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Linkage Disequilibrium Mapping Places the Gene Causing Familial Mediterranean Fever Close to *D16S246*

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Summary

This report presents refined genetic mapping data for the gene causing familial Mediterranean fever (FMF), a recessively inherited disorder of inflammation. We sampled 65 Jewish, Armenian, and Arab families and typed them for eight markers from chromosome 16p. Using a new algorithm that permits multipoint calculations for a dense map of markers in consanguineous families, we obtained a maximal LOD score of 49.2 at a location 1.6 cM centromeric to *D16S246*. A specific haplotype at *D16S283-D16S94-D16S246* was found in 76% of Moroccan and 32% of non-Moroccan Jewish carrier chromosomes, but this haplotype was not overrepresented in Armenian or Arab FMF carriers. Moreover, the 2.5-kb allele at *D16S246* was significantly associated with FMF in Moroccan and non-Moroccan Jews but not in Armenians or Arabs. Since the Moroccan Jewish community represents a relatively recently established and genetically isolated founder population, we analyzed the Moroccan linkage-disequilibrium data by using Luria-Delbrück formulas and simulations based on a Poisson branching process. These methods place the FMF susceptibility gene within 0.305 cM of *D16S246* (2-LOD-unit range 0.02–0.64 cM).

Introduction

Familial Mediterranean fever (FMF) is a recessively inherited disease occurring primarily in non-Ashkenazi Jews (Sohar et al. 1967), Armenians (Schwabe and Peters 1974), Turks (Ozdemir and Sokmen 1969), and Middle Eastern Arabs (Barakat et al. 1986). It is characterized by recurrent, self-limited attacks of fever with sterile peritonitis, pleurisy, and/or synovitis (Sohar et al. 1967). Patients with FMF may also develop systemic amyloidosis. The molecular basis of this disorder is not known.

We have mapped the FMF susceptibility gene, designated *MEF*, to chromosome 16p in non-Ashkenazi Jewish families from Israel (Pras et al. 1992) and in Armenian families from California (Shohat et al. 1992). Subsequent analysis of 31 non-Ashkenazi Jewish families placed *MEF* between *D16S94* and *D16S80*, a genetic interval of 9 cM (Aksentijevich et al. 1993*b*). Studies of Armenian (Fischel-Ghodsian et al. 1993) and Arab families (Pras et al. 1994) were consistent with this map location. Although there is no evidence for locus heterogeneity in FMF, allelic heterogeneity is possible, since there are clinical differences among the various affected populations (Sohar et al. 1967; Ozdemir and Sokmen 1969; Schwabe and Peters 1974; Barakat et al. 1986).

Part of our strategy for the refined genetic localization of *MEF* has relied on the use of homozygosity mapping (Smith 1953; Lander and Botstein 1987) in several consanguineous non-Ashkenazi Jewish families (Aksentijevich et al. 1993*b*). For rare recessive diseases, inbred families are more informative than comparable nonconsanguineous families, because the children of inbred marriages usually inherit two copies of the disease-causing mutation and closely linked markers from a common ancestor. Therefore, meioses that are inferred from inbreeding loops contribute to the overall LOD scores calculated for such families, and these meioses are a potential source of recombination events. Nevertheless, we have heretofore not been able to take full advantage of this powerful strategy for three reasons (Aksentijevich et al. 1993*b*): (*a*) the gene frequency of *MEF* is relatively high in the non-Ashkenazi Jewish population; (*b*) some of the markers in the candidate region have limited poly-

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morphism; and (*c*) there are significant computational constraints associated with multipoint linkage analysis in consanguineous families (Farral 1993; Terwilliger and Ott 1994). However, the latter problem has recently been reduced substantially by Kruglyak et al. (1995), who described a new algorithm for multipoint analysis in inbred families.

Allelic associations of marker loci with a disease are also sometimes helpful in refining the localization of the susceptibility gene. Theoretically, there is an inverse relationship between the degree of allelic association and the physical or genetic distance (Hill and Robertson 1968). In our previous studies of FMF, we have found highly significant haplotype associations with FMF in the Moroccan Jewish population (Aksentijevich et al. 1993*a*). Thus, in the Moroccan Jewish population it appears that most FMF is caused by a single founder chromosome. Undoubtedly the history of this population has favored the observation of this founder effect: Jews first settled in Spain ~2,000 years ago, and a series of persecutions led to the migration of a large part of the population to Morocco, especially in the 7th, 14th, and 15th centuries (Roth 1972; Goodman 1979). In Morocco the Jewish population has remained genetically isolated until their recent migration to Israel.

