Genetic linkage study of familial Mediterranean fever (FMF) to 16p13.3 and evidence for genetic heterogeneity in the Turkish population

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Abstract

Familial Mediterranean fever (FMF) is an autosomal recessive condition that is almost entirely restricted to the non-Askhenazi Jews, Arabs, Armenians, and Turks. Genetic linkage study of a large group of non-Turkish families has previously mapped the FMF locus to the 16p13.3 region and shown that this locus resides 0.305 cM distal to D16S246. Furthermore, allelic association has also been shown with D16S3070 (75%) and D16S3275 (66%). However, no genetic heterogeneity has been described for any of the three major reported groups of FMF families. Here, we describe the genetic linkage relationship of the fourth major group of Turkish families and report the first evidence for genetic heterogeneity of this condition. Two point linkage analysis and haplotype inspection of 15 DNA markers from the reported region of the FMF locus identified tight linkage in a group of six Turkish FMF families. A maximum lod score of 9.115 at θ=0.00 was observed for D16S3024. Nine other DNA markers provided similar evidence of linkage with lod score values of above 5.21. However, two other FMF families were completely unlinked to this region of chromosome 16. Haplotype construction of DNA markers in five consanguineous linked families showed that a segment of homozygosity has been conserved for D16S3070 and D16S2617. No other DNA markers showed any such conservation. Therefore, we suggested that these two markers reside in close proximity to the FMF locus. Furthermore, we observed 80% allelic association with D16S2617 but no association with D16S3070 or any other DNA markers from the FMF critical region. In summary, we conclude that our Turkish families are also linked to the reported FMF locus at 16p13.3, there is a genetic heterogeneity for this condition at least in our group of Turkish families, and D16S2617 is in linkage disequilibrium in the Turkish FMF families. Combination of this study with previously published observations suggests that the FMF locus resides between D16S246 and D16S3070/D16S2617 and within a region of about 250-300 kb.

(*J Med Genet* 1997;34:573–578)

Keywords: familial Mediterranean fever; genetic linkage; 16p13.3; Turkish population

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Received 15 November 1996
Revised version accepted for publication 21 February 1997

Familial Mediterranean fever (FMF), also known as recurrent polyserositis is an autosomal recessive disorder characterised by recurrent, self limiting attacks of fever accompanied by peritonitis, arthritis, or pleurisy.[1] The disease is almost completely restricted to non-Askhenazi Jews, Arabs, Armenians, and Turks.[2 3] Other subjects affected with this condition have also been reported from different populations, but for most of these patients the exact ancestry cannot be determined.[4] The fetal complication of FMF is the development of secondary amyloidosis. There is a striking difference in the frequency of amyloid nephropathy in different ethnic groups. It is known to be more frequent in Turks and non-Askhenazi Jews with a frequency of 60% and 12-30%, respectively.[5–7] However, the phenotypic expression of the disease is less severe, with a low incidence of amyloidosis in Iraqi Jews, Arabs, and Armenians.[8] Recurrent attacks and the development of amyloidosis can be prevented by colchicine treatment.[9 10] Despite numerous efforts to understand the basic mechanism underlying the FMF phenotype, the exact molecular mechanism of this condition is still unknown.

The FMF locus has been mapped to chromosome 16p13.3 within an interval that is flanked by D16S246 telomerically and D16S523/D16S423 centromerically.[11 12] Linkage disequilibrium between the FMF locus and a 2.5 kb fragment of D16S246 has been reported in Moroccan and non-Moroccan Jews, but not among Armenian or Arab families.[13] These linkage disequilibrium data have placed the FMF gene approximately 0.305 cM distal to D16S246.[13] In a more recent report, the FMF locus was shown to be tightly linked to two markers, D16S3070 and D16S3275, in non-Askhenazi Jews.[14] These two DNA markers are both reported to be localised to a 250 kb fragment.[14] To date, three out of the four major FMF groups, namely, Jewish, Armenian, and Arabs, have been extensively studied for both linkage and haplotype transmission analysis, but so far no locus heterogeneity has been reported for any of these populations. In this study, we aimed to evaluate the last major group, the Turks, by testing for linkage and genetic heterogeneity of

