MUTATION IN BRIEF

The West Side Story: *MEFV* Haplotype in Spanish FMF Patients and Controls, and Evidence of High LD and a Recombination "Hot-Spot" at the *MEFV* Locus

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Mutations at the *MEFV* gene cause, with various degrees of penetrance, familial Mediterranean fever (FMF). This disease is more prevalent in the Middle East than elsewhere, and most studies have focused on those populations. However, FMF occurs also in the Western Mediterranean and these populations should be taken into account for a complete view of FMF. We have analyzed intragenic *MEFV* SNPs in Spanish and Chueta (descendants of converted Jews) FMF patients and controls, and this constitutes the first systematic survey of normal *MEFV* SNP haplotype structure and variability. Our findings have allowed us to systematize the nomenclature of *MEFV* haplotypes and show that there is strong linkage disequilibrium (LD) at the *MEFV* locus and an intragenic recombination hot spot. The high local LD, regardless the recombination hot spot, is responsible for the limited diversity of the *MEFV* control haplotypes found in the Spanish population and it suggests that it may be a common feature to all Mediterranean populations. The *MEFV* mutation spectrum in Spain is quite diverse, and similar to those of France and Italy. On the contrary, the Chueta spectrum was poorer and closer to that of North African Jews, suggesting a direct connection with the Jewish diaspora. © 2004 Wiley-Liss, Inc.

KEY WORDS: MEFV; FMF; SNP; haplotype; linkage disequilibrium; recombination hotspot

INTRODUCTION

Familial Mediterranean Fever (FMF, MIM# 249100) is an autosomal recessive disease characterized by recurrent short episodes of fever and serositis which may lead to secondary Amyloid A (AA) amyloidosis and renal failure in some patients [Sohar et al., 1967]. FMF affects predominantly people from the Middle East: Non-Ashkenazi Jews (NAJ), Arabs, Armenians and Turks, which are often called "the ancestral populations". It is less frequent in other Mediterranean populations, and sporadical elsewhere [Touitou, 2001]. It has been shown that FMF is caused by mutations at the *MEFV* gene (*ME*diterranean *F*e*V*er), located in chromosome 16p13.3 [The French FMF Consortium, 1997; The International FMF Consortium, 1997].

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Four missense mutations — c.2040G>C (M680I), c.2080A>G (M694V), c.2082G>A (M694I) and c.2177T>C (V726A) — were found to be associated with FMF in Jewish, Arab, Armenian and Turkish FMF families, accounting for 85% of FMF chromosomes. Additionally, the M694V mutation was associated with the Med (A) haplotype and with the B haplotype. The finding that these two haplotypes converged at intragenic SNPs and the fact that were found in populations relatively isolated for centuries suggested that the M694V variant may be an ancient mutation, originating 2,500 years ago. To date, up to 40 *MEFV* mutations have been reported in the INFEVERS database (http://fmf.igh.cnrs.fr/infevers), being distributed in a wide range of non-ancestral populations [Sarrauste de Menthiere et al., 2003].

In the present study we have characterized the *MEFV* SNP haplotypes in Spanish and Chueta (descendants of Majorcan Jews) FMF patients and in Spanish controls in order to address a number of issues. Patterns of linkage disequilibrium (LD) in control haplotypes can be used to infer the genomic dynamics of the *MEFV* region, including the detection of a recombination hotspot in intron 2. The knowledge of SNP haplotype backgrounds in controls allows also to systematize its nomenclature in mutated chromosomes. This information can be then applied to the reconstruction of mutation history and, in particular, to the dispersion of FMF in the Mediterranean basin.

MATERIALS AND METHODS

Patients and control subjects

Both approval from the Institutional Review Board of the Hospital Clínic, Barcelona, and written-informed consent from controls and patients were obtained for the present study.

The control group consisted of 100 anonymous autochthonous mainland Spanish healthy donors. This sample is intended as a control for the general Spanish FMF patients rather than for our Chueta patients.