New quantitative methods have been described for estimating genetic distances on the basis of linkage disequilibrium in recently founded populations in which there is one major disease-associated haplotype. One approach is an adaptation of Luria and Delbrück's mathematical analysis of mutation rates in bacterial cultures (Luria and Delbrück 1943; Hästbacka et al. 1992), using allelic associations and information about the history of a population to estimate the genetic distance from a disease locus to closely linked markers. Luria-Delbrück analysis was recently used in the positional cloning of the gene causing diastrophic dysplasia (Hästbacka et al. 1994) and has also been employed in the refined mapping of several other recessively inherited disorders (Lehesjoki et al. 1993; Aaltonen et al. 1994; Haataja et al. 1994; Kestilä et al. 1994; Pigg et al. 1994; Sulisalo et al. 1994; Mitchison et al. 1995). Kaplan et al. (1995) have also recently described a method of simulating disease populations, based on a Poisson branching process, to estimate the recombination fraction between marker and disease loci. We reasoned that these strategies should be applicable to FMF in Moroccan Jews.

The present report summarizes our progress in refining the localization of *MEF*. By establishing a consortium of laboratories interested in FMF, we have assembled a panel of 65 families of Jewish, Armenian, or Arab ancestry. We genotyped these families for eight markers on chromosome 16p, including five not examined in previous reports. By using the algorithm described by Kruglyak et al. (1995), we have markedly improved our ability to perform multipoint linkage analysis on our consanguineous families. Moreover, we analyzed allelic associations for each ethnic group separately and performed Luria-Delbrück–type analyses and simulations of linkage disequilibrium in the Moroccan Jewish population.

Subjects and Methods

Patients and DNA Samples

Non-Ashkenazi Jewish and Arab families were recruited from a clinic at the Chaim Sheba Medical Center, Tel-Hashomer, Israel; Armenian families were recruited from a clinic at the Cedars-Sinai Medical Center, Los Angeles. FMF was diagnosed according to established clinical criteria (Sohar et al. 1967). This study was approved by the human experimentation committees at both institutions. Table 1 summarizes the ethnic composition of our panel. Informative meioses were determined for each family, on the basis of the assumption of a fully informative marker and a recessive disease susceptibility gene. Peripheral blood lymphocytes were immortalized with Epstein-Barr virus, and DNA was extracted from cell lines by standard techniques.

Southern Analysis

Southern blots were prepared as described (Pras et al. 1992). Plasmid probes 24-1 (*D16S80*) and 218EP6 (*D16S246*) were labeled with α-^{32}P-dCTP by random-priming (Oligolabeling Kit, Pharmacia). Hybridizations were performed at 42°C overnight in the presence of 0.25 mg of human placental DNA/ml and followed by two 30-min washes with 0.1 × SSC, 0.1% SDS at 60°C.

PCR Amplification

The following $(AC)_n$ markers were typed using previously published primers and conditions: *D16S283* (SM7, Harris et al. 1991); *D16S94* (VK5, Aksentijevich et al. 1993*b*); *D16S523* (13H1, Shen et al. 1993*a*); *D16S453* (301G12, Shen et al. 1993*b*); *D16S63* (CRI 0327, E. N. Levy and D. L. Kastner, unpublished information); and *D16S423* (AFM249yc5, Weissenbach et al. 1992). For each marker, one primer was end-labeled with γ-^{32}P-ATP by using polynucleotide kinase (New England Biolabs), and PCR amplification was carried out in a 10-μl volume containing 50 ng of genomic DNA.

Linkage Analysis

Pairwise LOD scores were calculated using the LINKAGE package of programs (Lathrop et al. 1984), using an FMF gene frequency and penetrances as described elsewhere (Pras et al. 1992). Recombination fractions were transformed to map distances by Kosambi's formula (Kosambi 1944).

Table 1

FMF Families

Ethnic Background	No. of Families	No. of Individuals	No. of Affecteds	No. of Informative Meioses
Jewish:				
Moroccan	18	108	47	78
Non-Moroccan	29	188	96	144
Total	47	296	143	222
Armenian	13	68	25	44
Arab	5	40	18	28
Grand total	65	404	186	294

Multipoint LOD scores were calculated using the HOMOZ program (Kruglyak et al. 1995). This program permits multipoint linkage analysis for both consanguineous and nonconsanguineous nuclear families, for a dense map of highly polymorphic markers. For the seven extended families in our panel, the pedigrees were broken into separate nuclear families. There were no recombinants in these families.

Allelic Associations and Haplotype Analysis

Parental alleles associated with disease susceptibility were identified in each pedigree. In consanguineous families, in which *MEF* and the flanking chromosomal region were derived from a single ancestral chromosome, the disease-associated allele was counted only once. Control allele distributions were determined from the noncarrier chromosomes in the same families. Haplotypes were inferred so as to minimize recombinants. The significance of allelic associations at single loci was determined by the χ^2 statistic for multiallelic loci (Hill 1975; Weir and Cockerham 1978), as described elsewhere (Aksentijevich et al. 1993*a*). We used the Bonferroni procedure (Weir 1990) to adjust for multiple comparisons.

