al., 2001; Cattan, 2005). Moreover, in the Western European countries, only a minority of patients compatible with FMF present *MEFV* mutations (Tchernitchko et al., 2005).

Although cumulative information is available concerning the spectrum and the distribution of *MEFV* mutations as well as the associated polymorphisms, the population genetics of FMF is still debatable as most data are dispersed and unexploited (Majeed et al., 2005; Sarkisian et al., 2005). The majority of the *MEFV* mutations are believed to be ancient. Based on the elevated frequency of healthy carriers of some common and widespread *MEFV* mutations, any of the four classically affected populations (Jews, Armenians, Arabs and Turks) might be proposed as the founder population for some of the *MEFV* mutations considered to be 'classical' (Aksentijevich et al., 1999).

The present study aims to perform a meta-analysis on data retrieved from the available literature of the last decade concerning the allelic and genotypic distribution of the 5 most

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Table 1 Distribution of *MEFV* mutations in the 14 populations studied; n is the number of alleles of FMF patients. O/U: Other/Unknown.

	n	M694V	V726A	M680I	M694I	E148Q	O/U
Arabs	706	141	99	49	85	42	290
Armenians	6000	2586	1140	955	20	112	1187
Cretans	142	39	7	0	10	20	66
Cypriots	68	12	17	0	2	5	32
French	86	4	0	0	4	6	72
Greeks	304	80	21	39	8	19	137
Italians	62	10	3	6	5	11	27
Jews	1302	847	39	13	0	65	338
Jordianians	110	27	15	10	2	5	51
Lebanese	1116	194	124	47	82	53	616
Spaniards	100	12	1	0	4	5	78
Syrians	166	76	23	16	8	10	33
Tunisians	278	29	5	34	14	19	177
Turks	1390	626	153	181	97	28	305
Total	11830	4683	1647	1350	341	400	3409

common *MEFV* mutations (M694V, V726A, M680I, M694I, and E148Q), in correlation to phenotypic outcome, in 14 different affected Mediterranean populations (Arabs, Armenians, Cretans, Cypriots, French, Greeks, Italians, Jews, Jordanians, Lebanese, Spaniards, Syrians, Tunisians, and Turks). This information, apart from providing a cumulative database for all researchers of the field for future diagnostic and research purposes, would enable the proposal of an evolutionary history model for *MEFV* locus through the ages using population genetics software analysis.

Materials and Methods

All published articles during the last decade describing *MEFV* mutation frequencies in 14 Mediterranean populations were retrieved to serve as primary data sources (Table 1). These populations comprised: Arabs (Touitou, 2001; Gershoni-Baruch et al., 2001; Gershoni-Baruch et al., 2002; Majeed et al., 2005); Armenians (Touitou, 2001; Sarkisian et al., 2005); Cretans (Fragouli et al., 2008); Cypriots (Deltas et al., 2002; Deltas, 2003); French (Touitou, 2001); Greeks (Giaglis et al., 2007); Italians (Touitou, 2001; La Regina et al., 2003); Jews (Touitou, 2001; Gershoni-Baruch et al., 2001; Gershoni-Baruch et al., 2002); Jordanians (Medlej-Hashim et al., 2005); Lebanese (Medlej-Hashim et al., 2005); Spaniards (Touitou, 2001; Aldea et al., 2004); Syrians (Mattit et al., 2006); Tunisians (Chaabouni et al., 2007), and Turks (Touitou, 2001; Yilmaz et al., 2001; Turkish FMF Study Group, 2005; Yigit et al., 2008). Whenever multiple data were available for the same population from various sources, the most complete data set was selected. Completeness was evaluated according to the availability of allelic and genotypic frequencies in both FMF patients and controls concerning the 5 most common mutations (M694V, V726A, M680I, M694I, and E148Q). Whenever more than one data set was considered complete, combined data were utilized. Identical-by-descent chromosomes were excluded from analysis when possible.

Two-way contingency tables were analyzed using the free on-line statistical tool at http://statpages.org/ctab2×2.html. Risk ratio (RR), positive predictive value (PPV), and number needed to diagnose (NND) were computed for every set of data. Additionally, Hardy-Weinberg equilibrium was checked using the statistical tool freely available on-line at http://www.graphpad.com/quickcalcs/chisquared1.cfm. The most accurate exact Hardy-Weinberg test, proposed by Guo & Thompson and incorporated in the ARLEQUIN 2.0 algorithm, was considered unnecessary as all samples tested were large and type I error was limited (Guo & Thompson, 1992).

The ARLEQUIN 2.0 software was used for the main population genetics analysis. In detail, based on allele frequencies of the 5 most common *MEFV* mutations (M694V, V726A, M680I, M694I, and E148Q), fixation index (F_{ST}, being a measure of inter- and intra-population variation), gene diversity (GD), theta estimation from expected homozygosity (θ, being a measure of the nucleotide polymorphism), and Watterson P-value (W-P, based on the Ewens–Watterson neutrality test, which is an application of the Ewens' sampling theory in a population at equilibrium) were computed for each of the 14 studied populations (Schneider, 2000).

At a second step, the PHYLIP 3.2 software was used for implementation of the UPGMA method on the mean number of pairwise differences, in order to construct (using the "neighbor" program) and draw (using the "drawgram" program) appropriate phylogenetic tree diagrams. This software carries out the UPGMA method with the assumption that all populations are contemporaneous and that there is an evolutionary clock. This means that branches of the tree cannot be of arbitrary length, but are constrained so that the total length from the root of the tree to any population is the same (Felsenstein, 1989).

