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

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Summary

Familial Mediterranean fever (FMF) is a recessive disorder characterized by episodes of fever with serositis or synovitis. The FMF gene (*MEFV*) was cloned recently, and four missense mutations were identified. Here we present data from non-Ashkenazi Jewish and Arab patients in whom we had not originally found mutations and from a new, more ethnically diverse panel. Among 90 symptomatic mutation-positive individuals, 11 mutations accounted for 79% of carrier chromosomes. Of the two mutations that are novel, one alters the same residue (680) as a previously known mutation, and the other (P369S) is located in exon 3. Consistent with another recent report, the E148Q mutation was observed in patients of several ethnicities and on multiple microsatellite haplotypes, but haplotype data indicate an ancestral relationships between non-Jewish Italian and Ashkenazi Jewish patients with FMF and other affected populations. Among ~200 anonymous Ashkenazi Jewish DNA samples, the *MEFV* carrier frequency was 21%, with E148Q the most common mutation. Several lines of evidence indicate reduced penetrance among Ashkenazi Jews, especially for E148Q, P369S, and K695R. Nevertheless, E148Q helps account for recessive inheritance in an Ashkenazi family previously reported as an unusual case of dominantly inherited FMF. The presence of three frequent *MEFV* mutations in multiple Mediterranean populations strongly suggests a heterozygote advantage in this geographic region.

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Introduction

Familial Mediterranean fever (FMF; MIM 249100) is a recessively inherited disorder characterized by acute, self-limited attacks of fever and dramatic but unexplained migration of granulocytes into the serosal or synovial spaces (reviewed in Samuels et al. 1998). Some patients also develop systemic amyloidosis because of the widespread tissue deposition of a product of the acute phase reactant serum amyloid A, most notably in the kidneys. Recently, the gene causing FMF (designated "*MEFV*") was identified on chromosome 16p13.3 by positional cloning (French FMF Consortium 1997; International FMF Consortium 1997). It encodes a novel protein, variously called "pyrin" or "marenostrin," that exhibits homology to a number of nuclear factors and is expressed predominantly in granulocytes. On the basis of the FMF phenotype, pyrin is likely to be a negative regulator of granulocyte-mediated inflammation. FMF is the prototype for several hereditary periodic fever syndromes (reviewed in Centola et al. 1998; Kastner 1998), including the recessively inherited hyperimmunoglobulinemia D with periodic fever syndrome (MIM 260920; also known as "Dutch-type periodic fever") and the dominantly inherited familial Hibernian fever (MIM 142680) and familial periodic fever, which were recently mapped to chromosome 12p13 (McDermott et al. 1998; Mulley et al. 1998).

Although FMF has been reported in many different countries, most large clinical series involve four Mediterranean populations: non-Ashkenazi Jews, Armenians, Arabs, and Turks. Carrier frequencies, when they have been estimated in these ethnic groups, are very high. Among various non-Ashkenazi North African Jewish populations, the carrier frequency has been estimated as 1/5–1/10 (Daniels et al. 1995; Yuval et al. 1995), whereas among Armenians in Los Angeles it is ~1/7 (Rogers et al. 1989). Such high frequencies in several

geographically related populations suggest that carriers of pyrin mutations might have a selective advantage, possibly because of heightened resistance to a pathogen endemic to the eastern Mediterranean.

The cloning of *MEFV* immediately broadened our understanding of FMF population genetics. Four missense mutations (M680I, M694V, M694I, and V726A), clustered within 46 amino acids of a predicted 781–amino acid protein, were responsible for a large percentage of mutations in the two initial study panels of families (French FMF Consortium 1997; International FMF Consortium 1997). Two of these mutations, M694V and V726A, are likely to date back at least to biblical times, because they were seen in association with specific microsatellite haplotypes in populations that have been separated for many centuries (International FMF Consortium 1997). Moreover, four different microsatellite haplotypes bearing the M694V mutation demonstrated convergence of single nucleotide–polymorphism (SNP) haplotypes within *MEFV.* In the case of the North African Jewish population, 83%–93% of FMF carrier chromosomes bore the M694V mutation in association with one microsatellite haplotype, strongly supporting genetic drift as an explanation for the high gene frequency in this ethnic group.

Since the cloning of *MEFV,* we and others have continued to screen for disease-associated mutations. Our goals have been to find mutations in the small number of individuals from our original study panel in whom they had not already been found and to assess the spectrum of mutations in a more ethnically diverse sample. It was unclear whether the four originally described mutations would account for a large percentage of new cases, because our initial panel of families was skewed with regard to both their ethnic background (predominantly non-Ashkenazi Jewish) and their disease severity (families with mild disease generally were not included). In an analysis of American periodic fever referrals (Samuels et al. 1998), we reported four previously undescribed *MEFV* mutations (E148Q, K695R, A744S, and R761H). A French group (Bernot et al. 1998) independently presented these same new mutations and four others (E167D, T267I, F479L, and I692del). Two additional mutations (T681I and M694del) were recently reported by an English group (Booth et al. 1998). The present study is a comprehensive analysis of patients and families from Israel, the United States, and Europe. In addition to identifying two novel *MEFV* mutations, this work presents the broadest FMF mutational screening to date in a large, ethnically diverse study population.

Overall, the second most common mutation in the recent American and French series was E148Q, occurring in all the major affected ethnic groups. Haplotype analysis of modern-day chromosomes bearing this mutation can provide evidence with respect to whether it is present on several independent lineages of carrier chromosomes, and is therefore a recurring mutation, or on a single common carrier chromosome, and is therefore caused by a single mutational event. On the basis of microsatellites flanking *MEFV,* Bernot et al. (1998) concluded that the former possibility is most likely. However, it should be noted that intragenic SNP analysis of four apparently distinct microsatellite haplotypes bearing another mutation (M694V) strongly suggests that they have a common, but relatively ancient, origin (International FMF Consortium 1997); we also found SNP convergence for two microsatellite haplotypes bearing M680I. The present study revisits the genesis of the E148Q mutation, on the basis of similar intragenic SNP haplotype analyses.

We were also interested in exploring the origins and frequency of mutations in populations in which these have not been previously examined. One such group is the Ashkenazi Jewish population. Previous data on the prevalence of FMF among Ashkenazi Jews are contradictory. Although the first recognizable case description of FMF involved an Ashkenazi Jewish girl (Janeway and Mosenthal 1908) and one of the first large American series of patients (under the name "familial paroxysmal polyserositis") was predominantly Ashkenazi Jewish (Siegal 1964), population-based studies from Israel have indicated a comparatively low carrier frequency of 1/135 (Yuval et al. 1995) among the Ashkenazim. If true, lower carrier frequencies in the Ashkenazi population could be attributable either to genetic drift or to the absence of a putative heterozygote advantage. In contrast, by means of a method based on the frequency of FMF among the first cousins of FMF probands, Daniels et al. (1995) estimated a carrier frequency of 1/11.4. These latter results, although determined on the basis of a small sample, suggest underascertainment of FMF among the Ashkenazi population. Consistent with such a possibility, FMF in Ashkenazi Jews is generally less severe than among Sephardic Jews, with a markedly lower rate of amyloidosis (Pras et al. 1982). Conceivably, this could be the result of differences in *MEFV* mutations, modifying genes, or nongenetic environmental effects. The present study provided an opportunity to examine more directly the frequency, origin, and penetrance of *MEFV* mutations in this population.

We also were able to obtain DNA for mutational analysis of an Israeli family of Ashkenazi ancestry that had previously been reported (Yuval et al. 1995) as probably exhibiting dominant inheritance of FMF. This was clearly of interest, because segregation analysis (Sohar et al. 1961) and initial mutation studies (French FMF Consortium 1997; International FMF Consortium 1997) support a recessive mode of inheritance. Other cases of possible dominant inheritance of FMF have been explained as pseudodominance because of the high car-

rier frequency for FMF in Mediterranean populations. The family studied here was not thought to be attributable to this phenomenon because four-generation inheritance of FMF was thought to be highly improbable in the face of a calculated low Ashkenazi gene frequency. Here we were able to test directly the mutational status of affected members of this family, as well as to determine more accurately the gene frequency that had served as the basis for the assertion of dominant inheritance.