Estimating Genetic Distances by Linkage Disequilibrium

The Luria-Delbrück analysis of bacterial mutations has been adapted to the study of linkage disequilibrium in human founder populations, as described elsewhere (Hästbacka et al. 1992; Lehesjoki et al. 1993; Mitchison et al. 1995). In brief, this method assumes that most present-day carrier chromosomes are descended from a single founder chromosome that has been expanding exponentially in number. For diastrophic dysplasia, the growth rate, d, of carrier chromosomes was estimated by $n = n_0e^{gd}$ (Hästbacka et al. 1992), where n is the total number of chromosomes in the present-day Finnish population, n_0 is the total number of chromosomes in the founding population, and g is the number of generations since the population was founded. Israeli census data allow us to estimate n as 10^6 (most Moroccan Jews emigrated to Israel in the 1950s), and historical accounts set g at 100 generations (assuming 20 years/generation), but there are insufficient historical data to allow an informed estimate of n_0. We therefore estimated d by simulating the expansion of a single founder chromosome carrying the mutation to N present-day *MEF* chromosomes (see below).

The fraction of FMF carrier chromosomes descended from a common ancestor is α, which is given by $\alpha = 1 - m/q$ (Mitchison et al. 1995). q is the gene frequency of the FMF gene in the Moroccan Jewish population, which, under Hardy-Weinberg equilibrium, is .038 (based on the total number of Moroccan Jewish cases at the central referral clinic for FMF in Israel). m is the likely number of *MEF* chromosomes carrying new mutations, which is given by $m = (\mu/d)\ln(10^6\mu/d)$, where μ, the average mutation rate for *MEF*, was taken as 5×10^{-6}/generation (Hästbacka et al. 1992). The quantity $\alpha\pi$ represents the fraction of *MEF* chromosomes descended from a common ancestor that have not had a recombination between *MEF* and a given marker locus.

The quantity $\alpha\pi$ can be estimated by the statistic p_{excess} (Lehesjoki et al. 1993), which is given by

$$p_{excess} = (p_{affected} - p_{normal})/(1 - p_{normal}) , \quad (1)$$

where $p_{affected}$ is the allele frequency of the *MEF*-associated allele at a given locus among FMF carrier chromosomes and p_{normal} is the frequency of the same allele among noncarrier chromosomes. Thus, following Hästbacka et al. (1992), we calculated iteratively the value of θ between *MEF* and marker loci by the equation

$$1 - \pi_{obs} = (\theta/d)\ln(38{,}000\theta/d) \pm 2(\theta/d) , \quad (2)$$

where $\pi_{obs} = p_{excess}/\alpha$ and 38,000 is the number of *MEF* chromosomes in the current Moroccan Jewish population.

Simulations

The growth of the disease population was modeled as a Poisson branching process (Kaplan et al. 1995). Starting with a single disease chromosome, we simulated the number of disease chromosomes in subsequent generations on which no recombination events between the marker and the disease have occurred, by choosing the number $X(t + 1)$ in generation $t + 1$ from a Poisson distribution with mean $(1 + d)(1 - \theta)X(t)$, where $X(t)$ is the number in generation t. The number of disease chromosomes on which a recombination event has occurred was generated similarly, with θ in place of $(1 - \theta)$. One hundred thousand simulations were carried out for each of a range of d and θ values. The simulations were run for 100 generations. We then recorded the total number of disease chromosomes, as well as the proportion of chromosomes on which no recombination between the marker and the disease has occurred, in the final generation (today's population).

In the current population, roughly 40,000 FMF chromosomes are observed, and 82% of these appear not to have undergone a recombination event with *D16S246*. We assumed that simulation outcomes within the range of 20,000–60,000 disease chromosomes and 80%–84% unrecombined chromosomes were consistent with these observations, although the actual choice of ranges does not significantly affect the results (unpublished data). We therefore took the number of simulations that fall in this range to be a measure of likelihood of the values of d and θ that were used in that set of simulations. The best joint estimate of d and θ was obtained by running simulations for d values between .05 and .17 and θ values between 0 and .01 and choosing the combination of d and θ that yielded the most simulations within the acceptable range. The 1-and 2-LOD-unit bounds were obtained by looking for parameters that gave 10-fold and 100-fold fewer simulations, respectively, within the acceptable range than the best joint estimate.

Results

Linkage Analysis

We genotyped a panel of 65 families (table 1) for eight markers mapped to the area of interest on chromosome 16p (fig. 1). Table 2 summarizes sex-averaged and sex-specific pairwise LOD scores, broken down by ethnic group. All markers demonstrated linkage to *MEF*. Maximal LOD scores over all families ranged from 14.56 to 40.7. LOD scores >3 were obtained for at least one marker in each of the ethnic groups we studied, confirming that the FMF susceptibility gene is encoded on 16p in both Jewish and non-Jewish populations.