The effective population size (the number of individuals that theoretically present the same genetic drift as the actual population) was estimated from the formula $N = \theta/4u$, where u stands for the mutation rate for the entire coding sequence of *MEFV* per generation. As the value of u has not been determined for *MEFV*, the value of 3.7×10^{-9} mutations per nucleotide per

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year, which has been attributed to the mutation rate of the coding sequence of the *HBB* gene encoding β-globin in humans, served as a rough but not untrue approach (Stamatoyannopoulos & Nute, 1984). Moreover, taking into consideration the length of the *MEFV* coding sequence (2343 nucleotides) and an arbitrary mean estimation of t_g (time in years per generation) to be around 25 years, N was found to equal the sum of $1153.52^{*}\theta$.

Furthermore, a coalescent unit (CU) in years is the product of 2N (as a diploid organism is involved) with t_g. These values are of special interest for users of programs concerning coalescence like GENETREE, whose time to most recent common ancestor (TMRCA) estimates are given in CU (Labate, 2000).

Whenever we refer to 'Cretans' and 'Greeks', we mean Greeks deriving from the isle of Crete and mainland Greece, respectively (Fragouli et al., 2008; Giaglis et al., 2007). Whenever we refer to 'Cypriots', we mean Greek-Cypriots (Deltas et al., 2002). Moreover, whenever 'Arabs' are referred to, we mean a mixed Middle Eastern/North African population as presented in the review of Touitou (2001). Finally, data from non-Ashkenazi Jews (of North African and Iraqui descent) have been preferentially used where possible, as the prevalence of FMF among Ashkenazi Jews might be low (Gershoni-Baruch et al., 2002); however, in the main population genetics analysis (Tables 1 and 5), a mixed Jewish population as presented in the review of Touitou (2001) has been taken into consideration.

Results

MEFV Mutations are Non-Uniformly Distributed Along the Mediterranean Sea

The distribution pattern of *MEFV* mutations along the Mediterranean Sea is non-uniform (Fig. 1). From this point of view, four populations, namely Arabs, Armenians, Jews, and Turks, are considered to be 'classically' affected populations, with a large number of patients and a high carrier rate.

In detail, when the overall FMF patients' chromosomes are considered, 71.2% (8,420/11,830) show mutated *MEFV* alleles.

The most frequent mutations detected among 5,915 analyzed FMF patients are M694V (39.6%), V726A (13.9%), M680I (11.4%), E148Q (3.4%), and M694I (2.9%), while 28.8% of their chromosomes carry unidentified or no mutations. Absolute and relative frequencies of these mutations, as well as other rare or unidentified mutations in the 14 populations studied are presented in Table 1 and Figure 1 accordingly.

The mean overall carrier rate is 0.186. Analytical data concerning carrier rates (proportion of normal individuals carrying a mutation) and mutation frequencies (ratio of mutated chromosomes to overall chromosomes in normal individuals) are presented in Table 2.

When FMF patients and controls are compared on the basis of carriage of *MEFV* mutations, the overall relative risk (RR), positive predictive value (PPV), and number needed to diagnose (NND) along with their 95% confidence intervals (in parentheses) are 3.372 (3.213–3.538), 0.900 (0.893–0.906), and 1.508 (1.468–1.551) respectively. Data for each population are presented in Table 3.

Many Common *MEFV* Mutations Might not be Neutral

When the Hardy-Weinberg rule was applied to nine populations where both allelic and genotypic data from FMF patients were available, namely Arabs (Gershoni-Baruch et al., 2002), Cretans (Fragouli et al., 2008), Cypriots (Deltas et al., 2002), Greeks (Giaglis et al., 2007), Non-Ashkenazi Jews (Gershoni-Baruch et al., 2002), Lebanese (Mansour et al., 2001), Syrians (Mattit et al., 2006), Tunisians (Chaabouni et al., 2007), and Turks (Yigit et al., 2008), a uniform and strong deviation was observed ($P < 0.0001$) (Table 4A). Nevertheless, as unifying data ignores the overall Mediterranean population infrastructure, a detailed table, including P-values of statistical significance concerning the deviation from Hardy-Weinberg law for each one of the 5 most common mutations has been added. In this table, only the V726A mutation is demonstrated to respect Hardy-Weinberg law and is thus presented neutral in all populations (Table 4B). Ideally, it would be desirable if data had been available for normal controls as well, in order to discriminate whether this deviation is a true evolutionary trend or a disease-related segregation.

Population-Specific *MEFV* Mutations

There are some rare *MEFV* mutations that tend to be linked with specific populations. Examples are T177I, S108R, and E474K identified in Lebanese (Medlej-Hashim et al., 2005), I591T found in Western Europeans (French/Spaniards) (Aldea et al., 2004), E225K identified in a Greek family with members suffering from FMF (Zonios et al., 2007), S702C identified in a Cretan FMF patient (Fragouli et al., 2008), and F479L, which is one of the prevailing *MEFV* mutations in Cypriot FMF patients, in strong linkage with E167D (Deltas et al., 2002). Similarly, R202Q homozygosity has been demonstrated to be disease-related in Greeks, implying a dosage-dependent detrimental effect and, perhaps, an evolutionary trend for pyrin protein through heterozygote advantage (Giaglis et al., 2007).