Table 1 Clinical presentation of eight FMF families used in this study

					Clinical manifestations				Laboratory findings				
FAM	*PID*	*Sex*	*Age*	*Fever*	*Abd pain*	*Chest pain*	*Joint pain*	*Skin erp*	*RSL*	*Leucocytosis*	*RSF*	*Amyloidosis*	*Colchicine treatment*
1	3	F	13	Yes	Yes	No	No	No	Yes	No	No	No	Responded
1	5	F	7	Yes	Yes	No	No	No	Yes	No	No	No	Responded
3	3	F	15	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Responded
3	4	M	13	Yes	Yes	No	Yes	No	Yes	Yes	Yes	? Mild proteinuria	Responded
3	5	F	9	Yes	Yes	No	No	No	No	No	Yes	No	Responded
4	11	F	17	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Responded
4	13	F	12	Yes	Yes	No	Yes	No	Yes	Yes	No	No	Responded
4	14	F	9	Yes	Yes	No	No	No	Yes	Yes	No	No	Responded
4	15	M	9	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	Responded
5	11	M	12	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Responded
5	12	F	9	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Responded
5	13*	F	7	Yes	Yes	No	No	No	ND	ND	ND	No	Recently started
5	14	M	9	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	Responded
6	14*	F	62	Yes	Yes	No	No	No	ND	ND	ND	No	No attack last 5 years
6	20	M	11	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Responded
6	22	F	4	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	Responded
7	3	F	17	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Responded
7	6	M	4	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Responded
8	11*	M	80	Yes	Yes	No	No	No	ND	ND	ND	No	No attack last 7 years
8	16	F	44	No	No	Yes	Yes	No	Yes	No	Yes	No	No colchicine
8	19	F	50	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Responded
8	20	M	40	Yes	Yes	Yes	No	No	Yes	No	Yes	No	Responded
8	22*	M	20	Yes	Yes	Yes	No	No	ND	ND	ND	No	Responded
8	25	M	24	Yes	Yes	No	No	No	Yes	No	Yes	No	Responded
8	28	M	11	Yes	No	Yes	Yes	No	Yes	No	Yes	No	Responded
12	3	M	20	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Haemodialysis
12	4	F	15	Yes	Yes	No	Yes	No	Yes	Yes	Yes	? Mild proteinuria	Responded
12	5	F	12	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Responded
12	6	M	9	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Responded

FAM=family number.
PID=person identification number as in figs 1 and 2.
Abd pain=abdominal pain.
Skin erp=skin eruption.
RSL=raised sedimentation level.
RSF=raised serum fibrinogen level.
*=diagnosis based on family history.
ND=not done.

a group of eight Turkish families with highly polymorphic DNA markers from the 16p13.3 region.

Materials and methods

FAMILIES

The families included in this study were ascertained from a FMF registry that is currently in operation at the Department of Paediatric Nephrology and Rheumotology, University of Hacettepe in Ankara, Turkey. This registry comprises 425 FMF patients who have been diagnosed and followed up for the last 20 years. The diagnostic criteria and clinical information of this database have already been reported.[3 10 15 16] From this registry, we selected a group of eight families with a minimum of two affected offspring and consanguinity in most of the parental generations (fig 1, pedigrees 1, 4, 5, 6, 8, and 12, and fig 2, pedigrees 3 and 7). All patients of either Armenian or Jewish ethnic origin were excluded from this analysis. Taken together, these eight families provided a total of 49 offspring, 28 of whom were affected with FMF. The clinical manifestation of the affected subjects in each pedigree is presented in table 1.