Multipoint linkage data, calculated with the HOMOZ program (Kruglyak et al. 1995), are shown in figure 2. This program permitted the computation of multipoint LOD scores for 65 families that included 10 first-cousin and 3 uncle-niece marriages, using eight markers as fixed points simultaneously. The maximal LOD score of 49.2 occurs ~1.6 cM centromeric to *D16S246*, and the 1-LOD-unit support interval lies entirely within the 4.1 cM between *D16S246* and *D16S523*.

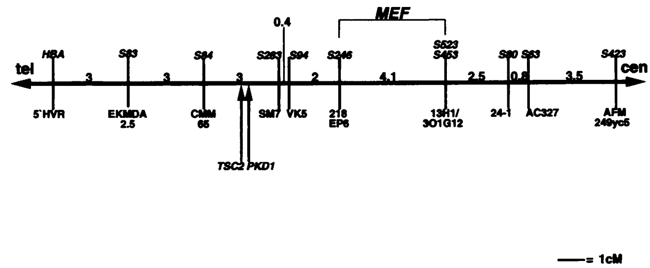

Figure 1 Map of polymorphic markers on chromosome 16p. Sex-averaged map distances are indicated between markers and are based on CEPH data (Kozman et al. 1995) or are estimated from the present study.

Analysis of Recombinant Families

Table 3 depicts each individual with at least one recombination event for the markers under study. Two individuals, 72-05 and 203-10, were recombinant from *HBA* (5'HVR) to *D16S246* (218EP6). Individual 203-10 was nonrecombinant at *D16S523* (13H1), whereas 72-05 was uninformative at this locus but nonrecombinant at informative loci centromeric to *D16S523*. This analysis places *MEF* centromeric to *D16S246*.

Conversely, individuals 25-06 and 25-07 both showed paternal recombinations at *D16S453* (301G12) and *D16S80* (24-1) but not at *D16S246*, placing *MEF* telomeric to *D16S453*. These recombinations were inferred on the basis of homozygosity mapping. Individual 25-06 is the single affected child of an uncle-niece marriage. He was homozygous for *D16S246* and more telomeric markers but was heterozygous for *D16S453* and more centromeric markers. Individual 25-07 is one of two unaffected children of the same marriage. This person was heterozygous for *D16S246* and more telomeric markers but homozygous for *D16S453* and more centromeric loci. The other unaffected child in this consanguineous family was heterozygous across the entire region.

Four other individuals (13-10, 112-13, 121-03, and 144-07) were recombinant at *D16S523*. Although they were uninformative at *D16S246*, their typings were consistent with the placement of *MEF* telomeric to *D16S523/D16S453*. Definite placement of *D16S523* telomeric to *D16S453* was not possible on the basis of our families alone, but CEPH data support that marker order (Kozman et al. 1995).

Table 2

Pairwise LOD Scores between *MEF* and Chromosome 16p Markers

Marker (locus) [heterozygosity] and Ethnic Group	LOD Scores at θ =						Maximal LOD Score	$\hat{\theta}_f$	$\hat{\theta}_m$
	.00	.01	.02	.05	.10	.15			
D16S283 (SM7) [.69]									
Jewish	32.88	35.20	35.22	33.70	29.77	25.28			
Armenian	−.57	0.56	1.01	1.53	1.64	1.46			
Arab	4.29	4.15	4.02	3.64	3.01	2.42			
Total	36.60	39.91	40.25	38.87	34.42	29.16	40.70	.006	.032
D16S94 (VK5) [.51]									
Jewish	16.04	17.02	17.00	16.18	14.14	11.89			
Armenian[a]	...	...	...	...	...	...			
Arab	3.02	2.93	2.84	2.57	2.13	1.71			
Total	19.06	19.95	19.84	18.75	16.27	13.60	19.96	.014	.011
D16S246 (218EP6/*Pvu*II) [.47]									
Jewish	20.11	21.04	20.74	19.39	16.73	13.94			
Armenian	−.20	.13	.30	.53	.62	.58			
Arab	3.16	3.10	3.04	2.84	2.47	2.06			
Total	23.07	24.27	24.08	22.76	19.82	16.58	24.47	.001	.019
D16S523 (13H1) [.72]									
Jewish	22.66	24.26	24.40	23.47	20.71	17.47			
Armenian	2.74	3.32	3.44	3.38	2.97	2.46			
Arab	1.58	1.55	1.50	1.39	1.17	.92			
Total	26.98	29.13	29.34	28.24	24.85	20.85	30.03	.001	.034
D16S453 (301G12) [.29]									
Jewish	18.00	18.66	18.54	17.56	15.32	12.85			
Armenian	.81	1.02	1.10	1.14	1.00	.79			
Arab[a]	...	...	...	...	...	...			
Total	18.81	19.68	19.64	18.70	16.32	13.64	19.94	.001	.027
D16S80 (24-1/*Taq*I) [.34]									
Jewish	7.99	11.78	12.58	12.96	11.96	10.35			
Armenian	−1.07	−.78	−.61	−.35	−.16	−.07			
Arab	1.59	1.54	1.49	1.34	1.09	.86			
Total	8.51	12.54	13.46	13.95	12.89	11.14	14.56	.001	.071
D16S63 (16AC327) [.92]									
Jewish	13.26	22.01	23.93	25.23	23.65	20.63			
Armenian	−1.14	−.01	.36	.92	1.26	1.29			
Arab	1.52	2.81	2.97	2.98	2.63	2.18			
Total	13.64	24.81	27.26	29.13	27.54	24.10	29.37	.033	.079
D16S423 (AFM249yc5) [.74]									
Jewish	−3.61	9.72	12.84	16.26	16.83	15.40			
Armenian[a]	...	...	...	...	...	...			
Arab[a]	...	...	...	...	...	...			
Total	−3.61	9.72	12.84	16.26	16.83	15.40	16.94	.089	.088