Population genetics analysis between different Mediterranean populations

The overall reduction in heterozygosity, as derived from the fixation index F_{ST} that compares the least inclusive to the most inclusive levels of the population hierarchy and

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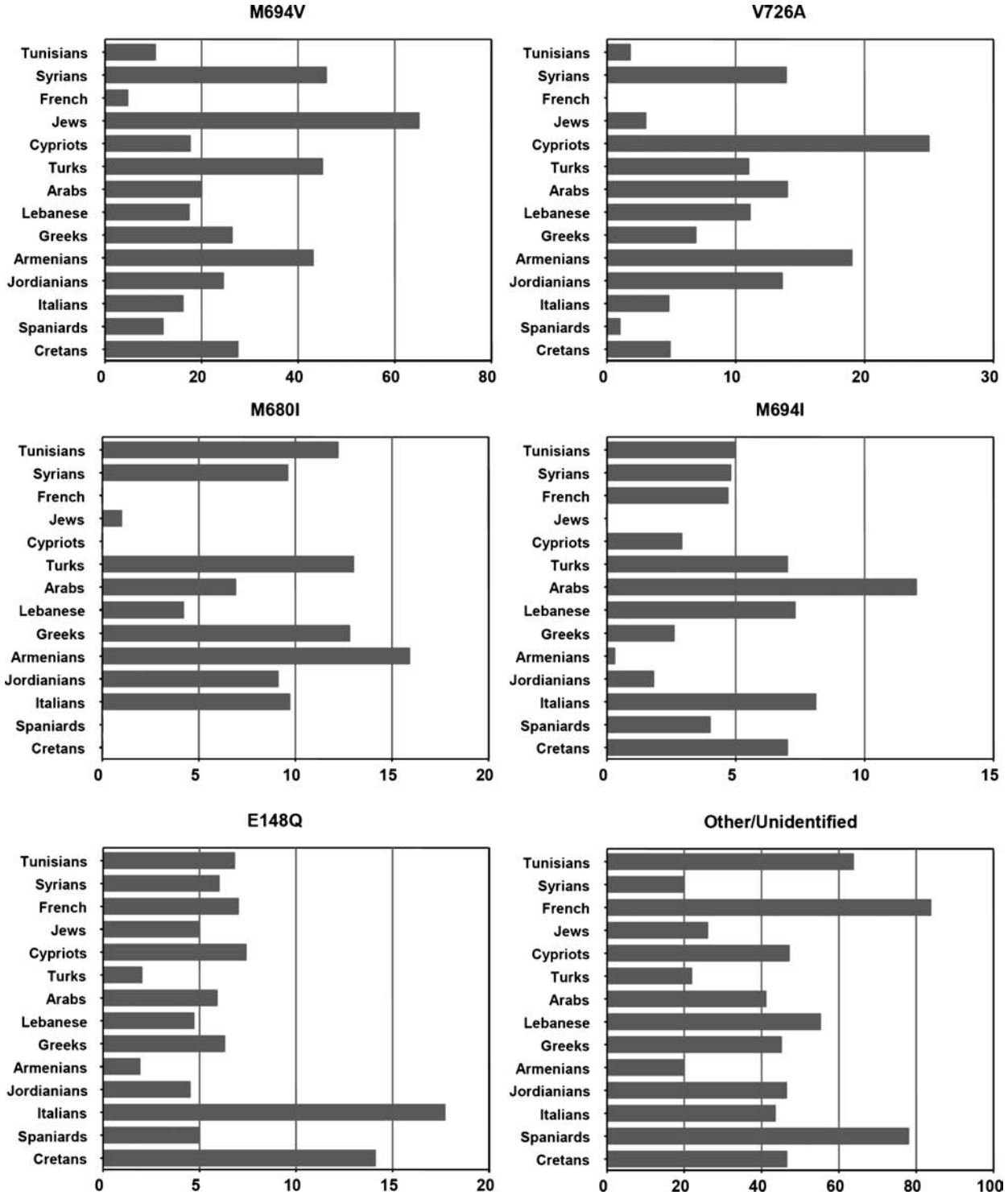

Figure 1 Distribution of the 5 most common *MEFV* mutation frequencies in the 14 populations studied.

measures all the effects of population substructure combined, has been computed to be 0.0944. This implies that a mere 10% reduction in overall heterozygosity can be explained by the breakoff in subpopulations.

Based on the exact test of population differentiation, the only pairs of populations that are not differentiated have been demonstrated to be Spaniards/Syrians ($P = 0.36 \pm 0.03$) and Greeks/Jordanians ($P = 0.35 \pm 0.02$), while the pairing

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	n	Overall carrier rate	Mutation frequencies				
			M694V	V726A	M680I	M694I	E148Q
Arabs	636	0.233	0.0031	0.0380	0.0128	?	0.0654
Armenians	500	0.210	0.0235	0.0230	0.0090	0	0.0170
Cretans	316	0.114	0	0.0060	0	0	0.0280
Cypriots	600	0.111	?	?	?	?	0.071
Greeks	280	0.014	0.0035	0	0.0035	0	0
Jews (Non-Ashkenazi)	1710	0.235	0.0193	0.0339	0	?	0.0304
Lebanese	200	0.172	0	0.0400	0.0050	0	0.0500
Syrians	484	0.175	0.0083	0.0227	0	0	0.0661
Turks	200	0.200	0.0150	0.0100	0.0250	0	0.0600
Total	4926	0.186					

Table 2 Overall *MEFV* mutation carrier rates and mutation frequencies concerning the 5 most common mutations in the *MEFV* gene in normal controls of the studied populations; n stands for the number of chromosomes derived from controls.

	FMF patients		Normal controls				
Population	Mutated	WT	Mutated	WT	RR	PPV	NND
Arabs	239	168	74	244	1.873	0.764	2.821
Armenians	2823	177	50	200	2.093	0.983	1.350
Cretans	59	12	18	140	9.706	0.766	1.395
Cypriots	30	4	33	267	32.262	0.476	1.295
Greeks	127	25	2	138	6.419	0.984	1.218
Jews (Non-Ashkenazi)	73	2	201	654	87.387	0.266	1.495
Lebanese	329	229	17	83	1.296	0.951	2.383
Syrians	74	9	42	200	14.814	0.638	1.393
Turks	346	104	20	80	1.673	0.945	1.758
Total	4100	730	457	2006	3.372	0.900	1.508

Table 3 Two-way contigency table analysis for the FMF phenotype in the 5 most common *MEFV* mutations in carriers of the studied populations. RR: relative risk, PPV: positive predictive value, NND: number needed to diagnose, WT: individuals carrying only wild type (unmutated) alleles.