Finally, the status of FMF among Mediterranean populations other than Jews, Arabs, Turks, and Armenians has been unclear. The only relevant published data are found in a small number of case reports, the most numerous of which concern Italians or Italian-Americans. In the past 30 years, there have been six such reports of so-called Italian FMF (Reich and Franklin 1970; Secchi et al. 1981; Passiu et al. 1984; Gentiloni et al. 1992; Marinone et al. 1992; Breda et al. 1998); all the patients were from southern Italy or Sicily. In one case, intestinal amyloidosis was documented (Reich and Franklin 1970) but occurred relatively late in life. There are also two reports of patients from Malta with FMF (Siegal et al. 1964; Rosenstein and Kramer 1994), an island lying south of Sicily in the Mediterranean. Nevertheless, patients of Italian ancestry with periodic fever and serositis were the third most common ethnic group (after Armenians and Ashkenazi Jews) among American patients (Samuels et al. 1998), and we have received additional Italian referrals from Sicily and Belgium. With the largest single experience with this ethnic group to date, we have investigated the spectrum of *MEFV* mutations and carrier haplotypes in Italian patients with FMF.

Families and Methods

Families and DNA Specimens

The present study included several Israeli families from our previous linkage and positional cloning projects (Balow et al. 1997; International FMF Consortium 1997). Seven of the 61 families from these earlier studies were chosen because we had not identified both *MEFV* mutations in affected individuals, whereas other families with known mutations were chosen for intragenic or extragenic haplotype analysis. All these families had been ascertained and sampled at the Chaim Sheba Medical Center in Tel-Hashomer, Israel, as described elsewhere (International FMF Consortium 1997). The diagnosis of FMF in all these families was made according to established clinical criteria (Sohar et al. 1967; Livneh et al. 1997). After obtaining informed consent, we collected blood samples from available family members. DNA was extracted from whole blood or from Epstein-Barr virus–transformed lymphocytes by standard techniques. Overall, we included nine Israeli families of non-Ashkenazi and two of Ashkenazi Jewish ancestry and five Israeli families of Arab/Druze ancestry.

Subsequent to the identification of *MEFV,* we performed mutational analysis on DNA samples derived from a total of 274 additional individuals. These included patients meeting clinical criteria for FMF who desired genetic confirmation of the diagnosis; individuals with unexplained fever, abdominal pain, or arthritis atypical of FMF who were seeking a diagnosis; and several asymptomatic relatives of patients. A total of 82 individuals were seen at the Clinical Center of the National Institutes of Health (NIH), 145 samples were sent from other sites in the United States, and an additional 47 samples were sent from outside the United States. The latter included samples from two Italian families, one living in Sicily and the other in Belgium. After subjects gave written informed consent, 5 ml of blood was drawn into tubes containing EDTA; samples from outside the NIH were express-shipped at ambient temperature. Genomic DNA was extracted from whole blood by means of a commercially available kit (Puregene, Gentra Systems).

Various control DNA samples were used in these studies. A total of 60 were from NIH Blood Bank donors, and 51 anonymous white controls were obtained at New York University. An additional 93 samples were from well-characterized Israeli FMF families; 12 were noncarriers and 81 were carriers, as determined by haplotype analysis.

In addition, *MEFV* mutational surveys were performed on a total of 213 anonymous American Ashkenazi Jewish DNA samples obtained from couples undergoing carrier screening for cystic fibrosis, Gaucher disease, and Tay-Sachs disease (Kronn et al. 1998). The frequencies of mutations in *CFTR,* glucocerebrocidase (Gaucher disease), hexosaminidase A (Tay-Sachs disease), *N*-aspartoacylase (Canavan disease), *BRCA1, BRCA2,* and *APC* observed in this population are similar to those observed in other series of Ashkenazi samples.

Microsatellite Analysis

Genotyping for *D16S3370, D16S2617, D16S3373, D16S3275,* and *D16S3376* was performed as described by Balow et al. (1997). Genotyping for *D16S3403, D16S3404,* and *D16S3405* (International FMF Consortium 1997) was performed by use of oligonucleotide primers and PCR conditions available through the Genome Database. Microsatellite haplotypes were constructed so as to minimize the number of intrafamilial recombinations.

Genomic DNA Sequencing

We amplified individual *MEFV* exons and flanking intronic sequences from genomic DNA by using M13-tailed oligonucleotide primers and PCR conditions, as described elsewhere (International FMF Consortium 1997), except as follows. In the present study, exon 2 was amplified in two overlapping PCR fragments. The forward and reverse oligonucleotide primers used to amplify the 5′ end of exon 2 were 5′-ATCATTTTGCATCTGGTTGTCCTTCC-3′ and 5′-TCCCCTGTAGAAATGGTGACCTCAAG-3′, respectively. The forward and reverse primers used to amplify the 3′ end of exon 2 were 5′-GGCCGGGAGGGGGCTGTCGAGGAAGC-3′ and 5′-TCGTGCCCGGCCAGCCATTCTTTCTC-3′, respectively. Exons 8 and 9 were amplified separately in the present study. The forward primer for exon 8 was 5′-GCATGCTCACTTCCTCCCTA-3′; and the reverse primer was 5′-CTTTGCTCCAGGTGTTTGGT-3′. The forward primer for exon 9 was 5′-TTAGACCACAGTCCCCAACA-3′; and the reverse primer was 5′-CAGGAAACAGGGACAGGGTA-3′.

Mutation detection was performed by fluorescent sequencing with dye-primer chemistry, as described elsewhere (International FMF Consortium 1997). Sequencing reactions were run on an ABI 377 automated sequencer, and data were analyzed with SEQUENCHER 3.0 (Gene Codes).

Amplification-Refractory Mutation Screening (ARMS)

We performed ARMS assays by amplifying 100 ng genomic DNA in a total volume of 25 μl, with 1 pmol of each primer and 0.2 mM dNTPs (Gibco BRL), in 1 × PCR buffer and 0.04 U AmpliTaq Gold (Perkin Elmer). The PCR amplification was performed under the following conditions: 94°C for 10 min, followed by 35 cycles of 94°C for 10 s, 60°C for 10 s, and 72°C for 30 s, with a final extension time of 10 min at 72°C. The annealing temperatures for the M694V and M694I assays were 55°C and 50°C, respectively. The amplified products were separated by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

Restriction Analysis of PCR Products from Genomic DNA

Restriction analysis was performed with the following steps: 50–100 ng genomic DNA in a total volume of 10 ml were amplified in 1.5 mmol $MgCl_2$ PCR buffer and 0.04 U AmpliTaq Gold. PCR began with 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with the final extension at 72°C for 10 min. The annealing temperature for the P369S amplification was 60°C. The amplified products were digested for ≥2 h with 10 U of the appropriate restriction enzymes (*Bst*NI, *Alu*I, or *Hin*fI). Positive and negative controls were included in each run. The samples were loaded on 4%–20% polyacrylamide gels (Novex) and stained with ethidium bromide.

SNP Analysis of FMF Carrier Chromosomes

MEFV intragenic SNP haplotypes were determined by DNA sequencing. In addition to known sites of mutations, 11 intragenic SNPs were evaluated, as described elsewhere (International FMF Consortium 1997). According to our previous nomenclature, exonic SNPs were designated by "x," and intronic SNPs by "i," with "x2.1" indicating the first SNP in exon 2. In order from telomere to centromere, including five known sites of mutations, the SNPs studied in this report were: V726A (T/**C**, nt 2177), M694V (A/**G**, nt 2080), M680I (G/**C/A**, nt 2040), x9.1 (A/G, nt 1764), i6.1 (C/T, nt 1610+95), x5.3 (C/T, nt 1530), x5.2 (A/G, nt 1428), x5.1 (A/G, nt 1422), i4.1 (A/G, nt 1356+43), P369S (C/**T**, nt 1105), x3.1 (C/T, nt 942), x2.4 (A/G, nt 605), x2.3 (A/C, nt 495), E148Q (G/**C**, nt 442), x2.2 (A/G, nt 414), and x2.1 (C/T, nt 306). Underlined sites are locations of mutations, with mutant alleles in boldface type.

