European Journal of Human Genetics (2000) 8, 242–246

www.nature.com/ejhg

ARTICLE

Familial Mediterranean fever in the 'Chuetas' of Mallorca: a question of Jewish origin or genetic heterogeneity

Cecile Domingo[1], Isabelle Touitou[1], Anne Bayou[2], Seza Ozen[3], Cecile Notarnicola[1], Marie Dewalle[1], Jacques Demaille[1], Rene Buades[4], Chalom Sayadat[2], Micha Levy[5] and Eldad Ben-Chetrit[5]

[1]A de Villeneuve Hospital, Montpellier; [2]ACT GENE Genopole, Evry, France; [3]Hacettepe University, Ankara, Turkey; [4]Juan March Hospital, Palma de Mallorca; [5]Hadassah University Hospital, Jerusalem, Israel

Familial Mediterranean fever (FMF) is a hereditary disease commonly found among Jews, Armenians, Turks and Arabs. Recently, FMF was found in the 'Chuetas', a unique community on the island of Mallorca (Spain). To address the question of their possible Jewish origin, we analysed markers known to be linked to the gene responsible for FMF in Jews (MEFV) in this population. We found that 1/3 of the 16p13.3 chromosomes of the 'Chuetas' FMF patients bore the major ancestral haplotypes (S,S2) and their corresponding M694V and E148Q mutations, displayed by Jews from North Africa. Furthermore, we also detected a novel mutation (L110P) in this community. Yet 2/3 of these patients bore S negative haplotypes and lack the mutations commonly known to cause FMF. These results confirm that at least some of the 'Chuetas' share a common origin with Jews. However, they also provide evidence for the possibility of genetic heterogeneity in this disorder. *European Journal of Human Genetics* (2000) **8**, 242–246.

Keywords: FMF; Chuetas; Jews; MEFV

Introduction

Familial Mediterranean fever (FMF) or recurrent polyserositis is a hereditary disease characterised by recurrent attacks of fever, transient peritonitis, pleuritis or synovitis, usually lasting from 1 to 3 days.[1] The disease occurs predominantly in populations inhabiting or originating from the Mediterranean basin. Recently we encountered a cluster of FMF patients among a unique closed community called Chuetas on the island of Mallorca.[2] The exact origin of this population is still obscure. It has been suggested that the Chuetas may be descendants of Sephardic (non-Ashkenazi) Jews converted in the Middle Ages. Although the disease is phenotypically very similar in the Chuetas and the North-African Jewish patients, a previous analysis of several enzymes and blood groups disclosed that, genetically, the Chuetas resembled more the natives of the island.[3,4]

Correspondence: Eldad Ben-Chetrit MD, FMF Clinic, Department of Medicine, Hadassah University Hospital, POB 12000, Jerusalem, Israel. Fax: +972 2 6777394; E-mail: Eldad@hadassah.org.il
Received 21 May 1999; revised 29 November 1999; accepted 6 January 2000

A gene (*MEFV*) responsible for the disease was located on chromosome 16p13.3,[5] refined to a 0 cM interval,[6] then identified independently by the International FMF Consortium[7] and by the French FMF Consortium.[8] We and others[9] have found two ancestral haplotypes (S and S2) in 71–93% of the FMF chromosomes in Jews from North Africa, which contain the *M694V* and *E148Q MEFV* mutations.[7,8] We have now analysed the genotype of a number of Chuetas FMF patients in order to determine if they share genetic features with North-African Jews who have FMF.

Patients and methods

Patients

Eight families (29 members, 16 FMF patients) were included in the present work (Figure 1). Criteria for diagnosis of FMF included typical history of recurrent febrile episodes accompanied by peritonitis, pleuritis, synovitis or erysipelas-like skin lesion with a family history of FMF or favourable response to colchicine treatment.[1] Some of their clinical