Tunisians/Turks ($P = 0.049 \pm 0.012$) lies in a borderline zone.

Population Genetics Analysis Between Patients and Controls

The overall F_{ST} between patients and controls is 0.64 ($P<0.001$); this means that a mere two thirds of the variation observed in *MEFV* mutation frequencies is observed among populations (patients and controls) and the remaining one third within them. The exact test of sample differentiation based on allele frequencies gives a $P = 0$; thus, there is absolute certainty over the fact that FMF patients carry *MEFV* mutations more frequently than healthy individuals.

Interestingly, patients and controls present comparable gene diversity (0.259 ± 0.007 and 0.271 ± 0.010, respectively), theta from homozygosity (0.261 ± 0.010 and 0.277 ± 0.013, respectively), and Tajimas' D (0.952 and 0.962, respectively) when *MEFV* is considered as a locus. These observations might imply a uniformly distributed evolutionary force over both patients and controls. When the five commonest *MEFV* mutations are taken into consideration within patients, the gene diversity observed is 0.726 ± 0.002, the theta from homozygosity is 2.040 ± 0.024, and the Tajima's D is 1.860. All CU values in years are given in Table 5.

Jews as a Candidate Founder Population for *MEFV* Mutations

The population genetics analysis showed that Jews present the most intense genetic isolation and drift among all classically affected populations and thus offer a suitable environment for accelerated evolution through increased survival of *de novo* mutations and subsequent increase of their frequency until fixation. French and Spaniards exhibit even more extreme values, but do not seem to be closely linked to the *MEFV*-related FMF phenotype. All other populations share more or less comparable effective population sizes of a few thousands. Moreover, Arabs and Turks are characterized by selective neutrality regarding the *MEFV* locus (Table 5).

From an evolutionary point of view, Jews might have been separated early from the main Mediterranean trunk, while three other groups might have followed distinct lines: the Asia Minor (Turkish/Armenian/Syrian), the Eastern European (Arab/Greek/Italian) and the Western European (Spaniard/French/Tunisian). The relevant

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Table 4 (A) Overall allelic and genotypic frequency data for the five most common *MEFV* mutations in FMF patients, demonstrating deviation from Hardy-Weinberg law. (B) P-values of statistical significance concerning deviation from Hardy-Weinberg law computed separately for Arabs, Cretans, Cypriots, Greeks, Non-Ashkenazi Jews, Lebanese, Syrians, Tunisians, Turks.

(A) Mutation	p	q	p^2 exp	q^2 exp	2pq exp	p^2 obs	q^2 obs	2pq obs	P
M694V	1820/2774 (0.6561)	954/2774 (0.3439)	0.4305	0.4513	0.1183	713/1387 (0.5141)	280/1387 (0.2019)	394/1387 (0.2841)	<0.0001
V726A	2537/2774 (0.9146)	237/2774 (0.0854)	0.8364	0.0073	0.1562	1168/1387 (0.8421)	35/1387 (0.0252)	184/1387 (0.1327)	<0.0001
M680I	2332/2774 (0.8407)	442/2774 (0.1593)	0.7067	0.0254	0.2678	1120/1387 (0.8075)	57/1387 (0.0411)	210/1387 (0.1514)	<0.0001
M694I	2703/2774 (0.9737)	71/2774 (0.0263)	0.9495	0.0007	0.0499	1321/1387 (0.9524)	15/1387 (0.0108)	51/1387 (0.0368)	<0.0001
E148Q	2621/2774 (0.9448)	153/2774 (0.0552)	0.8927	0.0030	0.1042	1248/1387 (0.8998)	14/1387 (0.0101)	125/1387 (0.0901)	<0.0001

(B)	M694V	V726A	M680I	M694I	E148Q
Arabs	**0.0002**	0.480	**0.010**	**0.0002**	0.730
Cretans	0.978	0.910	1.000	0.814	0.386
Cypriots	0.786	0.949	1.000	**<0.0001**	0.920
Greeks	0.565	0.599	1.000	0.935	**<0.0001**
Jews (Non-Ashkenazi)	**<0.0001**	0.728	1.000	0.993	0.856
Lebanese	0.012	0.774	0.170	**<0.0001**	**0.043**
Syrians	0.516	0.433	0.302	**<0.0001**	0.395
Tunisians	**<0.0001**	0.965	**<0.0001**	**<0.0001**	**0.008**
Turks	**<0.0001**	0.424	**<0.0001**	1.000	0.928
Total	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Table 5 Population genetics parameters in the 14 populations studied. n: number of alleles studied, GD: Gene diversity, E-W: Ewens-Watterson test of selective neutrality P: P-value, θ: theta estimation from expected homozygosity, N: effective population, CU: coalescent unit (in years).

	n	GD	W-P	θ	N	CU
Arabs	706	0.750 ± 0.011	0.011	2.327 ± 0.155	2684	134200
Armenians	6000	0.713 ± 0.003	0.062	1.910 ± 0.033	2203	110150
Cretans	142	0.686 ± 0.026	0.217	1.667 ± 0.208	2590	129500
Cypriots	68	0.689 ± 0.037	0.327	1.688 ± 0.308	1947	97350
French	86	0.293 ± 0.062	0.947	0.309 ± 0.093	356	17800
Greeks	304	0.704 ± 0.018	0.091	1.822 ± 0.163	2102	105100
Italians	62	0.747 ± 0.040	0.142	2.285 ± 0.514	2636	131800
Jews	1302	0.507 ± 0.012	0.449	0.767 ± 0.038	885	44250
Jordanians	110	0.702 ± 0.031	0.215	1.802 ± 0.278	2079	103950
Lebanese	1116	0.644 ± 0.013	0.151	1.370 ± 0.081	1580	79000
Spaniards	100	0.377 ± 0.058	0.888	0.450 ± 0.111	519	25950
Syrians	166	0.721 ± 0.026	0.099	1.985 ± 0.269	2290	114500
Tunisians	278	0.563 ± 0.032	0.454	0.967 ± 0.126	1115	55750
Turks	1390	0.715 ± 0.008	0.036	1.930 ± 0.084	2226	111300