Results

MEFV *Mutational Survey*

Since the initial identification of *MEFV,* we have used automated sequencing of PCR-amplified genomic DNA to screen for mutations. We have concentrated on new referrals and the small percentage of our original study panel, mostly patients of Arab or Druze ancestry, in whom we had not found any of the then four known *MEFV* mutations. After sequencing the entire coding region and splice junctions in several patients of Arab/Druze ancestry, we identified two disease-associated missense mutations in exon 2. These two mutations, which result in the substitution of glutamine for glutamic acid at codon 148 (E148Q) and of aspartic acid for glutamic acid at codon 167 (E167D), are depicted in figure 1*A* and *B*. Sequencing of the entire *MEFV* coding region and haplotype analysis in a family segregating E167D indicated that this mutation can exist as the only coding abnormality on a carrier chromosome, whereas a similar analysis of E148Q demonstrated that this substitution can occur either as the sole amino acid change on a carrier chromosome or as a complex allele with one of two other mutations (discussed below). Restriction endonuclease (E148Q) or ARMS (E167D) assays (fig. 1, *bottom panels;* table 1) permitted rapid screening of a panel of control chromosomes; E167D was not found in any of 100 control chromosomes, whereas E148Q was found in 1 of 246 controls. Bernot et al. (1998) also

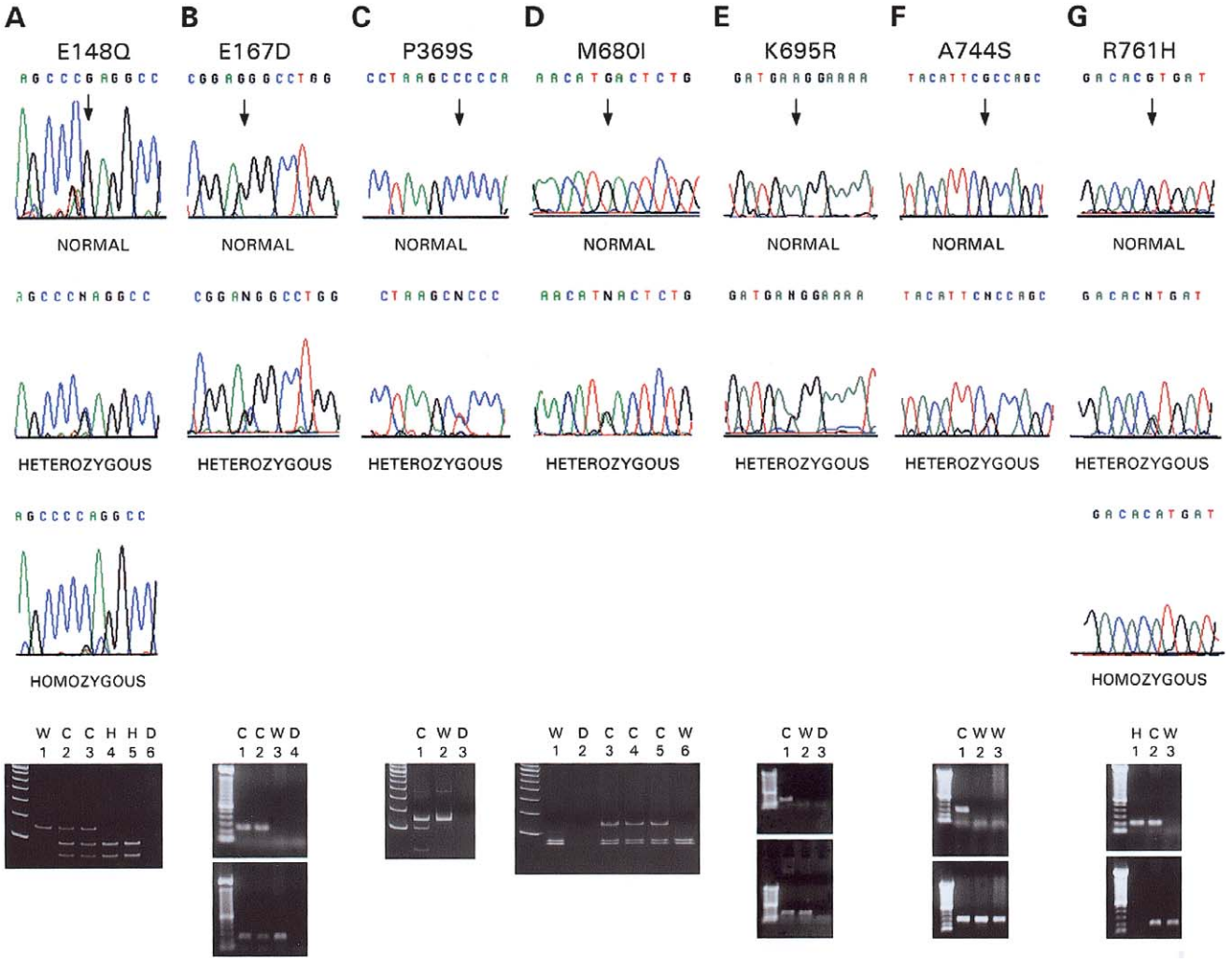

Figure 1 DNA sequence electropherograms and rapid-screening assays for seven missense mutations identified in the present survey. The P369S and M680I mutations are novel. The upper three panels depict individuals who were normal, heterozygous, and, where available, homozygous for each of these sites. The downward arrow indicates the point of the single-base mutation. The lower panels depict rapid-screening assays that were developed for each of the mutations. ARMS assays were developed for E167D, K695R, A744S, and R761H. For these assays, the upper photograph in the lower panel represents the product of the mutant-specific primer pair, and the lower photograph represents the product of the wild-type primer pair. Restriction endonuclease analyses of PCR products were used for E148Q, P369S, and M680I. "W" denotes wild-type, "C" denotes the carrier for the mutation, "H" denotes homozygous for the mutation, and "D" denotes the distilled-water control.

recently reported E148Q in their Druze population and found E167D in an Armenian patient.

Mutational screening was also performed on a set of >200 DNA samples from patients with periodic fevers. The clinical spectrum ranged from patients with classic histories of FMF to those with unexplained and sometimes very atypical constellations of symptoms. Clinical details of 100 of the referrals from the United States (47 with identifiable *MEFV* mutations) are presented elsewhere (Samuels et al. 1998). Because all four of the mutations initially described in *MEFV* were in exon 10, our mutational screen included sequencing of the coding region of this exon in its entirety for all 274 individuals. In addition to the initially described mutations, four other missense substitutions in this exon were identified among these patients (2040G→A, M680I, fig. 1*D;* 2084A→G, K695R, fig. 1*E;* 2230G→T, A744S, fig. 1*F;* and 2282G→A, R761H, fig. 1*G*). One of these mutations, 2040G→A, is novel and is the second reported single-nucleotide alteration leading to an M680I substitution (the first being a G→C transversion at the same position). For a R761H homozygote, sequencing of the entire coding region and splice junctions failed to identify any other sequence changes. For M680I