MOLECULAR STUDIES

Genomic DNA was amplified with sequence specific primers using the polymerase chain reaction (PCR) technique. We selected highly informative STRP (simple tandem repeat polymorphism) markers to cover the region of interest corresponding to data previously published by other groups.[13 14 17] DNA markers HBAP1, D16S3024, D16S3070, D16S523, and D16S510 were selected as anchor markers and were used to construct a correlation between different maps. Information on primer sequences, number of alleles, and band sizes were obtained from Genome Database (GDB),[18] Généthon,[19 20] Utah Marker Development Group,[21] and Cooperative Human Linkage Center (CHLC).[22] A total volume of 25 µl was used per reaction which included 1 × PCR buffer (10 mmol/l Tris-HCl with various concentration of $MgCl_2$, pH 8.4, 50 mmol/l KCl, 0.01% gelatin, and 0.1% Triton), 0.25 µmol/l of each primer, 100 ng template DNA, 0.2 mmol/l of each dNTPs, and 0.25 U *Taq* polymerase (Amplitaq-Perkin Elmer). We applied initial denaturation at 94°C for two minutes which was followed by three steps of amplification for a total of 28-30 cycles. Annealing temperatures were adjusted according to amplification information for the STRP markers as published previously; 6-7% denaturing polyacrylamide gels and silver staining[23] techniques were used to separate the amplified products.

LINKAGE ANALYSIS

Two point lod scores were calculated with the MLINK module of the LINKAGE package (FASTLINK, version 2.20) for an autosomal recessive condition.[24 25] DMS program (unpublished) was used for data entry, error checking, and preparation of input files for the LINKAGE package. Penetrance was set at 100%. Considering the age of onset of the