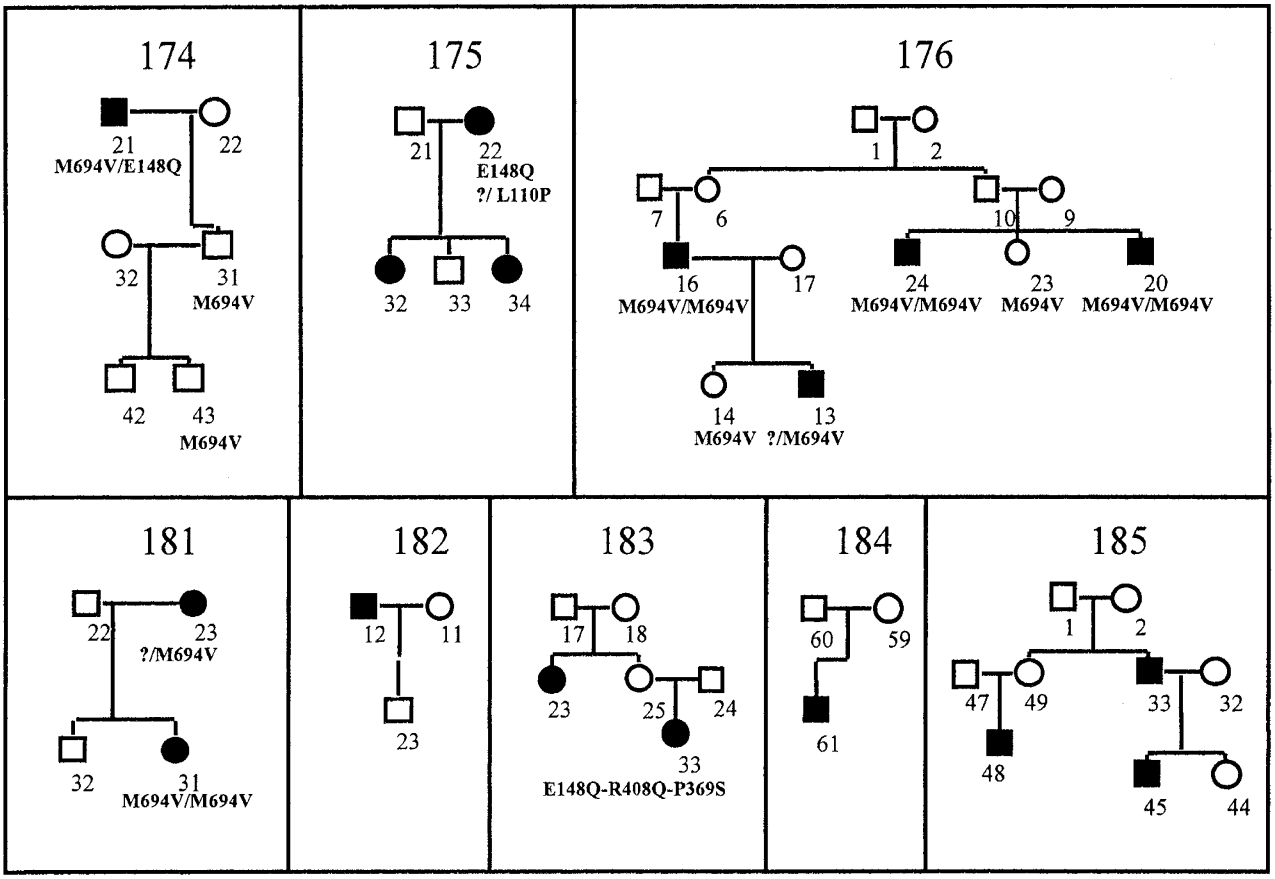

Figure 1 Pedigrees of eight Chuetas families analysed in the study. Filled symbols represent individuals with familial Mediterranean fever (FMF). Under each individual are the individual number, the mutation(s) he/she carries, (with a slash if they are on different chromosomes or one below the other if they are allelic). When DNA from a patient was sequenced and no mutation was found a ? was added. In patient 183-33 the mutations could not be phased so they were marked on the same line.

features are summarised in Table 1. None of the patients experienced headache or swollen lymph nodes during the acute attacks. They had neither conjunctivitis nor periorbital oedema as manifestations of their acute episodes. The average duration of a FMF attack in this population was about 2 days. All participants gave their informed consent to be included in the study.

DNA analysis

Blood was collected in EDTA, genomic DNA was extracted[10] and genotyping was carried out as previously described.[6] Information concerning the primers used for amplification of the microsatellite markers was obtained from the Genome Data Base (GDB). The common *MEFV* gene mutations were searched using appropriate primers and restriction enzymes.[8,11] When none of the previously known mutations was found the complete coding sequence of the gene and the intronic boundaries were sequenced in both directions as described previously.[11]

Results

The family pedigrees are depicted in Figure 1. A series of nine 16p13.3 microsatellite markers (D16S283, D16S3070, D16S3082, D16S3370, D16S2617, D16S3373, D16S3275, D16S475, D16S2622) were analysed. We found three families (six unrelated chromosomes) with the S haplotype, the major FMF haplotype in Jews from North Africa, and one family with the S2 haplotype, the second most frequent haplotype in this population (Figures 1 and 2). In the mutation-negative carrier chromosomes, we found haplotype sharing between 182 (11) and 183 (23) and between 183 (33) and 184 (61).