Table 1

PCR Primers Used in Mutation Assays

Mutation	Assay/Primer Type	Primer
442G→C (E148Q)	*Bst*NI Digest:	
	Forward	5′-GCCTGAAGACTCCAGACCACCCCG- 3′
	Reverse	5′-AGGCCCTCCGAGGCCTTCTCTCTG-3′
501G→C (E167D)	ARMS:	
	Mutant	5′-GGGCTCCGGGTCCGAGGCTTGCCCTGCGCGTCCAGGCCG-3′
	Normal	5′-GGGCTCCGGGTCCGAGGCTTGCCCTGCGCGTCCAGGCCC-3′
	Common	5′-TATTCCACACAAGAAAACGGCACAGATGATTCCGCAGCG-3′
1105C→T (P369S)	*Alu*I Digest:	
	Forward	5′-TCCCCGAGGCAGTTTCTGGGCACC-3′
	Reverse	5′-TGGACCTGCTTCAGGTGGCGCTTAC-3′
2040G→A (M680I)	*Hin*fI Digest:	
	Forward	5′-GAGGTTGGAGACAAGACAGCATGG-3′
	Reverse	5′-TCGGGGGAACGCTGGACGCCTGGTACTCATTTTCCT-3′
2084A→G (K695R)	ARMS:	
	Mutant	5′-TCGGGGGAACGCTGGACGCCTGGTACTCATTTTCCC-3′
	Normal	5′-TCGGGGGAACGCTGGACGCCTGGTACTCATTTTCCT-3′
	Common	5′-TTAGACTTGGAAACAAGTGGGAGAGGCTGC-3′
2230G→T (A744S)	ARMS:	
	Mutant	5′-GAAGATAGGTTGAAGGGGCCCAGAGAAAGAGCAGCTGAA-3′
	Normal	5′-GAAGATAGGTTGAAGGGGCCCAGAGAAAGAGCAGCTGAC-3′
	Common	5′-TGGAGGTTGGAGACAAGACAGCATGGATCC-3′
2282G→A (R761H)	ARMS:	
	Mutant	5′-ACAGATAGTCAGAGGAGCTGTGTTCTTCCCTCCATCTT-3′
	Normal	5′-ACAGATAGTCAGAGGAGCTGTGTTCTTCCCTCCATCTC-3′
	Common	5′-ACCCGCCTGCTAATAAAGGAGCCTCCCAAGCG-3′

(2040G→A), K695R, and A744S, we have not yet encountered a homozygous patient; analysis of coding and splice sequences in patients harboring these latter mutations revealed single additional heterozygous mutations, presumably on the opposite parental chromosome. Using restriction endonuclease and ARMS screening assays (fig. 1, *bottom panels;* table 1), we detected none of these substitutions in control panels comprising NIH Blood Bank volunteer samples or noncarrier chromosomes from FMF families.

Finally, in one of the Ashkenazi Jewish patients who was homozygous for the E148Q mutation, sequencing of entire coding region demonstrated that this individual was heterozygous for a novel C→T transition at nt 1105 (P369S, fig. 1*C*). By using an *Alu*I restriction endonuclease assay (fig. 1 and table 1), we subsequently identified P369S with E148Q in a second Ashkenazi Jewish patient and without E148Q in three other patients (two of whom had no known Jewish ancestry) but in only 1 of 222 control chromosomes.

Table 2 summarizes the frequencies of the various mutations identified in the panel of 274 referrals from the United States, Europe, and Israel. Screening consisted of the sequencing of the entire coding region of exon 10, plus ARMS/restriction endonuclease assays for E148Q, E167D, and P369S. When possible, the phase of complex alleles was assigned by genotyping parents or offspring. Consistent with the clinical heterogeneity of this cohort, only 90 unrelated individuals had at least one demonstrable mutation in *MEFV.* Of 180 carrier chromosomes in these individuals, we could account for mutations in 142 (79%). In contrast to the North African Jewish population, in which 93% of carrier chromosomes bore M694V (International FMF Consortium 1997), only 27% of chromosomes (48 of 180) from the present ethnically diverse cohort had this mutation. When carrier chromosomes bearing mutations alone or in combination were tabulated, V726A was the second most common mutation (36 [20%] of 180), followed by E148Q (26 [14%] of 180). It is also noteworthy that patients of Ashkenazi Jewish and Italian ancestry accounted for 20% and 16%, respectively, of the mutation-positive referrals.

Origins of E148Q-Bearing Chromosomes

Previously published microsatellite and SNP haplotype data suggest that most M694V and V726A alleles are descended from two ancestral chromosomes (French FMF Consortium 1997; International FMF Consortium 1997). We therefore asked whether there is also such a relationship among E148Q chromosomes, because, like M694V and V726A, this mutation is prevalent and cuts across ethnic boundaries. Table 3 presents microsatellite and SNP haplotypes deduced for five carrier chromosomes with only the E148Q mutation. Consistent with

Table 2

Distribution of *MEFV* Mutations among New Referrals, by Ethnic Group

	No. of Chromosomes with Mutation													
Ethnic Group[a]	E148Q	E167D	P369S	M680I[b]	M680I[c]	M694V	M694I	K695R	V726A	A744S	R761H	148/369	148/726	Total
Armenian (18)	4	1	1	6	0	11	0	0	5	0	2	0	0	30
Ashkenazi (18)	3	0	1	0	0	3	0	2	13	1	0	2	4	29
Italian (14)	7	0	0	1	1	6	1	0	2	0	1	0	1	20
Arab (14)	2	0	0	1	0	8	5	0	8	1	0	0	0	25
NAJ (9)	1	0	0	0	0	10	0	0	1	0	0	0	0	12
Turkish (8)	2	0	0	5	0	7	1	0	1	0	0	0	0	16
Spanish/Cuban (2)	0	0	0	0	0	2	0	0	0	0	0	0	0	2
Greek (1)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Iranian (1)	0	0	0	0	0	0	0	0	0	0	2	0	0	2
Other (5)	0	0	2	0	0	0	0	2	1	0	0	0	0	5
Total (90)	19	1	4	13	1	48	7	4	31	2	5	2	5	142

Note.—The T681I, I692del, and M694del mutations were not found in this set of patients.

[a] NAJ = non-Ashkenazi Jewish; numbers in parentheses represent numbers of unrelated patients.

[b] 2040G→C.

[c] 2040G→A.

recently published microsatellite data of Bernot et al. (1998), multiple microsatellite haplotypes were discernible. However, when these five carrier chromosomes were typed for sites of known mutations and 11 intragenic SNPs (International FMF Consortium 1997), we found that, despite the fact that there were four different SNP motifs on the 3′ end of the gene, all these haplotypes converged to G . . C . . **C** . . A . . T for the SNPs within exon 2 (x2.4, x2.3, E148Q, x2.2, and x2.1). Against the possibility that this might simply reflect a limited degree of polymorphism for these SNPs, the frequencies of the aforementioned alleles in a panel of 24 normal chromosomes of Jewish ancestry were 62.5% G at x2.4, 54% C at x2.3, 50% A at x2.2, and 50% T at x2.1 (International FMF Consortium 1997).

Table 3 also compares haplotypes of chromosomes bearing the complex E148Q-V726A carrier chromosome to those bearing these mutations individually. Chromosomes with V726A alone and those with the complex allele share microsatellite genotypes 3′ (telomeric) of *MEFV,* but diverge 5′ (centromeric) of the gene, suggesting that the complex allele is the result of recombination. Moreover, E148Q-V726A chromosomes share the **C** . . A . . G . . G . . T . . C . . G . . A . . G . . C . . T haplotype at the 3′ end of *MEFV,* with the SNP haplotype found in all V726A chromosomes. This suggests that the recombination giving rise to the complex allele may have occurred within *MEFV.* SNP data on the 5′ end of E148Q-V726A chromosomes are consistent with the common founder for all E148Q-bearing chromosomes, although the precise location of the putative intragenic recombination event would depend on the SNP haplotype of the ancestral E148Q-bearing chromosome that participated in this process.

Although the P369S mutation has been observed without E148Q in four individual samples, family data are not yet available to permit a haplotype comparison of chromosomes bearing P369S alone with those bearing the P369S-E148Q complex allele. However, intragenic SNP haplotype analysis of one Ashkenazi Jewish family segregating the complex allele demonstrated the conserved G . . C . . **C** . . A . . T motif in exon 2 of the complex chromosome and of a chromosome bearing E148Q alone, and heterozygosity at the exon 3 SNP in the one individual who was heterozygous for E148Q and E148Q-P369S.

FMF in the Ashkenazi Jewish Population

Because Ashkenazi Jewish patients with FMF represented a substantial percentage of the new referrals (table 2) and because FMF is thought to be relatively uncommon in this population, it was of interest to determine experimentally the frequencies of known *MEFV* mutations among the Ashkenazim. Table 4 shows the results of these studies in a cohort of Ashkenazi Jews undergoing carrier screening for other genetic disorders at the New York University Medical Center. Although it was not possible to contact members of this panel to inquire about symptoms of FMF, none of these individuals noted such complaints on a questionnaire administered at the time blood samples were obtained. The overall carrier frequency calculated from mutation frequencies was 21%, more than twice the 9% estimated from the frequency of FMF in the first cousins of affected probands (Daniels et al. 1995) and more than 25 × a recent estimate calculated on the basis of total numbers of Ashkenazi patients with FMF in Israel (Yuval et al. 1995).