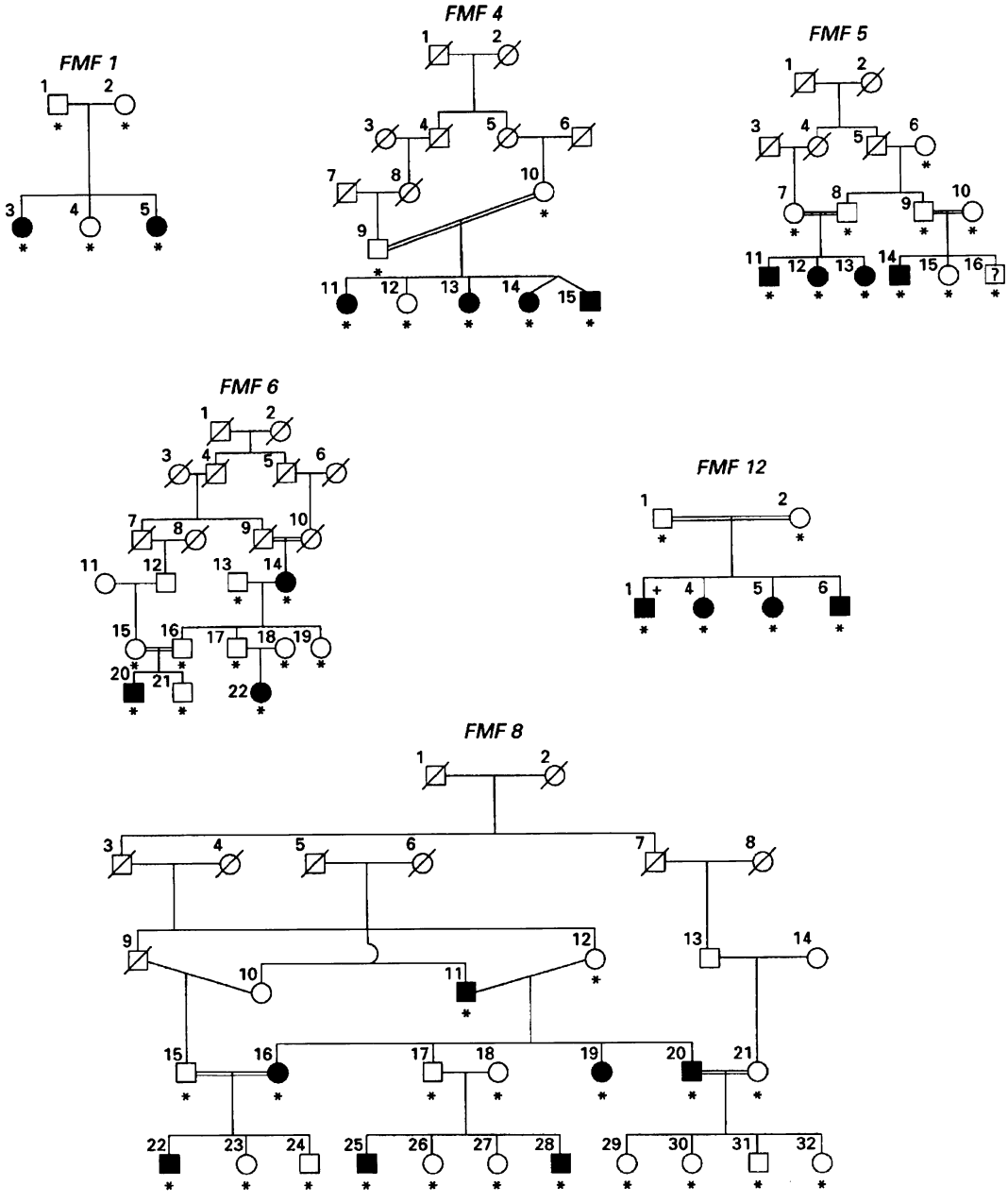

Figure 1 *Pedigree structure of six linked Turkish FMF families. *=DNA available.*

disease, normal subjects under 7 years were coded as an unknown.

Results

TWO POINT LINKAGE ANALYSIS

Previously, the FMF locus has been mapped between flanking markers D16S246 and D16S523/D16S453,[13] within a region of about 4 cM. However, using linkage disequilibrium information and homozygosity mapping data, the FMF gene was subsequently placed approximately 0.305 cM from D16S246 by the same group of researchers.[13] In this study, eight Turkish families (six of them consanguineous) were genotyped for 15 highly polymorphic markers that covered this 4 cM region on chromosome 16p13.3. All DNA markers tested in this study provided a positive lod score for six of our FMF families (families 1, 4, 5, 6, 8, and 12, fig 1). The lod score maximised with DNA marker D16S3024 ($Z=9.115$ at $\theta=0.00$) for these six families (table 2). The remaining two kindreds (families 3 and 7 in fig 2) showed no linkage to this region of chromosome 16 by two point linkage analysis. Construction and inspection of haplotypes also confirmed that the affected subjects in these two pedigrees have inherited completely different chromosomes from their respective parents and for an entire region that is flanked by HBAP1 and D16S510 (fig 2). Therefore, we excluded these two unlinked families from further analysis. However, in view of the fact that a few DNA markers from the region of 17q22-q24 have initially been shown to be linked to the FMF phenotype[26] and were subsequently proven to be a type 1 error,[27] we decided to test these two unlinked families with DNA markers from this region of chromosome 17. The two markers, D17S785 and D17S795, were selected and genotyped, but no evidence of linkage was observed in these two families (data not shown).

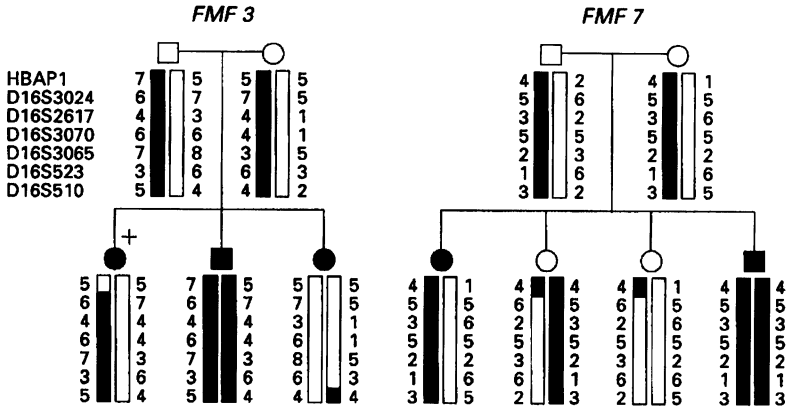

Figure 2 Pedigree structure and inherited haplotypes of two Turkish FMF families unlinked to 16p13.3.