A panel of 50 unrelated Spanish FMF patients was studied. No known consanguinity and no Jewish ancestry, or other ancestry in populations of high FMF incidence, was known for those patients. A group of 14 Chueta unrelated FMF patients from Mallorca, the largest of the Balearic Islands, was also studied. Chuetas are the Mallorcan individuals who carry one of the 15 surnames belonging to crypto-Jews who were persecuted and stigmatized by the Spanish Inquisition in the 17th century and whose descendants were marginalized from mainstream Mallorcan society ever since. Their genetic Sephardic Jewish origin has been shown by the affinity of their HLA haplotypes to those of other Jewish communities [Crespi et al., 2002]. All studied patients were diagnosed with FMF according to the clinical Tel-Hashomer diagnosis criteria.

MEFV haplotype determination

Determination of the *MEFV* intragenic SNP haplotypes was performed by DNA sequencing: all 10 *MEFV* exons as well as the immediately adjacent intronic sequences were analyzed as previously described [The International FMF Consortium, 1997]. Phase resolution of FMF patients was achieved by genotyping asymptomatic parents or siblings or, when not available, by cloning *MEFV* cDNA of each patient [Papin et al., 2000]. *MEFV* control haplotype frequencies were estimated on the basis of unphased genotype data, with the EM algorithm as implemented in the Arlequin software [Schneider et al., 2000].

The reference sequence and version number of MEFV cDNA used is NM_000243.1 (GenBank). The SNPs and mutations studied, in order from centromere to telomere, were: c.181C>T (x1/Y65Y), c.306C>T (x2.1/D102D), c.414G>A (x2.2/G138G), c.442G>C (*E148Q*), c.488A>C (*E163A*), c.495A>C (x2.3/A165A), c.501G>C (E167D), c.605G>A (x2.4/*R202Q*), c.800C>T (*T267I*), c.942C>T (x3.1/R314R), c.955A>G (*E319K*), c.1179C>T (x3.2 P393P), c.1356+43A>G (i4.1), c.1422A>G (x5.1 E474E), c.1428A>G (x5.2/Q476Q), c.1432C/T (*H478Y*), c.1437C>G (*F479L*), c.1530C>T (x5.3/D510D), c.1610+95C>T (i6.1), c.1759+325A>G (i8.1), c.1759+333T>A (i8.2), c.1764A>G (x9.1/P588P), c.1772T>C (*I591T*), c.2040G>C (M680I), c.2040G>A (M680I), c. 2040G>C>A (*M680I*), c.2080A>G (*M694V*), c.2082A>G (*M694I*), c.2084A>G (*K695R*), c.2177T>C (*V726A*), c.2230G>T (*A744S*), according to previous nomenclature [underlined; see The International FMF Consortium, 1997; Bernot et al., 1998]. Haplotypes were constructed with the nine informative SNPs underlined above.

Numerical analyses

LD among pairs of SNPs was measured with r^2 and tested for significance with Fisher's exact test, by using the Arlequin 2.000 software [Schneider et al., 2000]. An overall measure of LD at *MEFV* was obtained by computing the FNF ("Fraction Not Found") statistic [Mateu et al., 2001]. FNF at *MEFV* was compared to that in other genes by extracting data on haplotype frequencies deposited at ALFRED (http://alfred.med.yale.edu) [Rajeevan et al., 2003] on those genes for which five or more SNPs had been typed.

Reynolds' genetic distances [Reynolds et al., 1983] between populations were calculated using their relative *MEFV* mutation frequencies regardless of haplotype background (since this information was not available for many of those) using the Arlequin 2.000 software [Schneider et al., 2000]. The Spanish and Chueta were compared to French, Italians, Greeks, North African Arabs, Palestinians, Turks, Armenians, Ashkenazi Jews, and North African Jews [Dode et al., 2000; Shinawi et al., 2000; Touitou, 2001; La_Regina et al., 2003].