Approximately one-half of the carrier chromosomes we observed were attributable to E148Q, with P369S

Table 3

Microsatellite and Intragenic SNP Haplotypes of FMF Carrier Chromosomes

Individual[a]	Mutation	Allele Size[b] (bp)				Intragenic[c] (3′→ 5′)	Allele Size[b] (bp)		
		S3405	*S3404*	*S3370*	*S2617*		*S3373*	*S3275*	*S3376*
144-01A (Dr)	E148Q	165	114	149	110	TAGGTTAGACCGCCAT	185	190	221
S.T. (AJ)	E148Q	185	122	141	110	TAGACTAGAC(C/T)GCCAT	185	192	215
S.T. (AJ)	**E148Q-P369S**	**193**	**98**	**141**	**113**	**TAGACTAGAT(C/T)GCCAT**	**187**	**190**	**209**
43-02B (AJ)	E148Q	165	118	149	110	TAGGTCGAGCTGCCAT	185/187	194	205
146-07A (Arab)	E148Q	165	110	141	101	TAGGCCGAGCTGCCAT	187	194	223
Sicilian	E148Q	165	118	141	101	TAGGCCGAGCTGCCAT	187	194	217/219
43-02A (AJ)	V726A-E148Q	165	110	149	107	CAGGTCGAGCTGCCAT	185/187	186	215
76-06 (Dr)	V726A-E148Q	165	110	149	107	CAGGTCGAGCTGCCAT	187	186	201
33-02A (IrJ)	V726A	165	110	149	107	CAGGTCGAGCTGCGAT	185	194	209
72-01A (IrJ)	V726A	165	110	149	107	CAGGTCGAGCTGCGAT	185	194	209
146-19A (Arab)	V726A	165	98	149	107	CAGGTCGAGCTGCGAT	185	194	209
32-08A (AJ)	V726A	165	110	149	101	CAGGTCGAGCTGCGAT	185	194/200	215
K.X. (AJ)	V726A/V726A	165/185	110/98	149/141	101/101	CAGGTCGAGCTGCGAT	185/185	194/194	215/215
T.M. (AJ)	V726A/V726A	165/193	110/94	149/153	101/101	CAGGTCGAGCTGCGAT	185/185	194/194	215/213
Sicilian	M694V	165	118	153	104	TGGACTAGACCAAGGC	nd	184	nd
Sicilian	M680I	165	118	149	110	TACGTCGAGCTGAGGC	nd	186	nd

[a] Dr = Druze, AJ = Ashkenazi Jewish, and IrJ = Iraqi Jewish. Pedigree 43 is shown in figure 2; the Sicilian family is shown in figure 3.

[b] The order of the genetic markers in the table is the same as the order on the chromosome, with *D16S3405* the most telomeric and *D16S3376* the most centromeric. Underlined alleles represent a putative haplotype, determined on the basis of allele similarities with other known haplotypes. nd = not done.

[c] The order of intragenic polymorphisms is as follows: tel-3′-nt 2177 (amino acid [aa] 726)-nt 2080 (aa 694)-nt2040 (aa 680)-nt 1764 (x9.1)-nt 1610+95 (i6.1)-nt 1530 (x5.3)-nt 1428 (x5.2)-nt 1422 (x5.1)-nt 1356+43 (i4.1)-nt 1105 (aa 369)-nt 942 (x3.1)-nt 605 (x2.4)-nt 495 (x2.3)-nt 442 (aa 148)-nt 414 (x2.2)-nt 306 (x2.1). Underlined sites are locations of mutations, with mutant alleles in boldface type.

accounting for an additional 20%. It is noteworthy that E148Q was present on only 9 (25%) of 36 carrier chromosomes in Ashkenazi patients with FMF, whereas P369S was present in 3 (8%) of 36. Consistent with the hypothesis that E148Q and P369S are not fully penetrant in the Ashkenazi population, we identified one E148Q homozygote and one P369S homozygote from the screening panel of 200 asymptomatic individuals. The K695R mutation was also overrepresented in the general Ashkenazi sample, accounting for 12% of the carrier chromosomes, but was only observed on 2 (5%) of 36 Ashkenazi FMF patient chromosomes.

In contrast, the M694V and V726A mutations accounted for higher percentages of FMF patient chromosomes than would be expected from their relative frequencies in the overall Ashkenazi population. We observed M694V in 3 of 36 patient chromosomes but in none of 420 anonymous Ashkenazi chromosomes. Similarly, V726A was present in 17 (47%) of 36 of the Ashkenazi patients' chromosomes but comprised only 18% of the carrier chromosomes in the unselected Ashkenazi panel.

On the basis of low population-based estimates of the Ashkenazi Jewish carrier frequency, Yuval et al. (1995) suggested that an Ashkenazi family exhibiting four-generation inheritance of FMF might be because of a rare dominant mode of transmission in this family. The higher Ashkenazi carrier frequencies measured in the present study increase the possibility that asymptomatic carriers married into this family; therefore, DNA specimens were obtained from the family to test this hypothesis. The results are shown in figure 2. In both marriages in which it was at issue, there was evidence that asymptomatic carriers married into the family. Thus, the mother of 43-01 and 43-02 can be inferred to be a carrier for E148Q, and 43-04 is a carrier for V726A.

Haplotype analysis, presented in figure 2 and table 3, indicates that Ashkenazi Jewish FMF carrier chromosomes share common ancestors with carriers from other ethnic groups. For example, in figure 2, the E148Q-V726A complex allele is found on a microsatellite haplotype designated as the "C" (International FMF Consortium 1997) or "Arm3" (French FMF Consortium 1997) haplotype, which has been observed in several ethnic groups. As noted previously, Ashkenazi E148Q-bearing chromosomes share a common ancestral SNP haplotype with other chromosomes bearing this mutation. Table 3 also illustrates common SNP and microsatellite genotypes in V726A-bearing chromosomes of Ashkenazi Jewish, Iraqi Jewish, and Arab descent.

Table 4

***MEFV* Mutation Frequencies in the U.S. Ashkenazi Jewish Population**

Mutation	No. of Chromosomes Positive	No. of Chromosomes Scored	Gene Frequency	Carrier Frequency[a]
E148Q	23	420	.055	.104
E167D	0	172	.000	.000
P369S	10	426	.023	.045
M680I[b]/M680I[c]	0	225	.000	.000
M694V	0	420	.000	.000
M694I	0	122	.000	.000
K695R	6	402	.015	.030
V726A	9	400	.022	.043
A744S	1	146	.007	.014
R761H	0	105	.000	.000
Total	49	~400	.122	.214

[a] Calculated as twice the gene frequency times (1 minus the gene frequency). The total carrier frequency was calculated from applying this formula to the sum of the observed gene frequencies.
[b] 2040G→C.
[c] 2040G→A.

FMF in Patients of Italian Ancestry

Table 2 presents mutation data on 14 unrelated FMF patients of Italian ancestry. The E148Q mutation was observed without P369S or V726A in seven carrier chromosomes and as the E148Q-V726A complex allele once. Haplotype analysis in a Sicilian family segregating the E148Q mutation is shown in table 3 and figure 3. With the exception of a probable "slippage" mutation at D16S3404, the microsatellite and SNP haplotype associated with E148Q in this family was identical to that found in an Israeli Arab family (146), from *D16S3275* to at least *D16S3405*. In another patient of Italian ancestry, we observed the M694I mutation, which also occurs primarily in Arabs (table 2; French FMF Consortium, 1997).