phylogenetic tree involving all 14 populations studied, namely the ((((((ARABS:0.00464, (JORDANIANS: -0.00070, GREEKS:-0.00070):0.00534):0.00147, (ITALIANS:0.00289, CRETANS:0.00289):0.00322):0.00361, (LEBANESE: 0.00770, CYPRIOTS: 0.00770): 0.00203): 0.03885, ((SPANIARDS: 0.00140, FRENCH: 0.00140): 0.01771, TUNISIANS: 0.01910): 0.02947): 0.03360, ((ARMENIANS:0.00322,(TURKS:-0.00010,SYRIANS:-0.00010):0.00331):0.02568,JEWS:0.02889):0.05328); is depicted in Figure 2. A map chart with estimation of main gene flows between populations is given in Figure 3.

Discussion

The present meta-analysis, which is the first ever carried out in the field of population genetics of Familial Mediterranean

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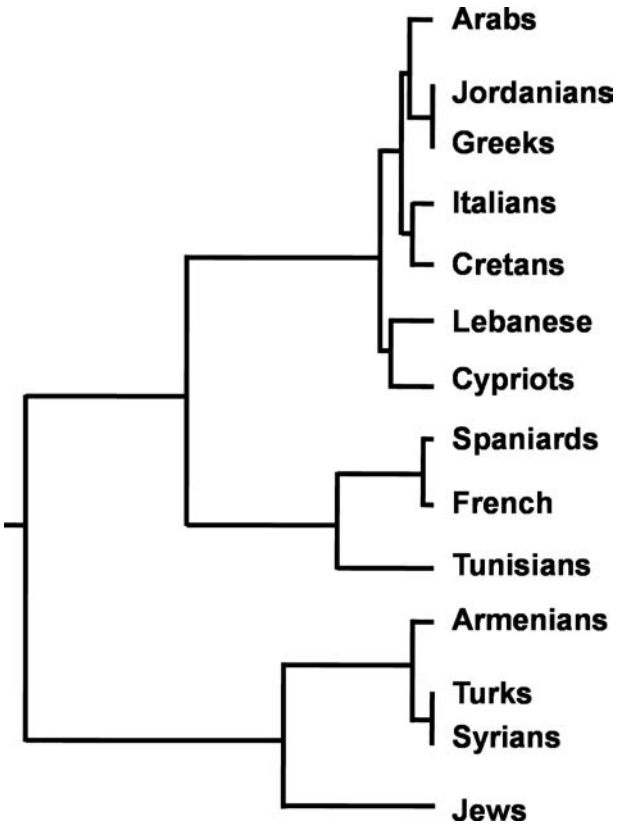

Figure 2 Phylogenetic tree constructed from F_{ST} values based on allele frequencies of the five major *MEFV* mutation frequencies (M694V, V726A, M680I, M694I, and E148Q) in 14 Mediterranean populations.

Fever, is based on up-to-date data and offers to researchers in this field a powerful tool for future studies. Detailed comprehensive information concerning the distribution of *MEFV* mutations along the Mediterranean Sea is provided. Furthermore, overall mutation frequencies, overall risk ratio, overall positive predictive value and overall number needed to treat are given. Also, Hardy-Weinberg law was applied over the most common mutations in FMF patients as a measure of neutrality and, subsequently, fixation. Finally, an extensive population genetics analysis over cumulative data was carried out, and suggested possible evolutionary links between FMF affected populations in relation to *MEFV* mutations. As proposed, Jews seem to have been separated early from the main Mediterranean trunk, while three other groups are well distinguished: the Asia Minor, the Eastern European, and the Western European.

Our data imply that the distribution pattern of *MEFV* mutations is not uniform and that their presence is more FMF-related in Eastern than in Western European countries. This is attributed to the fact that Western populations might present other autoinflammatory syndromes that mimic FMF but are not *MEFV*-related (Tchernitchko et al., 2005).

The value of *MEFV* mutational analysis also varies between populations. In Greeks, the presence of a single *MEFV* mutation, even with mild symptoms, is compatible with the diagnosis of FMF (Table 2). In contrast, in most classically affected populations, such as Jews, Arabs, Armenians, and Turks, the detection of a *MEFV* mutation, even in homozygosity or in compound heterozygosity, is not necessarily enough to diagnose FMF, due to the very high carrier rate observed.

The surprisingly increased normal carrier rates cannot be explained by intense inbreeding and increased genetic drift alone, as they are also observed in populations with high theta values like Arabs, Turks and Armenians (Table 5). Moreover, the presence of recurrent mutations in *MEFV* implies a strong selection pressure (Schaner et al., 2001). These data support the heterozygote advantage hypothesis (Touitou, 2001; Aksentijevich et al., 1999; Schaner et al., 2001). Thus, positive selection pressure favouring heterozygosity of *MEFV* mutations may reflect the need for better response to intracellular pathogens (Schaner & Gumucio 2005) or protection against diseases that are associated with an increased Th2 activity such as asthma (Sackesen, 2004).