RESULTS

Control haplotypes

Analysis of *MEFV* control haplotype frequencies revealed that 92% of chromosomes were accounted for by six major haplotypes (M1-M6). The remaining 8% of the chromosomes carried 12 minor haplotypes (m1-m12), in frequencies ranging from 0.5% to 1.5% (Fig.1). Besides their high frequency, major haplotypes show a peculiar structure: they consist of two blocks, separated by intron 2, and the six haplotypes are the combinations resulting from taking subhaplotypes from each block. That is, the exon 2 SNPs show three subhaplotypes: TACG, CGAA, and CGAG (the latter may have arisen by a rare within-block recombination or by back-mutation at position R202Q; Fig.1), and SNPs in exons from 3 to 9 form subhaplotypes TAGCG and CGATA. The haplotype frequencies are at linkage equilibrium with respect to the subhaplotype frequencies in each block (χ^2=4.637, 2 d.f., p=0.0984), suggesting that either an ancient recombination event or a high recombination rate at intron 2 maintains linkage equilibrium between the blocks. Most minor haplotypes can be explained as intrablock recombinants.

	Intragenic SNPs									
	E2				E3	E5			E9	
Haplotypes	D102D	G138G	A165A	R202Q	R314R	E474E	Q476Q	D510D	P588P	Frequency %
M1	T	A	C	G	T	A	G	C	G	27.5
M2	T	A	C	G	C	G	A	T	A	27.5
M3	C	G	A	A	T	A	G	C	G	14
M4	C	G	A	A	C	G	A	T	A	7
M5	C	G	A	G	T	A	G	C	G	7
M6	C	G	A	G	C	G	A	T	A	9
										92 total M
m1	T	A	C	G	T	A	G	C	A	1.5
m2	T	A	C	G	T	G	A	T	A	0.5
m3	T	A	C	G	T	n	n	n	A	0.5
m4	T	A	C	G	C	n	n	n	A	0.5
m5	C	G	A	A	C	G	A	T	G	1
m6	C	G	A	A	T	G	A	T	A	1
m7	C	G	A	A	C	G	A	C	A	0.5
m8	C	G	A	A	n	n	n	n	n	0.5
m9	C	G	A	G	T	A	G	C	A	0.5
m10	T	A	A	n	C	G	A	T	A	0.5
m11	T	G	C	A	T	A	G	C	G	0.5
m12	C	G	C	n	C	G	A	T	A	0.5
										8 total m

Figure 1. MEFV SNP haplotypes in the control Spanish population and their frequencies (200 chromosomes). M, major haplotypes. m, minor haplotypes. E, exon. n, undetermined.

LD was considered at two levels: as pairwise tests and jointly for the whole gene. Measures and tests of LD showed extremely high within-block LD, with r^2 = 0.81-1 (median, 0.94) in pairs not including c.605G>A (R202Q), and r^2 = 0.44-0.48 in pairs including R202Q. In both groups of cases, all Fisher's exact tests yielded

significances ~0. In contrast, between-block LD was nonexistent, with r^2 = 0.0008 - 0.054 (median, 0.002) and Fisher's exact tests were not significant (except for R202- P588, p=0.027).

The overall extent of LD at *MEFV* was assessed through FNF, that is, the fraction of the number of different haplotypes expected under linkage equilibrium that was not found. In a sample of 200 chromosomes, we found 18 different haplotypes at *MEFV*, while the expectation under linkage equilibrium was 155.98, and FNF=0.896. Then, even if the two blocks within *MEFV* are essentially in linkage equilibrium from each other, LD within the blocks is sufficient to deplete the overall haplotype diversity. In order to provide some context for this result, we selected all genes in the ALFRED database for which ≥ 5 SNPs had been typed and haplotypes inferred. The results showed that among *ADH*, *ATM*, *D4S10*, *HOXB6*, *PAH* and *VW* genes, only *ATM* presents a higher FNF than *MEFV*.

Non-synonymous SNPs in the control population. Carrier frequency

Three non-synonymous changes were detected in the control sample: c.442G>C (E148Q), R202Q and c.2230G>T (A744S). Since their relation to FMF is controversial for some of them, we compared their frequencies in cases and controls to detect any possible association.