The M694V mutation was seen in a total of six unrelated Italian carrier chromosomes, including one in the aforementioned Sicilian family. Haplotype analysis in this family (fig. 3) places this mutation on the A "(Med)" haplotype, the major founder chromosome associated with M694V in several Mediterranean populations. The third mutation segregating in this family, M680I, is also seen in patients of Armenian and Turkish ancestry. The M680I Sicilian haplotype appears to be the same as one previously found in the Armenian population, designated either as the "K" haplotype (International FMF Consortium 1997) or the "Arm2" haplotype (French FMF Consortium 1997). The area of identity extends at least from *D16S3405* (fig. 3) through the intragenic SNPs (data not shown), with the exception of a probable "slippage" mutation at *D16S2617*.

Discussion

The present report adds substantially to an emerging body of data indicating a much more complex mutational picture in FMF than had been appreciated with the initial cloning of *MEFV*. Whereas four mutations in a single exon accounted for a large percentage of the mostly North African Jewish carrier chromosomes in the first reports (French FMF Consortium 1997; International FMF Consortium 1997), our present data from a more diverse sample indicate a larger pool of disease-associated mutations that are not restricted to a single domain of the pyrin/marenostrin protein, with the prospect that there are yet more mutations to be found. Moreover, in performing the first reported population-based *MEFV* mutational screen, we have obtained evidence for relatively common mutations with reduced penetrance among Ashkenazi Jews, thus blurring the distinction between *MEFV* mutations and polymorphisms. Finally, the SNP and microsatellite haplotype data presented here suggest intriguing ancestral relationships among FMF carrier chromosomes.

Table 5 summarizes the 16 *MEFV* mutations identified at the time of this writing, two of which are described for the first time in this report. For each mutation, we have listed the combined experience from our own laboratories and the French FMF Consortium, with regard to gene frequencies in panels of DNA samples representing the general North American/northern European population and among chromosomes identified by segregation analysis as being noncarriers for FMF. For 13 of the 16 mutations, no positives were found in an aggregate experience of as many as several hundred control chromosomes. It is interesting to note that the three mutations that were detected at low frequency among the control panels (E148Q, P369S, and K695R) were relatively common in our Ashkenazi Jewish sample. The positives in the general population therefore may be carriers with partial ancestry from one of the high-risk eth-

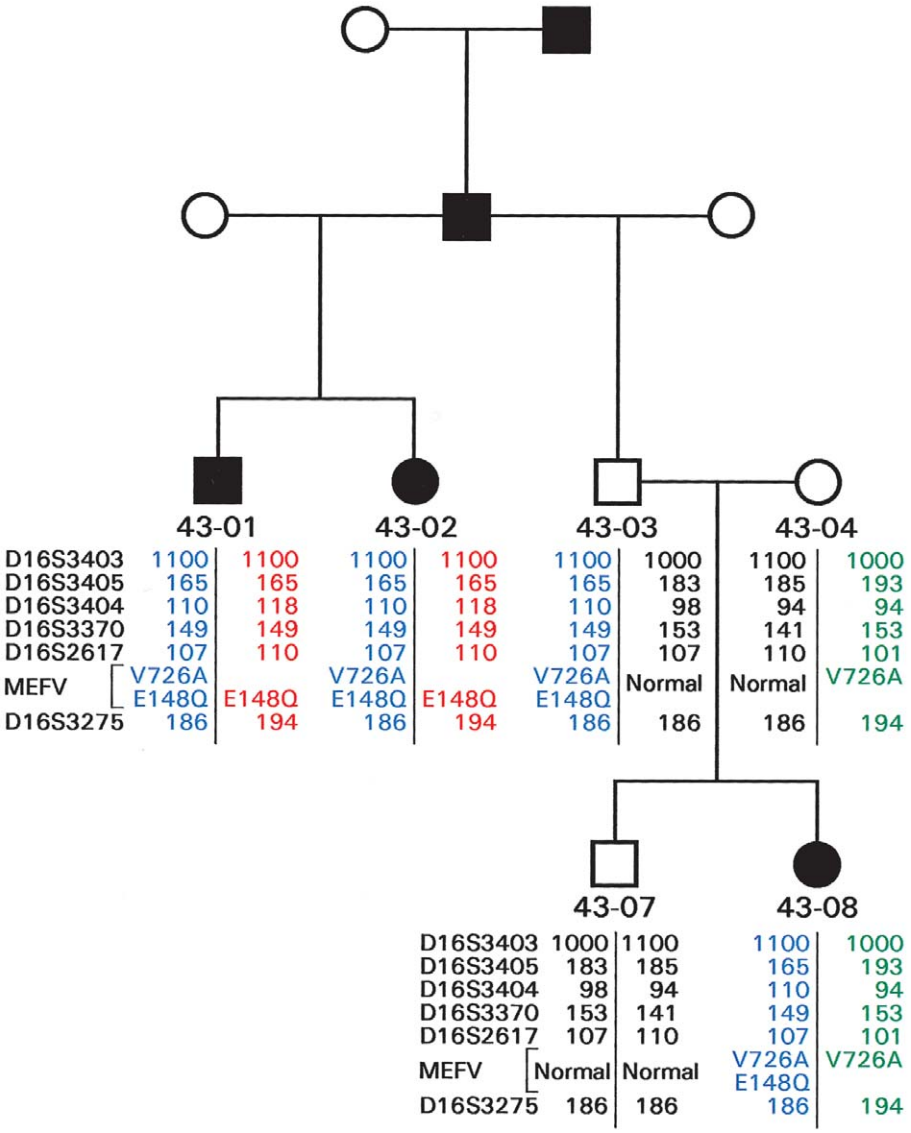

Figure 2 Pedigree of an Ashkenazi Jewish family previously thought to exhibit dominant inheritance of FMF. The diagram is adapted from the report by Yuval et al. (1995). Microsatellite typings are listed from telomere (*top*) to centromere (*bottom*), with *MEFV* residing between *D16S2617* and *D16S3275*. Affected individuals in the first two generations were not available for genotyping. In the report by Yuval et al. (1995), individual 43-03 was explained as a nonpenetrant carrier of a dominant mutation.

nic groups. Table 1 and figure 1 also present rapid-screening assays for the two novel mutations described here, as well as for five other recently published mutations.

Eleven of the 16 mutations listed in table 5 are located in exon 10 and would be predicted to affect the B30.2 ret finger protein C-terminal domain of pyrin. E148Q and E167D are not part of any recognizable motif or domain in the protein (International FMF Consortium 1997), but T267I falls within the bZIP basic domain, P369S is 6 amino acids N-terminal to the B-box zinc finger domain, and F479L resides within the α-helical (coiled-coil) domain. As yet, no *MEFV* mutation has been identified in FMF that would be predicted to truncate the pyrin protein; thus, the data continue to be consistent with the hypothesis that more profound loss-of-function mutations may lead to a different phenotype (International FMF Consortium 1997).

Our ability to account for only 79% of the carrier chromosomes of the patients presented in table 2 sets an approximate upper limit to the sensitivity of current site-specific testing for the identification of *MEFV* mutations. Because we sequenced exon 10 for all these patients, our survey would only miss T267I and F479L,