HAPLOTYPE ANALYSIS

In order to localise the disease gene further, haplotypes were constructed and analysis performed according to the observed recombination events. The information on the order of DNA markers was obtained from previously published genetic linkage maps. Two recombination events (one affected and one normal subject) positioned the disease locus centromeric to marker HBAP1. We observed another recombination with D16S3027 in a normal subject (family 6, subject 21). This subject is 8 years old and his clinical examination was completely normal. However, previously the mean age of onset in Turkish families has been reported as 5.5 ± 3.4.[10] Therefore, this recombination event cannot be used to place the FMF gene more precisely, as the possibility of this person developing the condition in the near future still exists. Similarly, another normal subject (family 5, subject 16) has inherited two copies of the entire affected chromosome from his carrier parents. Since he is only 1 year old and therefore too young to develop the disorder as yet, his status was coded as unknown for linkage purposes. No other recombinations were observed in the linked families. Thus, we could not reduce the FMF critical region any further.

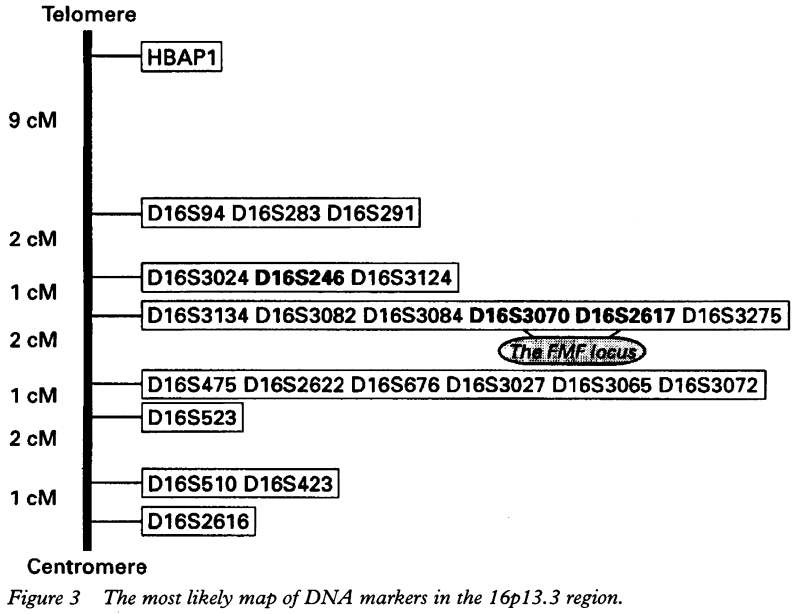

Figure 3 The most likely map of DNA markers in the 16p13.3 region.

LINKAGE DISEQUILIBRIUM

Inspection of the genotypic data obtained showed that a 104 bp fragment of D16S2617 has a higher frequency in the affected subjects than in normal members of our linked families. D16S2617 is a trinucleotide repeat marker with a 69% heterozygosity ratio (CEPH and GDB databases) and eight allele sizes that range between 92 and 113 bp. Table 3 shows the comparative frequency and allelic distribution of D16S2617 from the CEPH database and those obtained in the Turkish population. As seen in this table, the 104 bp fragment (allele 4) is not a common allele in either of these two general populations. However, this fragment is over-represented among the FMF affected subjects with an incidence of 80%, suggesting linkage disequilibrium with this marker. The observed difference between the affected and normal chromosomes in the FMF families is statistically significant (χ=14.08, p=0.0002). We did not observe linkage disequilibrium with any other markers used in this study, including D16S3070.

HOMOZYGOSITY MAPPING

Five of the linked families used in this study were the result of consanguineous marriages which allowed us to construct the common inherited haplotypes. As shown in table 4, the smallest segment of homozygosity was observed in families 4 and 8, suggesting that D16S3070 and D16S2617 are the two minimum segments of DNA that have been conserved in these families. None of the markers above or below these two markers showed the same homozygosity in these consanguineous families. Therefore, we were able to position D16S2617 in very close proximity to D16S3070. This homozygosity in the affected subjects also suggests that FMF may reside very close to these two markers.