At codon 148, allele frequencies were 98.5% E and 1.5% Q in the control population, whereas in the 142 chromosomes of the Spanish FMF group they were 85% E and 15% Q. The frequency of E148Q is significantly higher in the FMF cases (χ^2= 32.23, p= 1.4×10^{-8}), which can be interpreted as E148Q carrying a relative risk at FMF of 16.4 times to carriers (95% CI: 4.7 to 56.8). These data replicate a previous report which described E148Q as a mutation with low penetrance since it was detected at frequencies of about 2% in CEPH controls and 24% in European and Middle Eastern FMF cases [Bernot et al., 1998; Aksentijevich et al., 1999].

At codon 202, allele frequencies in the control and FMF population were exactly the same: 75% R and 25% Q. Since these frequencies were obviously not significantly different from each other, R202Q is not associated with FMF in the Spanish population and does not confer a significant risk at suffering the disease, as previously reported [Bernot et al., 1998].

The A744S substitution has sporadically been found both in a few FMF patient and in controls, and its status in relation to the disease has not been firmly established [Bernot et al., 1998; Aksentijevich et al., 1999]. We have found it in two out of 200 Spanish control chromosomes, in two healthy relatives of FMF patients, but not in patients themselves. Thus, the role of A744S remains unclear, and larger numbers of patients and controls may be needed to determine whether it can be understood as a polymorphism, a risk factor, or a true mutation.

No other FMF-causing mutations, such as those found in the Spanish patients, were detected in the sample of 200 Spanish control chromosomes. Thus, the carrier rate would be 2.5% if E148Q and A744S are considered, but 0 if only highly-penetrant mutations such as M680I, M694V, M694I, and V726A are taken into account. These figures are much lower than those found in Middle Eastern populations, where carrier rates are 37% in Armenians, over 20% in Turks, Non-Ashkenazi and Ashkeani Jews, and 10-23% in Arabs [Touitou, 2001].

Spanish FMF haplotypes

The exons and flanking intron sequences of *MEFV* were sequenced in 100 chromosomes of 50 Spanish FMF patients. In 32 of them, nine different mutations were found; these are given in figure 2, along with their associated haplotypes. M694V is the most frequent variant in the Spanish mutated chromosomes (12/32, 37%). It was linked to the background haplotypes M6 (eight chromosomes) and M4 (four chromosomes). The M694V-M4 haplotypes showed an identical SNP haplotype to the previously reported founder Med(A) haplotype [The French FMF Consortium, 1997; The International FMF Consortium, 1997]. The M694V-M6 haplotypes only differed from the Med(A) haplotype at the R202Q position. Consistent with its high frequency and wide distribution, the M694V-M4 haplotype could be the founder from which the M694V-M6 haplotype had appeared (probably by recombination at the intron 2 hotspot), and genetic drift would explain the inverted founder versus recombinant haplotype proportion of the M694V-bearing chromosomes within the Spanish FMF chromosomes.

E148Q was found in five Spanish FMF chromosomes (5/32, 16%), three with an M1 background and two with M2. This mutation has been frequently reported in complex alleles with several mutations such as V726A [Bernot et al., 1998; Aksentijevich et al., 1999], M694I [Booth et al., 2001] or P369S [Aksentijevich et al., 1999]. However, no complex alleles were found in our Spanish FMF patients; patients carrying E148Q and a different mutation were demonstrated to be compound heterozygotes by family and cloning analysis. The E148Q-M1 intragenic SNP haplotype was previously reported in patients of these Jewish groups and in others of diverse

ethnicities. The two E148Q-bearing haplotypes of our study group converged at exon 2, within the TACG motif, according to previous data [Aksentijevich et al., 1999], supporting the hypothesis of a common founder haplotype.