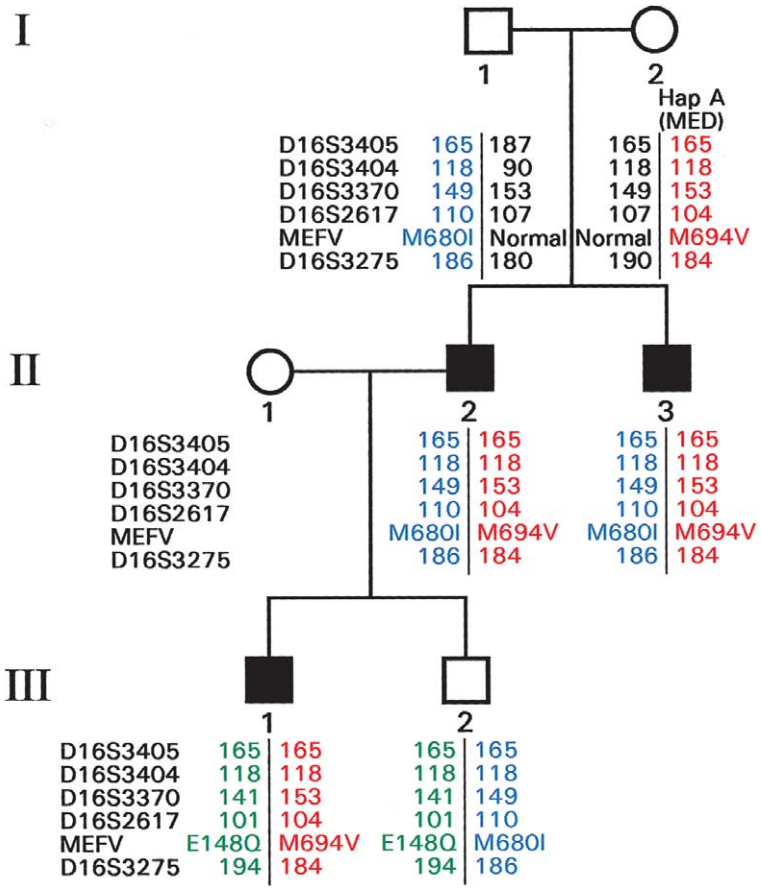

Figure 3 Haplotype analysis of a Sicilian family with FMF. Microsatellite marker order is the same as in figure 2. Three different *MEFV* mutations, with associated haplotypes, segregated in this family. The haplotype with the M694V mutation has been observed in several Mediterranean populations; the haplotypes associated with E148Q and M680I have been observed in Arab and Armenian families, respectively. Individual III-2 is age 13 years and has experienced no symptoms of FMF to date.

which are reportedly infrequent mutations (Bernot et al. 1998). Conversely, the sensitivity could be somewhat lower if there are patients with FMF for whom we have not yet detected mutations on either chromosome. Our clinical analysis of 100 American periodic fever referrals indicates that such patients probably exist (Samuels et al. 1998), although it is difficult to estimate their number in the present series, since complete clinical data were not available for each of the samples received. Assuming that the current screening assays identify only ~75% of FMF carrier chromosomes, then 6% (0.25 × 0.25) of patients with FMF would be predicted to be completely negative for these assays. It is possible that these patients may have mutations in regions of *MEFV* that were not screened or that their periodic illness is caused by another locus (Akarsu et al. 1997).

Perhaps the most interesting and provocative finding of this report is the demonstration, by direct mutational screening in a panel of unselected DNA samples, of an FMF carrier frequency of ~1/5 in the Ashkenazi Jewish population. Previous estimates of the FMF gene and carrier frequency in this ethnic group have been lower and were inferred from enumerations of symptomatic individuals either in the Israeli population (Yuval et al. 1995) or in Ashkenazi families with FMF (Daniels et al. 1995). There is no evidence to suggest that the Ashkenazi Jewish population of the United States differs substantially from that of Israel, and the allele frequencies for several genetic disorders determined from the New York University Ashkenazi population are consistent with those of other studies of Ashkenazi Jews (Kronn et al. 1998; H. Ostrer, unpublished data). None of these individuals gave any history of symptoms compatible with FMF. Against the possibility that there was a significant degree of unrecognized Sephardi Jewish ancestry among our panel, the M694V *MEFV* mutation was not detected.

Another way of reconciling our Ashkenazi frequency data with previous estimates would be to propose that at least some of the putative mutations tested here are actually population-specific polymorphisms in linkage

Table 5

Summary of *MEFV* Mutations

Nucleotide	Amino Acid	Exon	Control Chromosomes: Present Study, North American	Control Chromosomes: Present Study, NC[a]	Control Chromosomes: French FMF Consortium, CEPH	Control Chromosomes: French FMF Consortium, NC[a]	Reference
442G→C	E148Q	2	1/206	0/40	1/148	2/131	Present study; Bernot et al. (1998)
501G→C	E167D	2	0/94	0/26	0/148	0/57	Present study; Bernot et al. (1998)
800C→A	T267I	2	…	…	0/148	0/57	Bernot et al. (1998)
1105C→T	P369S	3	1/222	…	…	…	Present study
1437C→G	F479L	5	…	…	0/120	0/51	Bernot et al. (1998)
2040G→C	M680I	10	0/222	0/105	0/146	0/>250	International FMF Consortium (1997); French FMF Consortium (1997)
2040G→A	M680I	10	0/222	0/105	…	…	Present study
2042C→T	T681I	10	0/190	0/105	…	…	Booth et al. (1998)
2076-2078del	I692del	10	0/190	0/105	0/146	0/52	Bernot et al.(1998)
2078-2080del	M694del	10	0/190	0/105	…	…	Booth et al. (1998)
2080A→G	M694V	10	0/190	0/105	0/146	0/>250	International FMF Consortium (1997); French FMF Consortium (1997)
2082G→A	M694I	10	0/190	0/105	0/146	0/>250	French FMF Consortium (1997)
2084A→G	K695R	10	0/190	0/105	0/146	1/52	Present study; Bernot et al. (1998)
2177T→C	V726A	10	0/178	0/105	0/146	0/>250	International FMF Consortium (1997); French FMF Consortium (1997)
2230G→T	A744S	10	0/140	…	0/146	0/52	Present study; Bernot et al. (1998)
2282G→A	R761H	10	0/137	…	0/146	0/52	Present study; Bernot et al. (1998)

[a] Noncarrier chromosomes (NC), as determined by segregation analysis in FMF families.

disequilibrium with "real" mutations elsewhere in *MEFV*. By this hypothesis, perhaps only a small fraction of the chromosomes bearing E148Q, for instance, might have the associated bona fide mutation. If, in table 4, we were to consider only the observed frequency of V726A to estimate the FMF carrier frequency in the Ashkenazi population, the resulting figure of 1/23 would fall within the range of previous estimates (1/135–1/11), although definitely on the high end of the range. Moreover, it could be argued that there are already a substantial number of other known sequence variants within the coding region of *MEFV* that definitely are polymorphisms (determined on the basis of the frequent observation of both variants in haplotype-proven noncarrier chromosomes). In our original positional cloning paper, we identified nine of these polymorphic sequence variants, all but one being third base, synonymous substitutions: D102 (x2.1), G138 (x2.2), A165 (x2.3), R202Q (x2.4), R314 (x3.1), E474 (x5.1), Q476 (x5.2), D510 (x5.3), and P588 (x9.1). Bernot et al. (1998) have subsequently identified four additional silent substitutions: Y65, P393, S683, and P706.

It is, however, important to point out the differences between the putative mutations found at high frequency in the Ashkenazi population and the aforementioned polymorphisms. The two most frequent substitutions, glutamine for glutamic acid (E148Q) and serine for proline (P369S), could readily lead to conformational changes in the pyrin protein; K695R and A744S would be predicted to cause more subtle changes but reside within a domain already known to be sensitive to relatively conservative amino acid substitutions. Moreover, for each of these putative mutations, we have sequenced the entire coding region and splice junctions of *MEFV* without finding other substitutions (except in the case of the complex alleles described). Bernot et al. (1998) performed similar analyses of E148Q, E167D, T267I, and F479L. It is unlikely, on the basis of these arguments, that the variants screened in the Ashkenazi population are polymorphisms in linkage disequilibrium with as yet unknown true mutations.

Instead, the variants presented in table 4 are likely to be mutations, with several exhibiting relatively low penetrance. Reduced penetrance in women has long been invoked to explain the 1.5–2:1 male-to-female ratio in FMF, but the discordance between gene frequencies inferred from symptomatic cases and those determined by molecular analysis suggest a quantitatively more substantial effect among the Ashkenazim. On the basis of the disproportion among the frequencies of E148Q, P369S, and K695R in the general Ashkenazi population and among Ashkenazi FMF carrier chromosomes, it is likely that these three mutations are not fully penetrant in this population. Moreover, we directly observed E148Q and P369S homozygotes among the asymptomatic Ashkenazi screening panel. This latter observation emphasizes the need for caution in making the diagnosis of FMF on the basis of DNA testing alone and indicates

the importance of similar mutational screens in other Mediterranean populations. Available data suggest that M694V is highly penetrant in the North African Jewish population (French FMF Consortium 1997; International FMF Consortium 1997).