Since

(1) examination of the pedigrees was not always consistent with linkage to chromosome 16,

(2) none of the known mutations were detected in about 70% of the 16p13.3 chromosomes in the affected patients and

(3) mild still unknown mutations could account for these findings,

Table 1 Phenotype–genotype correlation in Chuetas FMF patients

Patient	*Fever*	*Peritonitis*	*Pleuritis*	*Arthritis*	*Skin*[a]	*Age of onset*[b]	*Colchicine 1.0mg/day*[c]	*Amyloidosis (per biopsy)*
Homozygous for the M694V mutation								
176–16	+	+	+	+	–	6	+	+
176–20	+	+	+	+	+	7	+	–
176–24	+	+	+	+	–	12	+[d]	+
181–32	+	+	+	+	–	21	+[d]	–
Heterozygous for the M694V or E148Q mutations								
176–14	+	±	–	–	–	18	+[d]	–
174–21	+	±	+	–	–	15	+	–
181–22	+	±	+	+	–	45	+	–
175–22	+	+	+	+	–	7	+	–
183–33	+	+	–	–	–	18	+	–
No mutation detected in MEFV								
175–32	+	+	–	–	–	16	+	–
175–34	+	+	–	–	–	18	+	–
182–11	+	+	+	–	–	20	+	–
183–23	+	+	–	–	–	12	+	–
184–61	+	+	+	–	–	10	+	–
185–32	+	+	+	–	–	4	+[d]	–
185–44	+	+	+	+	–	2	+	–

[a]erysipelas-like rash; [b]in years; [c]good response (fewer than three attacks per year); [d]more than three attacks per year.

we sequenced the *MEFV* gene in patients from families 175, 176 (individual 14 only), 181 (individual 22 only), 182, 183, 184 and 185. The sequencing results confirmed that the new haplotypes (families 182–184) converged at the level of SNPs. No new mutation was detected in most of these patients. However, in patient 175-22 we found a substitution L110P in exon 2, which was on the same allele as the E148Q mutation. Two other mutations (R408Q and P369S located in exon 3) were found in individual 183-33, who also bore E148Q mutation (Figure 2).

To determine whether these three substitutions were true mutations or polymorphisms, we analysed a wider series of other FMF chromosomes with no mutation in exon 10, and normal chromosomes ascertained through segregation analysis. These chromosomes were mostly chosen from non-Ashkenazi Jews, which was the best matched population in the present haplotype study. L110P created an Ava1 restriction site, P369S an Alu1 site and R408Q destroyed an HpaII site. Thus, these mutations could easily be screened by means of RFLP. P369S and R408Q were found once in 28 FMF and once in 87 normal chromosomes. In the normal chromosome (from a non-Ashkenazi Jew) these two sequence variants were on the same allele. L110P was found in two out of 110 FMF chromosomes (the second was from a Turkish patient, also in a complex allele associating E148Q) but not in 150 normal chromosomes.

Discussion

The majority of Israeli patients are descendants of Jewish refugees who emigrated to North Africa from the Iberian peninsula at the end of the 15th century following persecution and expulsion. The finding of a cluster of FMF patients exclusively among the Chuetas while the disease is unknown among non-Chuetas inhabitants of Mallorca, raised the question of their possible Jewish origin. We genotyped eight Chuetas FMF families and showed that three of them had the S haplotype, which is specific for the North-African FMF Jewish patients. One family bore the S2 haplotype, which is also common among FMF Jews. Therefore, it is likely that at least part of these two populations (Chuetas and Jews) acquired the FMF gene defect from a common Spanish ancestry. Nevertheless, some Chuetas patients bore an S negative haplotype and this finding needs to be explained.

One possibility is that some of the S negative and positive FMF chromosomes were derived from ancient Jewish sailors who came from the Middle East to North Africa, Palma de Mallorca and Spain in Biblical times.[7] Another possibility is that *de novo* mutations arose in these chromosomes among the Chuetas. Indeed, we sequenced the complete coding region of the gene in those patients and detected three substitutions, two of which, *P369S* and *R408Q*, were recently reported by Aksentijevich *et al*[12,13] and by Cazeneuve *et al*,[14] respectively. The third substitution, *L110P*, is a novel mutation which has not yet been described.