Spanish and Chueta FMF chromosomes

MEFV Mutation	Background haplotype	Mutant haplotype frequency (c)		Intragenic SNPs									Previous nomenclature
				E2				E3	E5			E9	
		Spanish	Chueta	D102D	G138G	A165A	R202Q	R314R	E474E	Q476Q	D510D	P588P	
M694V	M6	.66 (8)	.1 (1)	C	G	A	G	C	G	A	T	A	-
	M4	.33 (4)	.9 (10)	C	G	A	A	C	G	A	T	A	Med(A)[a]
	M2	0	0	T	A	C	G	C	G	A	T	A	B[b]
E148Q	M1	.60 (3)	1 (1)	T	A	C	G	T	A	G	C	G	-
	M2	.40 (2)	0	T	A	C	G	C	G	A	T	A	-
K695R	M2	.80 (4)	0	T	A	C	G	C	G	A	T	A	-
	M4	.20 (1)	0	C	G	A	A	C	G	A	T	A	-
M694I	M5	.50 (2)	0	C	G	A	G	T	A	G	C	G	[c]
	M1	.25 (1)	0	T	A	C	G	T	A	G	C	G	-
	M2	.25 (1)	0	T	A	C	G	C	G	A	T	A	Ara2[d]
I591T	M3	1 (2)	0	C	G	A	A	T	A	G	C	G	-
V726A	M1	1 (1)	0	T	A	C	G	T	A	G	C	G	Arm3(C)[a]
E319K	M1	1 (1)	0	T	A	C	G	T	A	G	C	G	-
E163A	M6	1 (1)	0	C	G	A	G	C	G	A	T	A	-
H478Y	M5	1 (1)	0	C	G	A	G	T	A	G	C	G	-

Figure 2. Mutation bearing FMF chromosomes, their background haplotypes and frequencies found in the Spanish and Chueta FMF patients. The vertical red bar indicates the genomic position of the mutation relative to the intragenic SNPs. [a]Bernot et al. [b]The International FMF Consortium. [c]Aksentijevich et al. [d]The French FMF Consortium. M, major haplotypes. E, exon. c, chromosomes.

K695R comprises 5/32 (15.6%) of the mutated chromosomes in the Spanish cases. It is associated in our sample with haplotypes M2 (four chromosomes) and M4 (one). These two backgrounds can be explained by a recombination at intron 2, where most recombination within *MEFV* occurs; this makes recombination much likelier than recurrent mutation as the explanation of the presence of K695R in two different haplotype backgrounds. In fact, this is the first description of the haplotypes associated with K695R.

M694I was detected in 4/32 mutated chromosomes (12.5%), associated to three different haplotypes: M5, M1, and M2. The M694I-M2 haplotype corresponds to the Ara2 founder haplotype, previously reported in two Arab and one Jewish patients [The French FMF Consortium, 1997; Bernot et al., 1998]. All three haplotypes can be connected easily by recombination (either at intron 2 or at intron 9) with frequent haplotypes; however, given the small sample size, an ancestral haplotype cannot be inferred.

The V726A mutation was found in a single FMF chromosome harbouring the background haplotype M1, which corresponds to the haplotype named Druze (C) [The French FMF Consortium, 1997; The International FMF Consortium, 1997; Booth et al., 2001] found in several ethnicities.

A group of rare mutations, including three private mutations, is found in 16% of mutated Spanish chromosomes: I591T, in exon 9, was first described in a French patient [Touitou, 2001]. Here, we show that the I591T mutation found in two unrelated Spanish patients [Aldea et al., 2002] is associated with the same background haplotype (M3), which suggests a common founder haplotype. The private mutations E319K, E163A, and H478Y [Aldea et al., 2003] mutations were found associated with the M1, M6 and M5 haplotypes respectively.

Chueta FMF haplotypes

In a sample of 14 unrelated Chueta FMF patients the M694V and E148Q mutations were found. The associated *MEFV* SNP haplotypes are shown in Figure 4.

M694V was associated with the background haplotype M4 (ten chromosomes) previously reported as Med-(A) [Domingo et al., 2000] but also with the M6 haplotype (one chromosome). Both M694V-bearing haplotypes are also found in the group of Spanish FMF chromosomes, but, contrary to the latter population, in Chueta M694V chromosomes the presumably ancestral M4 background is, by far, the most frequent. The E148Q mutation was associated with the M1 haplotype (one chromosome).