The concept of reduced penetrance among Ashkenazi Jews is consistent with clinical studies suggesting that this population may have somewhat milder disease than Sephardi Jews, with a much lower incidence of amyloidosis (Pras et al. 1982). Several Ashkenazi patients in our mutation panel have relatively mild disease: one K695R/(E148Q-V726A) heterozygote experienced a 30-year spontaneous remission when not taking colchicine; a E148Q/(E148Q-P369S) heterozygote experienced typical attacks as a child but has been attack-free without colchicine for 20 years, as an adult; and another V726A/(E148Q-V726A) compound heterozygote has been attack-free for 20 years while taking colchicine. The disorder in the first two patients could have easily escaped diagnosis. The present data do not exclude the possibility that part of the reduction in penetrance and severity of disease could be because of non-*MEFV* modifier genes or environmental factors in the Ashkenazi population. Alternatively, reduced penetrance may be a property of certain of the more frequent mutations in the Ashkenazim, such as E148Q.

SNP haplotype data presented in this report suggest that chromosomes bearing E148Q from several ethnic groups probably share a common progenitor, thus implicating a founder effect. Bernot et al. (1998) defined several microsatellite haplotypes associated with E148Q in their study population. A novel founder haplotype, designated "S2," was observed only among North African Jewish carriers of the E148Q mutation. The authors concluded that E148Q is a recurrent mutation, on the basis of the observation of other distinct microsatellite haplotypes, although six of the seven independent E148Q FMF chromosomes reported in their article had the same SNP motif presented here. The fact that these haplotypes only converge at the level of intragenic SNPs suggests a relatively ancient founder. Together with the previous intragenic SNP haplotype analysis of the M694V and M680I mutations (International FMF Consortium 1997), our E148Q data underscore the utility of these relatively stable polymorphisms in studying very old mutations. A hypothetical E148Q progenitor common to all the populations harboring this mutation would most likely have lived $\geq$1,500–2,000 years ago (Goodman 1979).

Other haplotype data from Ashkenazi and Italian families in the present report also support the view that many FMF carrier chromosomes from disparate ethnic groups are descended from a limited number of ancestral chromosomes. Apparent ancestral ties between Sicilian and Arab, Jewish, and Armenian FMF carriers are especially noteworthy and probably reflect Sicily's historic status as a crossroads of Mediterranean commerce.

Nevertheless, the high frequency of several mutations in different populations of Mediterranean descent suggests a role for evolutionary selection. Demonstrating the precise nature of an FMF selective advantage may not be easy, given that antibiotic therapy and modern public health measures are likely to obscure the effects of infectious diseases and that even a small advantage compounded over many generations may give rise to a high carrier frequency. One recent study (Brenner-Ullman et al. 1994) proposed a reduced rate of asthma in obligate FMF heterozygotes, but the differences between groups was of borderline statistical significance. Animal models currently under development may contribute substantially to the resolution of these issues.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, http://www.gdb.org/ (for oligonucleotide primers and PCR conditions)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FMF [MIM 249100], hyperimmunoglobulinemia D with periodic fever syndrome [MIM 260920], and familial Hibernian fever [MIM 142680])

References

Akarsu AN, Saatci U, Ozen S, Bakkaloglu A, Besbas N, Sarfarazi M (1997) Genetic linkage study of familial Mediterranean fever (FMF) to 16p13.3 and evidence for genetic heterogeneity in the Turkish population. J Med Genet 34: 573–578

Balow JE Jr, Shelton DA, Orsborn A, Mangelsdorf M, Aksentijevich I, Blake T, Sood R, et al (1997) A high-resolution genetic map of the familial Mediterranean fever candidate region allows identification of haplotype-sharing among ethnic groups. Genomics 44:280–291

Bernot A, da Silva C, Petit J-L, Cruaud C, Caloustian C, Castet V, Ahmed-Arab M, et al (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

Booth DR, Gillmore JD, Booth SE, Pepys MB, Hawkins PN (1998) Pyrin/marenostrin mutations in familial Mediterranean fever. QJM 91:603–606

Breda L, Magri M, Morgese G, Chiarelli F (1998) Familial Mediterranean fever in two Italian brothers. Panminerva Med 40:157–160

Brenner-Ullman A, Melzer-Ofir H, Daniels M, Shohat M (1994) Possible protection against asthma in heterozygotes for familial Mediterranean fever. Am J Med Genet 53: 172–175

Centola M, Aksentijevich I, Kastner DL (1998) The hereditary periodic fever syndromes: molecular analysis of a new family of inflammatory diseases. Hum Mol Genet 7:1581–1588

Daniels M, Shohat T, Brenner-Ullman A, Shohat M (1995) Familial Mediterranean fever: high gene frequency among the non-Ashkenazic and Ashkenazic Jewish populations in Israel. Am J Med Genet 55:311–314

French FMF Consortium (1997) A candidate gene for familial Mediterranean fever. Nat Genet 17:25–31

Gentiloni N, Gabbrielli N, Caradonna P, Schiavino D, Anti M, David M, Segni G, et al (1992) Familial Mediterranean fever and electroencephalographic changes: a clinical case. Minerva Med 83:307–310

Goodman RM (1979) Genetic disorders among the Jewish people. Johns Hopkins University Press, Baltimore, pp 1–26

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

Janeway TC, Mosenthal HO (1908) An unusual paroxysmal syndrome, probably allied to recurrent vomiting, with a study of the nitrogen metabolism. Trans Assoc Am Phys 23: 504–518

Kastner DL (1998) Familial Mediterranean fever: the genetics of inflammation. Hosp Pract 33:131–158

Kronn D, Jansen V, Ostrer H (1998) Carrier screening for cystic fibrosis, Gaucher disease, and Tay-Sachs disease in the Ashkenazi Jewish population. Arch Intern Med 158: 777–781

Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T, Migdal A, et al (1997) Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 40:1879–1885

Marinone C, Bisbocci D, Pellacani A, Chiandussi L (1992) Familial Mediterranean fever: a report of 2 cases of Italian origin. Ann Ital Med Int 7:114–117

McDermott MF, Ogunkolade BW, McDermott EM, Jones LC, Wan Y, Quane KA, McCarthy J, et al (1998) Linkage of familial Hibernian fever to chromosome 12p13. Am J Hum Genet 62:1446–1451

Mulley J, Saar K, Hewitt G, Rüschendorf F, Phillips H, Colley A, Sillence D, et al (1998) Gene localization for an autosomal dominant familial periodic fever to 12p13. Am J Hum Genet 62:884–889

Passiu G, Perpignano G, La Nasa G, Carcassi U (1984) Familial Mediterranean fever: description of a case observed by us. Minerva Medica 75:1147–1152

Pras M, Bronshpigel N, Zemer D, Gafni J (1982) Variable incidence of amyloidosis in familial Mediterranean fever among different ethnic groups. Johns Hopkins Med J 150: 22–26

Reich CB, Franklin EC (1970) Familial Mediterranean fever in an Italian family. Arch Intern Med 125:337–340

Rogers DB, Shohat M, Petersen GM, Bickal J, Congleton J, Schwabe AD, Rotter JI (1989) Familial Mediterranean fever in Armenians: autosomal recessive inheritance with high gene frequency. Am J Med Genet 34:168–172

Rosenstein ED, Kramer N (1994) Familial Mediterranean fever in an individual of Maltese extraction: history is destiny. Clin Exp Rheumatol 12:96

Samuels J, Aksentijevich I, Torosyan Y, Centola M, Deng Z, Sood R, Kastner DL (1998) Familial Mediterranean fever at the millennium: clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. Medicine 77:268–297

Secchi GC, Negri E, Monti MA (1981) Periodic disease of twenty-five years' duration in an Italian subject: efficacy of long-term colchicine prophylaxis. Panminerva Med 23: 249–252

Siegal S (1964) Familial paroxysmal polyserositis: analysis of fifty cases. Am J Med 36:893–918

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

Sohar E, Pras M, Heller J, Heller H (1961) Genetics of familial Mediterranean fever (FMF). Arch Intern Med 107:529–538

Yuval Y, Hemo-Zisser M, Zemer D, Sohar E, Pras M (1995) Dominant inheritance in two families with familial Mediterranean fever (FMF). Am J Med Genet 57:455–457