Interestingly, when the overall FMF patients are considered, a mere one third of alleles (3,409/11,830) carry no identifiable *MEFV* mutations. Not all investigators used exhaustive mutation detection methods, such as sequencing or NIRCA (Ritis et al., 2004), therefore the percentage of FMF patients without any mutation at all might be slightly smaller. Nevertheless, in two recent studies (Fragouli et al., 2008; Giaglis et al., 2007), where the whole *MEFV* coding sequence was examined, this percentage was again considerable: 16.9% (for Greeks) and 16.4% (for Cretans). In one of our previously published studies, R202Q homozygosity has been demonstrated to explain the FMF phenotype in patients lacking any classical *MEFV* mutations after exhaustive analysis (Giaglis et al., 2007). In detail, R202Q, which was initially proposed to be a benign non-synonymous polymorphism, conserves a high carrier rate in Greeks and presents neutrality in normal individuals. Nevertheless, its allelic distribution disrupts Hardy-Weinberg equilibrium in patients with the FMF phenotype but no classical *MEFV* mutations, as merely half of them are R202Q homozygotes. These data imply that R202Q might carry a heterozygote advantage, which turns out to be disease-related in the homozygous state, underlining a potent dosage effect.

Additionally, the present study offers new evidence that Jews might be the most likely founder population of several common *MEFV* mutations. Their limited effective population size, counting to some hundreds, offers an evolutionary opportunity through intense genetic drift. This environment favours the survival and the fixation of *de novo* mutations, which in a second step, through gene flow, will be exposed

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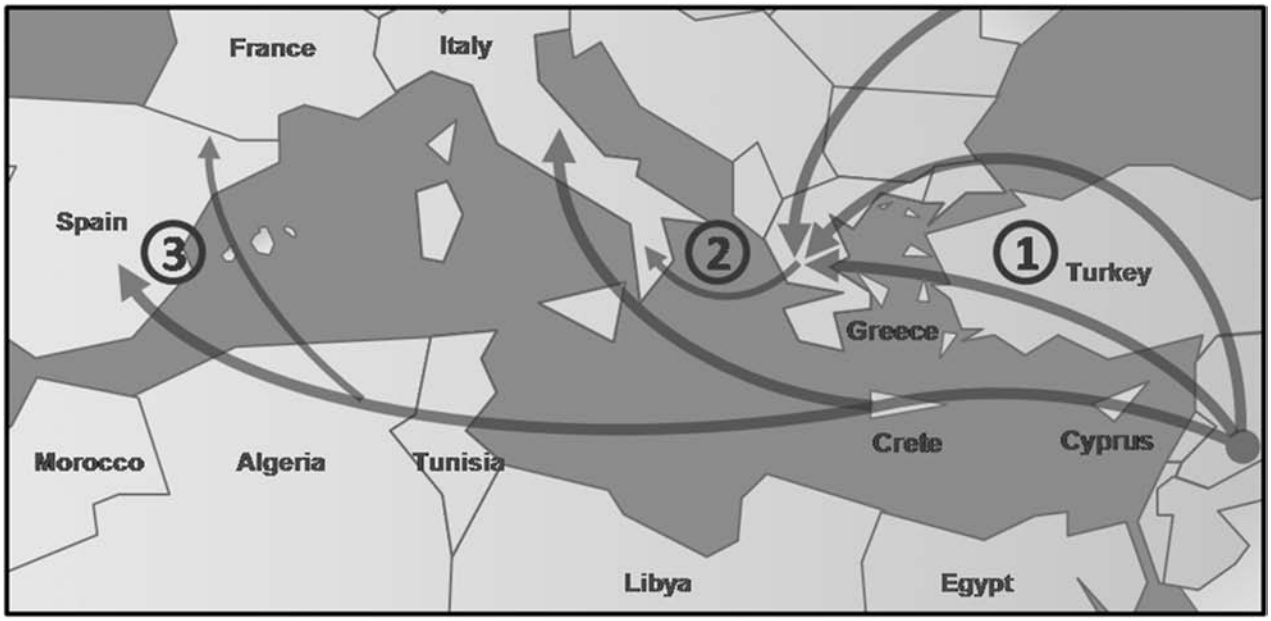

Figure 3 Map of the Mediterranean Sea depicting the three major possible gene flows through populations.

to evolutionary forces. Moreover, as there is historical evidence that Jews have undergone severe fluctuations in their total population size through the ages, the possibility of a bottleneck effect could further enhance the already increased evolutionary trends. These observations may explain why Jews are presented in a unique evolutionary line in the phylogenetic tree (Fig. 2). Interestingly, this clearly separated line is not necessarily the oldest one: In the case of Jews, the limited population size has a direct effect in proportionally lowering the coalescent unit, which in turn can be interpreted as a 'condensation' of evolution.

Nevertheless, populations other than Jews could have nested *de novo* mutations, as in the case of Cypriots and the closely related F479L mutation found in 20.6% of FMF patients. This population-specific mutation is also found in Armenians, though in much lower frequency (1.3%), thus constituting a historically interesting genetic link between the Cypriots and Armenians (Deltas, 2004).

The proposed phylogenetic approach is not identical with a previous one published by our team (Giaglis et al., 2007). Nevertheless, the data used for this purpose included 4 additional populations and incorporated rare and unidentified mutations and thus is believed to be more representative.

Concerning the ages of the *MEFV* mutations, Jalkh et al., using microsatellite data analyzed elegantly by the ESTIAGE program, estimated the ages of the most recent common ancestor (MRCA) for M694V, M694I, V726A, M680I and E148Q to be 7000, 8500, 15000, 23000 and 30000 years, respectively (Jalkh et al., 2008). Thus, the commonest *MEFV* mutations might have emerged after the 'out-of-Africa' dispersal, which is estimated to be 50,000–65,000 years old (Behar et al., 2008). Nevertheless, Rohde et al., using a non-genetic model, proposed that the MRCA of all living humans may have lived within historical times, namely from 3000 BC to 1000 AD (Rohde et al., 2004). From this point of view, the suggestions concerning *MEFV* mutation ages in the recently published work of Jalkh et al., might have been overestimated and at least reflect only a single population. Moreover, in the present study, the V726A mutation has been demonstrated to be the most neutral one among patients (Table 4B); thus, a hint on this mutation being universally the most ancient one is again provided, in contrast with proposals made by Jalkh et al.