In patients in whom no mutations were found a number of theories could be invoked. First, some of the patients may suffer from one of the periodic syndromes resembling FMF rather than from true FMF.[15–18] However, Chuetas patients did not have features typical of TNFR1-associated periodic syndromes (TRAPS) or hyper IgD syndrome (HIDS).[16–18] Second, the mutations could lie elsewhere in sites other than in the coding region, eg in the promoter, within an intron, or in the 3' UTR. If such as yet unknown mutations do exist, it is still possible that some of these families are linked to

CEN	D16S283	D16S3070	D16S3082	D16S3370	D16S2617	Exon 1	Exon 2								Exon 3				Exon 5				Exon 9	Exon 10						D16S3373	D16S3275	D16S475	D16S2622
						Y65	D102	L110P	G138	E148Q	A165	E167D	R202Q	T267I	R314	P369S	P393	R408Q	E474	Q476	F479L	D510	P588	M680I	M694V	K695R	V726A	A744S	R761H				
nomenclature in (7,12)							x2.1		x2.2		x2.3		x2.4		x3.4				x5.1	x5.2		x5.3	x9.1										
175 (22)	9	7	3	5	6	C	C/T	T	A/G	G	A/C	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	6	7	9	4
	9	8	10	9	6	C	C/T	C	A/G	C	A/C	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	4	7	4	5
175 (32)	7	5	2	3	6	C	C/T	T	A/G	G	A/C	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	4	7	13	4
	9	7	3	5	6	C	C/T	T	A/G	G	A/C	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	6	7	9	4
175 (34)	8	5	11	5	5	C	nd	T	nd	G	nd	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	6	7	9	5
	9	7	3	5	6	C	nd	T	nd	G	nd	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	6	7	9	4
176 (14)	7	3	9	9	6	C	T	T	A	G	C	G	G	C	T	C	C	G	A	G	C	C	A	G	A	A	T	G	G	5	6	3	4
	7	3	3	3	7	C	C	T	G	G	A	G	A	C	C	C	C	G	A	G	C	T	G	G	G	A	T	G	G	5	9	11	4
182 (11)	5	3	8	9	7	C	T	T	A	G	C	G	G	C	C/T	C	C	G	A/G	A/G	C	C/T	A/G	G	A	A	T	G	G	5	7	10	nd
	7	5	17	9	5	C	T	T	A	G	C	G	G	C	C/T	C	C	G	A/G	A/G	C	C/T	A/G	G	A	A	T	G	G	5	11	3	nd
183 (23)	10	3	5	9	5	C	T	T	A	G	C	G	G	C	C/T	C	C	G	A/G	A/G	C	C/T	A/G	G	A	A	T	G	G	3	8	3	4
	7	5	17	9	5	C	T	T	A	G	C	G	G	C	C/T	C	C	G	A/G	A/G	C	C/T	A/G	G	A	A	T	G	G	5	11	3	4
183 (33)	7	7	11	9	6	C	T	T	A	G	C	G	G	C	T	C	C	G	A	G	C	C	G	G	A	A	T	G	G	5	6	4/9	4/5
	6	8	8	9	6	C	T	T	A	C	C	G	G	C	T	T	C	A	G	A	C	T	A	G	A	A	T	G	G	3	6	4/9	4/5
184 (61)	7	7	11	9	6	C	T	T	A	G	C	G	G	C	T	C	C	G	A	G	C	C	G	G	A	A	T	G	G	5	7	5	4
	8	5	2	9	6	C	C	T	G	G	A	G	G	C	T	C	C	G	A	G	C	C	G	G	A	A	T	G	G	6	7	11	4
185 (32)	5	8	8	9	6	C	C	T	G	G	A	G	G	C	C/T	C	C	G	G	A/G	C	C/T	A/G	G	A	A	T	G	G	6	7	12	4
	8	8	2	9	6	C	C	T	G	G	A	G	G	C	C/T	C	C	G	A	A/G	C	C/T	A/G	G	A	A	T	G	G	6	9	11	3
185 (44)	5	8	8	9	6	C	T	T	G	G	C	G	G	C	C/T	C	C	G	A	A/G	C	C/T	A/G	G	A	A	T	G	G	6	7	12	4
	4	8	10	9	5	C	C	T	A	G	A	G	G	C	C/T	C	C	G	A	A/G	C	C/T	A/G	G	A	A	T	G	G	5	11	13	4

Figure 2 Sequence alterations detected by sequencing 10 Chuetas FMF patients. The first line depicts the Marenostrin/Pyrin gene exon positions. The second line depicts the sequence variations within the gene (centre), or in flanking genetic markers (left: centromeric markers, right: telomeric markers). The third line depicts the nomenclature used elsewhere[7,12] for the intragenic polymorphisms. The lines below show the sequence variations observed in the 10 individuals. In individual 175-34, the complete coding sequence of the paternal chromosome was obtained from the father. When the phase is not determined, the corresponding markers are shown with a slash. Mutations are boxed.