[a] This ethnic group was not genotyped for this marker.

In summary, the most likely position of *MEF* is within the interval flanked by *D16S246* and *D16S523*. There were two definite and four possible recombinants (7-06, 38-07, 125-05, and 213-05) at *D16S246*. Moreover, there were four definite and three possible recombinants (25-06, 25-07, and 102-05) at *D16S523*.

Allelic Associations

We have determined the allele distributions for marker loci among FMF carrier and noncarrier chromosomes, for Moroccan Jews, non-Moroccan Jews, Armenians, and Arabs (table 4). Adjusting for multiple comparisons by the Bonferroni procedure, a p value of .002 is statistically significant.

The data confirm, in a larger panel of families, our previous observation of a founder effect in the Moroccan Jewish population (Aksentijevich et al. 1993*a*). There was an overrepresentation of the 93-bp allele for *D16S283* in Moroccan Jews but not in non-Moroccan Jews, Armenians, or Arabs.

Moving toward *MEF*, the 86-bp allele for *D16S94* was overrepresented both in the Moroccan Jews and in

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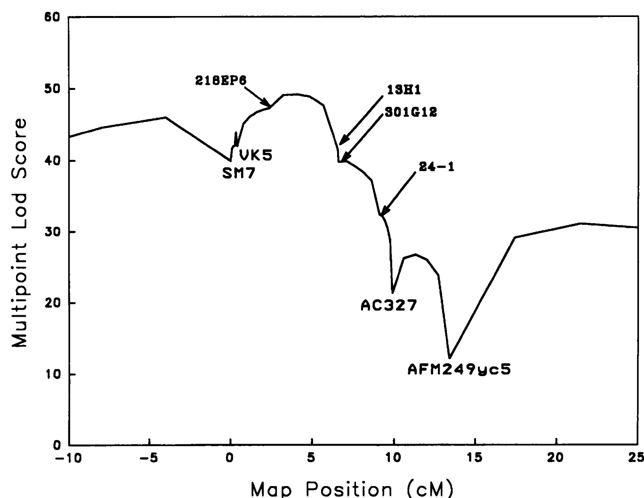

Figure 2 Multipoint linkage analysis between *MEF* and markers on chromosome 16p for 65 families. SM7 (*D16S283*) was arbitrarily assigned position 0; multipoint LOD scores were calculated at each of the marker loci and at four equally spaced points within each intermarker interval. The maximal multipoint LOD score was obtained 1.6 cM centromeric to 218EP6 (*D16S246*).

the non-Moroccan Jews. In light of the high frequency of the 86-bp allele in the noncarrier chromosomes, these associations did not reach significance.

At *D16S246,* the distal flanking marker for the *MEF* interval, the 2.5-kb *Pvu*II fragment was significantly associated with FMF susceptibility in both the Moroccan and non-Moroccan Jewish populations. The 2.8-kb and 2.4-kb alleles for *D16S246* were also overrepresented in the Armenian and Arab populations, respectively, although these associations were not statistically significant, perhaps because of the small sample size.

The Moroccan Jewish carrier chromosomes were also notable for a modest increase in the frequency of the 83-bp and 125-bp alleles of *D16S523* and *D16S453,* respectively, although these associations did not reach significance. Taken together, the linkage disequilibrium data suggest that *MEF* is located closer to *D16S246,* at the telomeric end of the candidate interval.