A map chart of the Mediterranean Sea, where three possible main gene flows between populations have been represented as solid curved lines, is given in Figure 3. This proposal was concieved by combining the basic tree characteristics (Fig. 2) with the most prominent historical data, mainly of the last two millennia (Rohde et al., 2004). The Byzantine Empire, the Arab conquests, the Ottoman dominance, the dispersal of the Armenian nation and the Jewish Diaspora might all have contributed, in as yet unclarified ways, to the historical background of *MEFV* mutations in terms of their distribution pattern and extrapolated phylogeny.

In conclusion, the *MEFV* mutational pattern is characterized by non-uniformity regarding distribution, phenotypic expression, neutrality and population genetics characteristics. Jews, being genetically isolated, might represent the candidate population for the greatest number of founder effects in *MEFV*, while three other distinct evolutionary groups are

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proposed. These meta-analysis data are believed to provide a most valuable diagnostic and research tool for further studies, thought to be of special interest nowadays, where ethnic, cultural, religious and linguistic barriers fall beyond worldwide migration and assimilation of peoples.

References

Aksentijevich, I., Torosyan, Y., Samuels, J., Centola, M., Pras, E., Chae, J. J., Oddoux, C., Wood, G., Azzaro, M. P., Palumbo, G., Giustolisi, R., Pras, M., Ostrer, H. & Kastner, D. L. (1999) Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population. *Am J Hum Genet* **64**, 949–962.

Aldea, A., Calafell, F., Arostegui, J. I., Lao, O., Rius, J., Plaza, S., Masó, M., Vives, J., Buades, J. & Yagüe, J. (2004) The west side story: *MEFV* haplotype in Spanish FMF patients and controls, and evidence of high LD and a recombination ''hotspot'' at the *MEFV* locus. *Hum Mutat* **23**, 399–406.

Behar, D. M., Villems, R., Soodyall, H., Blue-Smith, J., Pereira, L., Metspalu, E., Scozzari, R., Makkan, H., Tzur, S., Comas, D., Bertranpetit, J., Quintana-Murci, L., Tyler-Smith, C., Wells, R. S. & Rosset, S., Genographic Consortium. (2008) The dawn of human matrilineal diversity. *Am J Hum Genet* **82**, 1130–1140.

Bernot, A., da Silva, C., Petit, J. L., Cruaud, C., Caloustian, C., Castet, V., Ahmed-Arab, M., Dross, C., Dupont, M., Cattan, D., Smaoui, N., Dodé, C., Pêcheux, C., Nédelec, B., Medaxian, J., Rozenbaum, M., Rosner, I., Delpech, M., Grateau, G., Demaille, J., Weissenbach, J. & Touitou, I. (1998) Non-founder mutations in the *MEFV* gene establish this gene as the cause of familial Mediterranean fever (FMF). *Hum Mol Genet* **7**, 1317–1325.

Cattan, D. (2005) *MEFV* mutation carriers and diseases other than familial Mediterranean fever: proved and non-proved associations; putative biological advantage. *Curr Drug Targets Inflamm Allergy* **4**, 105–112.

Chaabouni, H. B., Ksantini, M., M'rad, R., Kharrat, M., Chaabouni, M., Maazoul, F., Bahloul, Z., Ben Jemaa, L., Ben Moussa, F., Ben Chaabane, T., Mrad, S., Touitou, I. & Smaoui, N. (2007) *MEFV* mutations in Tunisian patients suffering from familial Mediterranean fever. *Semin Arthritis Rheum* **36**, 397–401.

Deltas, C. C. (2003) Discovery of old diseases: the molecular approach. *Eur J Hum Genet* **11**, 3–4.

Deltas, C. C. (2004) Inherited Diseases and Cyprus Reality. A Historico-Genetic Approach. *Annals of Cyprus Research Center* **30**, 457–489.

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.

Felsenstein, J. (1989) PHYLIP – Phylogeny Inference Package (Version 3.2). *Cladistics* **5**, 164–166.

Fragouli, E., Eliopoulos, E., Petraki, E., Sidiropoulos, P., Aksentijevich, I., Galanakis, E., Kritikos, H., Repa, A., Fragiadakis, G., Boumpas, D. T. & Goulielmos, G. N. (2008) Familial Mediterranean Fever in Crete: a genetic and structural biological approach in a population of 'intermediate risk'. *Clin Genet* **73**, 152–159.

Gershoni-Baruch, R., Shinawi, M., Leah, K., Badarnah, K. & Brick, R. (2001) Familial Mediterranean fever: prevalence, penetrance, and genetic drift. *Eur J Hum Genet* **9**, 634–637.

Gershoni-Baruch, R., Brik, R., Shinawi, M. & Livneh, A. (2002) The differential contribution of *MEFV* mutant alleles to the clinical profile of Familial Mediterranean Fever. *Eur J Hum Genet* **10**, 145–149.

Giaglis, S., Papadopoulos, V., Kambas, K., Doumas, M., Tsironidou, V., Rafail, S., Kartalis, G., Speletas, M. & Ritis, K. (2007) *MEFV* alterations and population genetics analysis in a large cohort of Greek patients with familial Mediterranean fever. *Clin Genet* **71**, 458–467.

Guo, S. W. & Thompson, E. A. (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Infevers: an online database for autoinflammatory mutations. Available at *http://fmf.igh.cnrs.fr/ISSAID/infevers/ Accessed* 2008, March 14.

Jalkh, N., Génin, E., Chouery, E., Delague, V., Medlej-Hashim, M., Idrac, C. A., Mégarbané, A., Serre, J. L. (2008) Familial Mediterranean Fever In Lebanon: Founder Effects For Different *MEFV* Mutations. *Ann Hum Genet* **72**, 41–47.