chromosome 16 but that reduced penetrance obscure this linkage. Third, perhaps on a permissive genetic background, a single *MEFV* mutation may be sufficient to cause clinical symptoms. Lastly, large deletions or locus heterogeneity for FMF cannot be excluded. Indeed, a Turkish group of investigators has shown such heterogeneity in a small subset of Turkish families.[19] No other gene involved in FMF has yet been identified and it is possible that another unknown gene is cosegregating in some of the families presented here.

Acknowledgements

This work was supported by the Association Française pour la Myopathie, The Centre Hospitalier de Montpellier and the Adolfo and Evelin Blum Fund for arthritis research. We thank Dr Weissenbach and all the other members of the French FMF Consortium for helpful discussions.

References

1 Eliakim M, Levy M, Ehrenfeld M: *Recurrent Polyserositis (Familial Mediterranean Fever)*. Elsevier/North Holland Biomedical Press: Amsterdam, 1981.
2 Buades RJ, Ben-Chetrit E, Levy M: Familial Mediterranean fever in the 'Chuetas' of Mallorca - origin in inquisition? *Isr J Med Sci* 1995; **31**: 497–499.
3 Picomell A, Miguel A, Castro JA, Ramon MM: Enzymatic polymorphism in the Jewish community (Chuetas) from the Majorca Island. *Gene Geogr* 1990; **45**: 165–171.
4 Picomell A, Casro JA, Ramon MM: Blood groups in the Chueta community (Majorcan Jews). *Hum Hered* 1991; **41**: 35–42.
5 Pras E, Aksentijevich I, Gruberg L *et al*: Mapping of a gene causing Familial Mediterranean fever to the short arm of chromosome 16. *New Engl J Med* 1992; **326**: 1509–1513.
6 French FMF Consortium: Localization of the Familial Mediterranean fever (*FMF*) gene to a 250 kb interval in non-Ashkenazi Jewish haplotypes. *Am J Hum Genet* 1996; **59**: 603–612.
7 International FMF Consortium: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* 1997; **90**: 797–807.
8 French FMF Consortium: A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; **17**: 25–31.

9 Balow JE Jr, Shelton DA, Osborn A *et al*: A high resolution genetic map of the familial Mediterranean fever candidate region allows identification of haplotype-sharing among ethnic groups. *Genomics* 1997; **44**: 280–291.
10 Sambrook J, Fritsch EF, Maniatis J: *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.
11 Bernot A, da Silva C, Petit J-L *et al*: Non-founder mutations in the *MEFV* gene establish this gene as the cause of familial Mediterranean fever (FMF). *Hum Mol Genet* 1998; **7**: 1317–1325.
12 Aksentijevich I, Pras E, Gruberg L *et al*: Familial Mediterranean fever (FMF) in Moroccan Jews: demonstration of a founder effect by extended haplotype analysis. *Am J Hum Genet* 1993; **53**: 644–651.
13 Aksentijevich I, Torosyan Y, Samuels J *et al*: Mutation and haplotype studies in familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in Ashkenazi Jewish population. *Am J Hum Genet* 1999; **64**: 949–962.
14 Cazeneuve C, Sarkisian T, Pecheux C *et al*: MEFV-Gene analysis in Armenian patients with familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype-genetic and therapeutic implications. *Am J Hum Genet* 1999; **65**: 88–97.
15 Knockaert DC, Vanneste LJ, Bobbaers JJ: Recurrent or episodic fever of unknown origin: review of 45 cases and survey of the literature. *Medicine* 1993; **72**: 184–196.
16 Drenth JPH, Cuisset L, Grateau G *et al*: Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. *Nat Genet* 1999; **22**: 178–181.
17 Houten SM, Kuis Wietse, Dura M *et al*: Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinemia D and periodic syndrome. *Nat Genet* 1999; **22**: 175–177.
18 McDermott MF, Aksentijevich I, Galon J *et al*: Germline mutations in the extracellular domains of the 55kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* 1999; **97**: 133–144.
19 Akarsu AN, Saatci U, Ozen S, Bakkaloglu A, Besbas N, Sarfarazi M: Genetic linkage study of familial Mediterranean fever (FMF) to 16p13.3 and evidence for genetic heterogeneity in the Turkish population. *J Med Genet* 1997; **34**: 573–578.