Discussion

In this report, we present evidence that in a group of Turkish FMF families the FMF gene is also linked to the same reported region of 16p13.3. Thus, we confirmed that this location is a major locus responsible for the FMF phenotype that occurs in different populations with various ethnic backgrounds. Six out of eight families studied here provided positive lod scores for all the STRP markers from this region of chromosome 16. A group of tightly linked markers did not show any recombination with the FMF locus. A maximum lod score of 9.115 at θ=0.00 was obtained for DNA marker D16S3024. Other DNA markers from this region of chromosome 16 also showed strong linkage with all the six linked families. We have also observed 80% allelic association with marker D16S2617 in our group of linked families. Furthermore, all of our consanguineous family members showed homozygosity for this marker.

Previously, another allelic association (76%) was also reported with closely linked marker D16S246 in the Moroccan Jewish population, and in a recent report, 75% and 66% allelic association has been described for D16S3070

Table 2 Two point lod scores between six Turkish FMF families and 15 DNA markers from the 16 p13.3 region

	Recombination fraction (cM)												
	0.000	0.050	0.100	0.150	0.200	0.250	0.300	0.350	0.400	0.450	Zmax	θ	−1 lod CI
HBAP1	−∞	2.989	3.046	2.831	2.492	2.078	1.615	1.130	0.662	0.266	3.073	0.080	0.010 -> 0.260
D16S3024	9.115	8.194	7.229	6.224	5.183	4.118	3.049	2.018	1.093	0.381	9.115	0.000	0.000 -> 0.060
D16S3134	4.809	4.283	3.740	3.183	2.613	2.040	1.475	0.943	0.482	0.149	4.809	0.000	0.000 -> 0.100
D16S3082	2.056	1.866	1.664	1.450	1.226	0.992	0.753	0.517	0.300	0.120	2.056	0.000	0.000 -> 0.240
D16S3084	5.566	4.954	4.324	3.676	3.016	2.351	1.694	1.070	0.529	0.143	5.566	0.000	0.000 -> 0.090
D16S3070	6.117	5.476	4.803	4.104	3.387	2.662	1.949	1.279	0.695	0.251	6.117	0.000	0.000 -> 0.080
D16S2617	8.974	8.102	7.182	6.215	5.204	4.158	3.095	2.057	1.116	0.388	8.974	0.000	0.000 -> 0.060
D16S475	8.832	7.917	6.967	5.982	4.966	3.929	2.891	1.892	1.002	0.330	8.832	0.000	0.000 -> 0.060
D16S2622	5.212	4.621	4.015	3.396	2.770	2.146	1.539	0.976	0.495	0.151	5.212	0.000	0.000 -> 0.090
D16S676	7.755	6.998	6.205	5.374	4.508	3.612	2.701	1.809	0.995	0.356	7.755	0.000	0.000 -> 0.070
D16S3027	−∞	3.850	3.554	3.118	2.618	2.086	1.542	1.013	0.539	0.179	3.870	0.040	0.000 -> 0.180
D16S3065	7.327	6.588	5.817	5.016	4.187	3.338	2.485	1.660	0.915	0.333	7.327	0.000	0.000 -> 0.070
D16S523	5.559	5.000	4.416	3.807	3.174	2.524	1.869	1.233	0.663	0.226	5.559	0.000	0.000 -> 0.090
D16S510	2.658	2.391	2.111	1.819	1.517	1.209	0.901	0.605	0.341	0.131	2.658	0.000	0.000 -> 0.180
D16S2616	6.245	5.598	4.923	4.222	3.499	2.763	2.031	1.332	0.715	0.248	6.245	0.000	0.000 -> 0.080

Zmax=maximum value of lod score, θ=corresponding maximum recombination fraction for Zmax, CI=confidence interval.