Haplotype Analysis

We also performed haplotype analysis on the carrier and noncarrier chromosomes deduced in each ethnic group. Among the Moroccan Jewish population, the haplotype comprised of the 93-bp allele at *D16S283,* the 86-bp allele at *D16S94,* and the 2.5-kb fragment at *D16S246* was observed in 76% (26/34) of carrier chromosomes, but in only 11% (4/36) of noncarrier chromosomes. The 93-86-2.5 haplotype was also seen in 32% (19/59) of non-Moroccan carrier chromosomes and 6% (3/53) of non-Moroccan noncarrier chromosomes.

We found no evidence for overrepresentation of this founder chromosome in either the Armenian or Arab population. The 93-86-2.5 haplotype was observed in 2/29 Armenian carrier chromosomes and in 2/26 Armenian noncarrier chromosomes. This haplotype was not observed in 14 Arab carrier chromosomes or 8 Arab noncarrier chromosomes. Moreover, no other haplotype was significantly overrepresented among Armenian or Arab carrier chromosomes.

Estimating Genetic Distances by Linkage Disequilibrium

The haplotype data strongly support the view that a high proportion of Moroccan Jewish FMF carrier chromosomes descend from a common ancestor, a critical assumption in applying Luria-Delbrück equations to the study of linkage disequilibrium. For individual marker loci, the statistic p_{excess}, calculated from allele frequencies in affected and normal chromosomes, approximates $\alpha\pi$, where α is the proportion of chromosomes that descend from the common ancestor and π is the proportion of disease-causing chromosomes descended from the common ancestor that have not undergone recombination between *MEF* and the marker locus (Lehesjoki et al. 1993). Among the markers we studied, p_{excess} was highest at *D16S246,* with a value of .82. For all values of d (see below), α was calculated to be >.99, and thus π_{obs} is .82 for *D16S246.*

Once π_{obs} is determined, an estimate of the recombination fraction θ, as well as confidence bounds, may be obtained either from the Luria-Delbrück equations (Hästbacka et al. 1992, 1994) or from simulations (Kaplan et al. 1995). In both cases, two parameters are important: g, the number of generations since the original mutation, and d (denoted as λ by Kaplan et al. [1995]), the growth rate of the number of disease chromosomes.

The parameter g is usually estimated from historical records. Although a minimal estimate for the age of the Moroccan Jewish population is 500 years (based on the last major migration of Jews from Spain and Portugal to Morocco in 1492), a more ancient founding date appears more appropriate. Jewish traders are known to have settled in both Spain and Morocco in Biblical times (Roth 1972), and at the Council of Bishops in Spain in 301 A.D. there were decrees forbidding Christians from associating with the local Jewish population (Goodman 1979). Since FMF is seen today in other Jewish populations that remained in the Middle East, it is likely that it was brought by an ancient settler, rather than having arisen anew in Spain or Morocco. Religious persecutions over the centuries forced the migration of the larger Spanish Jewish community to Morocco, among other places. Since the 93-86-2.5 haplotype can be seen in non-Moroccan FMF patients descended from Spanish ancestors, the mutation associated with this haplotype

Table 3

Recombinations between *MEF* and Chromosome 16p Loci

Individual	Status[a]	Recombinant[b]	*HBA* 5′HVR	*S83* EKMDA	*S84* CMM65	*S291* AC2.5	*S283* SM7	*S94* VK5	*S246* 218EP6	*S523* 13H1	*S453* 301G12	*S80* 24-1	*S63* O327	*S423* AFM249
7-06	U	P	?	+	+	+	+	+	?	–	?	?	?	–
10-06	A	P	+	?	–	–	–	?	–	?	?	?	–	–
10-06	A	M	–	?	?	–	–	?	?	–	–	?	–	+
11-03	A	P	–	–	–	?	–	?	?	–	–	?	–	+
13-10	A	P	–	–	–	–	–	?	?	+	?	+	+	+
15-06	A	M	?	?	+	+	+	+	–	–	?	?	–	–
21-05	A	M	?	–	–	–	–	?	?	?	?	?	+	+
23-09	A	P	+	+	?	–	–	–	–	–	–	–	–	–
25-06	A	P	–	–	–	–	–	?	–	?	+	+	?	+
25-07	U	P	–	–	–	?	–	?	–	?	+	+	?	+
27-12	A	P	–	?	?	–	–	–	–	?	?	+	+	+
31-05	A	M	–	–	–	–	–	?	–	–	?	?	–	+
33-04	A	P	+	?	+	+	+	?	–	?	?	–	–	–
35-02	A	P	?	–	?	–	–	–	?	?	–	?	–	+
38-07	U	M	+	?	+	+	+	?	?	–	?	–	–	?
42-05	A	P	–	–	–	–	?	?	?	–	–	–	+	?
46-06	A	P	?	?	?	?	?	–	?	?	?	?	?	+
62-06	A	P	–	?	–	?	?	?	–	?	?	?	+	+
72-05	A	P	+	?	+	+	+	?	+	?	?	?	–	–
75-05	A	P	+	–	–	–	–	–	–	–	?	?	–	?
102-05	A	P					–	–	?	?	?	+	+	+
104-11	U	M					–	?	?	–	?	–	–	+
112-13	A	P					?	?	?	+	?	+	+	+
117-03	A	M					–	?	–	–	?	–	+	+
121-03	A	P					–	?	?	+	+	?	+	+
121-03	A	M					–	?	?	–	–	?	–	+
125-05	A	P					?	+	?	–	–	–	–	–
203-10	U	P	+	?	?	+	+	?	+	–	–	?	–	
203-07	U	M	–	?	?	?	–	?	–	–	–	?	+	
213-05	U	P	+	?	?	+	+	?	?	–	–	–	–	
218-03	A	P	+	?	?	–	?	?	–	?	?	?	–	
144-07	A	M					–	–	?	+	?	?	+	