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**, 50–56.

Labate, J. A. (2000) Software for Population Genetic Analyses of Molecular Marker Data. *Crop Sci* **40**, 1521–1528.

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**, 813–818.

Mansour, I., Delague, V., Cazeneuve, C., Dodé, C., Chouery, E., Pêcheux, C., Medlej-Hashim, M., Salem, N., El Zein, L., Levan-Petit, I., Lefranc, G., Goossens, M., Delpech, M., Amselem, S., Loiselet, J., Grateau, G., Mégarbane, A. & Naman, R. (2001) Familial Mediterranean fever in Lebanon: mutation spectrum, evidence for cases in Maronites, Greek orthodoxes, Greek catholics, Syriacs and Chiites and for an association between amyloidosis and M694V and M694I mutations. *Eur J Hum Genet* **9**, 51–55.

Mattit, H., Joma, M., Al-Cheikh, S., El-Khateeb, M., Medlej-Hashim, M., Salem, N., Delague, V. & Mégarbané, A. (2006) Familial Mediterranean fever in the Syrian population: gene mutation frequencies, carrier rates and phenotype-genotype correlation. *Eur J Med Genet* **49**, 481–486.

Medlej-Hashim, M., Serre, J. L., Corbani, S., Saab, O., Jalkh, N., Delaque, V., Chouery, E., Salem, N., Loiselet, J., Lefranc, G. & Mégarbané, A. (2005) Familial Mediterranean fever (FMF) in Lebanon and Jordan: a population genetics study and report of three novel mutations. *Eur J Med Genet* **48**, 412–420.

Ritis, K., Giaglis, S., Spathari, N., Micheli, A., Zonios, D., Tzoanopoulos, D., Deltas, C. C., Rafail, S., Mean, R., Papadopoulos, V., Tzioufas, A. G., Moutsopoulos, H. M. & Kartalis, G. (2004) Non-isotopic RNase cleavage assay for mutation detection in *MEFV*, the gene responsible for familial Mediterranean fever, in a cohort of Greek patients. *Ann Rheum Dis* **63**, 438–443.

Rohde, D. L., Olson, S. & Chang, J. T. (2004) Modelling the recent common ancestry of all living humans. *Nature* **431**, 562–566.

Sackesen, C., Bakkaloglu A., Sekerel B. E., Ozaltin F., Besbas N., Yilmaz E., Adalioglu G., Ozen S. (2004) Decreased prevalence of atopy in paediatric patients with familial Mediterranean fever. *Ann Rheum Dis* **63**, 187–190.

Sarkisian, T., Ajrapetyan, H. & Shahsuvaryan, G. (2005) Molecular study of FMF patients in Armenia. *Curr Drug Targets Inflamm Allergy* **4**, 113–116.

Schaner, P., Richards, N., Wadhwa, A., Aksentijevich, I., Kastner, D., Tucker, P., & Gumucio, D. (2001) Episodic evolution of pyrin

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in primates: human mutations recapitulate ancestral amino acid states. *Nat Genet* **27**, 318–321.
Schaner, P. E. & Gumucio, D. L. (2005) Familial Mediterranean fever in the post-genomic era: how an ancient disease is providing new insights into inflammatory pathways. *Curr Drug Targets Inflamm Allergy* **4**, 67–76.
Schneider, S., Roessli, D. & Excoffier, L. (2000) *Arlequin ver. 2.000: A software for population genetics data analysis*. Switzerland: Genetics and Biometry Laboratory, University of Geneva.
Sohar, E., Gafni, J., Pras, M. & Heller, H. (1967) Familial Mediterranean Fever: A survey of 470 cases and review of the literature. *Am J Med* **43**, 227–253.
Stamatoyannopoulos, G. & Nute, P. E. (1984) Cases of unstable hemoglobin and methemoglobin produced by de novo mutation. *Hemoglobin* **8**, 85–95.
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**, 307–310.
Tchernitchko, D., Moutereau, S., Legendre, M., Delahaye, A., Cazeneuve, C., Lacombe, C., Grateau, G. & Amselem, S. (2005) *MEFV* analysis is of particularly weak diagnostic value for recurrent fevers in Western European Caucasian patients. *Arthritis Rheum* **52**, 3603–3605.
The French FMF consortium. (1997) A candidate gene for familial Mediterranean fever. *Nat Genet* **17**, 25–31.
The international FMF consortium. (1997) Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* **90**, 797–807.
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., Dodé, 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**, 194–198.
Tunca, M., Kirkali, G., Soytürk, M., Akar, S., Pepys, M. B. & Hawkins, P. N. (1999) Acute phase response and evolution of familial Mediterranean fever. *Lancet* **353**, 1415.
Turkish FMF Study Group. (2005) Familial Mediterranean Fever (FMF) in Turkey. Results of a Nationwide Multicenter Study. *Medicine* **84**, 1–11.
Yigit, S., Bagci, H., Ozkaya, O., Ozdamar, K., Cengiz, K. & Akpolat, T. (2008) *MEFV* mutations in patients with familial Mediterranean fever in the Black Sea region of Turkey. *J Rheumatol* **35**, 106–113.
Yilmaz, E., Ozen, S., Balci, B., Duzova, A., Topaloglu, R., Besbas, N., Saatci, U., Bakkaloglu, A. & Ozguc, M. (2001) Mutation frequency of Familial Mediterranean Fever and evidence for a high carrier rate in the Turkish population. *Eur J Hum Genet* **9**, 553–555.
Zonios, D., Mavragani, C. P., Giaglis, S. & Ritis, K. (2007) A novel mutation of the familial Mediterranean fever gene in a Greek family related to a non-classical, variably expressed FMF phenotype. *Rheumatol Int* **28**, 167–169.

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