Table 3 Comparison of D16S2617 allelic frequencies in the CEPH and Turkish populations

Allele No	Band sizes (bp)	CEPH data (n=20)	Turkish population (n=56)	FMF families (affected, n=10)	FMF families (normal, n=23)
1	113	0.08	0.04	—	0.13
2	111	0.08	0.32	—	0.26
3	107	0.67	0.52	0.20	0.39
4	104	0.08	0.05	0.80*	0.13
5	101	—	0.07	—	0.04
6	98	—	—	—	0.04
7	95	—	—	—	—
8	92	0.08	—	—	—

n=number of chromosomes, *= statistically significant, p=0.0002.

Table 4 Conserved segment of homozygousity in five consanguineous FMF families

	Family 8	Family 12	Family 6	Family 4	Family 5	
HBAP1	3–3	ND	5–5	5–2	7–7	
D16S3024	3–7	2–1	6–6	5–7	4–5	
D16S3070	7–7	7–7	6–6	6–6	1–1	FMF
D16S2617	4–4	4–4	4–4	4–4	4–4	region
D16S475	9–7	3–3	9–9	2–3	6–6	
D16S2622	2–3	3–3	ND	3–2	3–3	
D16S676	9–7	6–6	7–7	3–5	7–7	
D16S3065	5–3	ND	3–3	5–6	3–3	
D16S523	3–3	6–2	6–6	3–3	5–1	

and D16S3275, respectively. We did not use D16S3275 in our study, but our homozygosity mapping suggested that D16S3070 is part of a conserved haplotype and, therefore, is expected to be one of the closest DNA markers to the FMF locus. Combining our results with the two previously observed linkage disequilibrium results suggests that FMF is located between D16S246 and D16S3070/D16S2617, within a region of about 250-300 kb.

Although a large number of STRP markers are now available for this region of chromosome 16, their map order is still unknown. Inspection and construction of linkage haplotypes in the CEPH pedigrees, comparison of different published maps, and evaluation of YAC and contig information provided the most likely map order for this region as shown in fig 3.

Surprisingly, two Turkish families showed no linkage to this region of chromosome 16, thus providing the first evidence of genetic heterogeneity in this disorder (fig 2). The affected members of these two families were revisited and their members were resampled and subsequently regenotyped for all the DNA markers studied. The diagnosis in the two unlinked families was confirmed and was based on their recurrent, self-limiting attacks of fever and synovitis. These attacks have responded well to colchicine with resolution of these attacks in all the affected members. One distinctive phenotypic feature in these two families is that they have had pronounced attacks of arthritis. Rheumatoid arthritis was excluded by the lack of joint deformity, the short duration of the arthritis, and spontaneous resolution of the joint findings. Behcet's disease was also excluded as the result of lack of clinical criteria.[28] The IgD levels of the affected subjects from these two unlinked families were found to be normal based on the differential diagnosis of periodic fever with hyperimmunoglobulinaemia D syndrome.[29] Therefore, the clinical diagnosis and genotypic data were confirmed in these two families. In addition, no indication for linkage was obtained with markers from other candidate regions on chromosome 17q.

Phenotypic variation between people of different ethnic origin is well known in this condition. For example, in the Arab population, FMF is less severe with low incidences of arthritis, amyloidosis, and erysipeloid erythema.[4] Similarly, while amyloidosis and arthritis are less common in Armenians, pleuritis seems more frequent in this population.[30] In Turks, amyloidosis is relatively more common. However, erysipelis-like lesions are very rare in the Turkish population (unpublished observations). In this study, two families (families 3 and 12) showed complications of amyloidosis (table 1). However, one of them (family 3) showed no linkage to chromosome 16, while the other one showed tight linkage to all the DNA markers studied from this region. Therefore, this locus heterogeneity cannot be attributed to the phenotypic variation. A study is currently under way to screen other kindreds, aiming to identify more families unlinked to this region of chromosome 16.

We are indebted to the FMF families who participated in this study. We would like to thank Dr M Ozguc, director of the DNA/Cell Bank and Gene Research Laboratory of the Scientific and Technical Council of Turkey (TUBITAK) for extraction and banking of the FMF DNA samples. This work is partially supported by "The State Planning Organization of the Turkish Republic".

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