NOTE.—Data for *HBA* (5′HVR), *D16S83* (EKMDA), *D16S84* (CMM65), and *D16S291* (AC2.5) are from previous reports (Aksentijevich et al. 1993*b;* Fischel-Ghodsian et al. 1993). Families numbered up to 125 are Jewish. Families 203, 213, and 218 are Armenian. Family 144 is Arab. A plus sign (+) = recombinant between *MEF* and the marker in question; a minus sign (–) = nonrecombinant between *MEF* and the marker in question; a question mark (?) = uninformative; and a blank = not done. Recombinants place *MEF* between *D16S246* (218EP6) and *D16S523* (13H1).

[a] U = unaffected individual; and A = affected individual.

[b] P = paternal recombinant; and M = maternal recombinant.

Table 4

Allelic Associations between *MEF* and Chromosome 16p Flanking Markers

Locus	Population	Allele	Carrier Chromosomes	Noncarrier Chromosomes	χ^2	*p* (df)
D16S283 (SM7)	Moroccan	93 bp	27/35 (77%)	5/35 (14%)	32.03	.0014 (12)[a]
	Non-Moroccan	91 bp	26/61 (43%)	25/55 (46%)	17.16	.14 (12)
	Armenian	91 bp	15/31 (48%)	11/29 (38%)	6.62	.88 (12)
	Arab	91 bp	6/13 (46%)	5/8 (63%)	2.37	.99 (12)
D16S94 (VK5)	Moroccan	86 bp	27/33 (82%)	19/35 (54%)	7.28	.20 (5)
	Non-Moroccan	86 bp	46/55 (84%)	34/53 (64%)	7.97	.16 (5)
	Arab	88 bp	7/12 (58%)	3/8 (38%)	3.61	.61 (5)
D16S246 (218EP6)	Moroccan	2.5 kb	30/33 (91%)	17/35 (49%)	14.44	.00073 (2)[a]
	Non-Moroccan	2.5 kb	50/56 (89%)	30/53 (57%)	14.89	.00058 (2)[a]
	Armenian	2.8 kb	14/31 (45%)	6/28 (21%)	3.70	.16 (2)
	Arab	2.4 kb	5/13 (39%)	1/8 (13%)	1.78	.41 (2)
D16S523 (13H1)	Moroccan	83 bp	24/35 (69%)	19/35 (54%)	3.67	.72 (6)
	Non-Moroccan	83 bp	31/57 (54%)	30/54 (56%)	3.79	.71 (6)
	Armenian	83 bp	16/31 (52%)	19/29 (66%)	7.47	.28 (6)
	Arab	77 bp	6/13 (46%)	4/8 (50%)	.73	.99 (6)
D16S453 (301G12)	Moroccan	125 bp	28/34 (82%)	20/35 (57%)	5.52	.063 (2)
	Non-Moroccan	125 bp	36/56 (65%)	42/52 (81%)	6.61	.037 (2)
	Armenian	125 bp	23/31 (74%)	18/29 (62%)	2.43	.30 (2)
D16S80 (24-1)	Moroccan	3.8 kb	25/36 (69%)	25/35 (71%)	.04	.98 (2)
	Non-Moroccan	3.8 kb	34/51 (67%)	38/47 (81%)	4.12	.13 (2)
	Armenian	3.8 kb	26/28 (93%)	21/24 (88%)	.56	.76 (2)
	Arab	3.8 kb	9/12 (75%)	6/8 (75%)	.83	.66 (2)
D16S63 (16AC327)	Moroccan	229 bp	13/36 (36%)	4/35 (11%)	16.53	.28 (14)
	Non-Moroccan	229 bp	25/63 (40%)	12/57 (21%)	18.17	.20 (14)
	Armenian	225 bp	9/31 (29%)	4/31 (13%)	19.87	.13 (14)
	Arab	229 bp	5/13 (39%)	0/8 (0%)	12.52	.56 (14)
D16S423 (AFM249)	Moroccan	127 bp	10/34 (29%)	12/31 (39%)	10.34	.67 (13)
	Non-Moroccan	129 bp	15/62 (24%)	10/55 (18%)	19.07	.12 (13)

[a] Statistically significant at the .05 level, adjusted for multiple comparisons by the Bonferroni procedure.

probably dates to the ancient Spanish Jewish community. Therefore, we estimated the population age as 2,000 years, with $g = 100$ (assuming 20 years/generation).