DISCUSSION

MEFV genomic dynamics

The general description of the *MEFV* haplotypes for the first time in a control population has allowed to establish a systematic nomenclature for *MEFV* haplotypes (Fig. 2) which can be used as a reference framework for mutation studies. Six major haplotypes were found, with frequencies ranging from 7% to 27.5%; these were estimated with the EM algorithm, which has been shown to be much more precise in estimating the frequencies of common rather than rare haplotypes [Tishkoff et al., 2000]. Thus, we do not expect that the use of the EM algorithm has biased our description of haplotype structure at *MEFV*.

The *MEFV* SNP haplotype study in the Spanish general population clearly showed two regions separated by intron 2 with a limited number of subhaplotypes each. The six most frequent *MEFV* haplotypes in the control population are found at frequencies that would be expected under a random association of subhaplotypes. That is, there is strong LD between exon 2 SNPs and between exon 3 to exon 9 SNPs, but these two blocks are essentially at equilibrium. There are two possible explanations for this situation: i) continuous recombination at intron 2 (which would be a recombination hotspot), or ii) an ancient recombination event, again at intron 2. The first hypothesis would be much more efficient in maintaining haplotype frequencies at equilibrium between the two blocks, and could explain why LD is almost complete between SNPs R314 and P588 (which are ~5.9 Kb apart) but nonexistent between R314 and R202, which are physically closer (~4.5Kb). An ancient recombination event, and low recombination thereafter, would have allowed haplotype frequencies to drift from equilibrium independently in different populations. Then, although a recombination hotspot explains best haplotype frequencies in the Spanish population, determining haplotype frequencies in other populations would help in resolving the question.

The structure of LD in *MEFV* can be used to define a set of three tag SNPs; R202Q, plus one of any other SNP at exon 2 (D102, G138 or A165), plus one of any of the SNPs between exons 3 to 9 (R314, E474, Q579, D510 or P588). This set would capture any of the haplotypes that are found at frequencies over 5% in the control Spanish population and that add up to 92%.

The natural history of *MEFV* mutations

Nine different MEFV mutations were found in 32 Spanish FMF chromosomes; three of those were private. Two features of the Spanish MEFV mutation spectrum seem more prominent: the presence of K695R at relatively high frequencies, and the fact that most of the M694V mutants fall on an M6 SNP background rather than on M4, which is the most frequent elsewhere and presumed to be ancestral; both findings can be easily explained by drift.

We compared the Spanish *MEFV* mutation spectrum with those of 10 other populations and found that the Spanish are closest to the French and Italian *MEFV* spectra (Reynold's genetic distance equals 0.04 in both cases); this genetic distance is half of that to next closest population, the Turks. Fisher's exact tests showed that mutation frequencies are significantly different between Spanish and any other populations at the p=0.01 level, except for the comparisons between Spain and France and Spain and Italy. Then, a Western Mediterranean *MEFV* mutation spectrum can be defined, which is at least quantitatively distinct from that of the so-called ancestral populations. Probably, other mechanisms besides the Jewish diaspora can explain the diffusion of *MEFV* mutations in the Western Mediterranean. In particular, gene flow associated with personal mobility may have spread mutations from the Middle East in the at least 100 generations since the appearance of the most common *MEFV* mutations. Obviously, haplotype backgrounds with a uniform set of markers would help in reconstructing the history of *MEFV* mutations, but this information is not widely available.

The Chueta mutation spectrum is poorer than the Spanish spectrum, and does not contain any private mutation, as expected from a much smaller population. Contrary to the Spanish sample, the Chuetas carry M694V most often on the ancestral M4 background. They are closest to the mutation spectrum of the North African Jews (Reynold's distance ~0), while their genetic distance to the general Spanish population is 0.18. In this case, it is much clearer

that the Chueta owe their *MEFV* mutation spectrum (and their HLA haplotypes as well, [Crespi et al., 2002]) to the Jewish diaspora.

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