Estimating d is less straightforward. In general, $d = r + s$, where r is the growth rate of the population and s is the possible selective advantage of carriers over normals (Kaplan et al. 1995). If we assume a single founder chromosome carrying the mutation, and if there are N disease chromosomes in the present-day population, d may be estimated from $N = (1 + d)^g/2d$ (Kaplan et al. 1995). Simulations show that this estimate of d has high variance. In the case of FMF, if we assume $N = 38{,}000$ and $g = 100$, the best estimate of d was .091, but values in the range of .07 to .13 were within 1 LOD unit, and values in the range of .06 to .16 were within 2 LOD units. In the absence of additional information about the value of d, we decided to estimate d and θ jointly in our simulations. We show below that the choice of d has a clear effect on estimates of θ.

If we assume no selective advantage for carriers, d is equal to r, the growth rate of the population. In this case d may be estimated from $n = n_0(1 + d)^g$, where n_0 is the total number of chromosomes in the founding population and n is the number in the present-day population, provided n_0 is not too small (Kaplan et al. 1995; for practical purposes, $n_0 d \gg 1$). However, the size of the original population is usually unknown and can only be estimated roughly from historical records. Alternatively, once d is determined from the growth of the FMF chromosomes as above, this relation may be used to estimate the total size of the founding population. For example, in the absence of selection, a d value of .091 corresponded to a founding population of 83, on the basis of 100 generations and a present-day population of 500,000 (10^6 chromosomes). In light of the relatively high gene frequency in affected populations, it is possible that a selective advantage to carriers exists for FMF; that would affect the estimates of the founding population size (moving them upward) but not the estimates of θ.

We estimated the recombination fraction θ between *MEF* and *D16S246* assuming a single ancestral disease

Table 5

Estimation of the Genetic Distance between *MEF* and *D16S246*, on the Basis of Luria-Delbrück Calculations for the Moroccan Jewish Population

d[a]	$n_0/2$[b]	θ[c]	Range[d]
.060	1,474	.00151	.00120–.00201
.070	576	.00177	.00140–.00236
.091	83	.00232	.00185–.00310
.130	...[e]	.00335	.00267–.00447
.160	...[e]	.00415	.00330–.00552

[a] Growth rate of the no. of disease chromosomes, estimated by simulating the expansion of one founder chromosome to 38,000 present-day *MEF* chromosomes, assuming g = no. of generations = 100.

[b] No. of individuals in founding population, calculated by $n = n_0(1 + d)^g$, assuming n = total no. of present-day chromosomes = 10^6, $g = 100$, and no selective advantage for *MEF*.

[c] Moment estimate for θ, by solving for θ:

$$1 - p_{\text{excess}}/\alpha = (\theta/d)\ln[38{,}000(\theta/d)] \, .$$

[d] Range for θ, by solving for θ:

$$1 - p_{\text{excess}}/\alpha = (\theta/d)\ln[38{,}000(\theta/d)] \pm 2(\theta/d) \, .$$

[e] In the absence of selection, the value is sufficiently small that an accurate estimate is not possible.

chromosome 100 generations ago that expanded to the currently observed 38,000 disease chromosomes. Table 5 gives estimates of θ from the Luria-Delbrück formulas. Calculations were based on the maximum-likelihood estimate of d (.091) as well as the 1-LOD-unit and 2-LOD-unit ranges for d. Based on the assumption of no selective advantage for FMF, the values of $n_0/2$ (the number of individuals in the founding population) corresponding to each value of d are also shown; higher values of d led to implausible n_0's, unless there is selection for FMF. For $d = .091$, the moment estimate for θ was .00232 (.232 cM), and the two-sigma bounds for θ were .00185–.00310 (.185–.310 cM). The Luria-Delbrück bounds broadened if the 2-LOD-unit range of d values was considered.

Figure 3 shows the results of simulations, under the same assumptions as the Luria-Delbrück analysis, estimating d and θ jointly. Our best joint estimate was $d = .091$, $\theta = .00305$ (.305 cM). The 1-LOD-unit range for d was .07–.13, and the 2-LOD-unit range was .06–.16, although, in the absence of selection, the higher values of d may be unlikely for historical reasons, as noted above. The 1-LOD-unit range for θ was .001–.005 (.1–.5 cM), and the 2-LOD-unit range was .0002–.0064 (.02–.64 cM). For any given d, the 2-LOD-unit range for θ by simulations was broader than the two-sigma range by Luria-Delbrück formulas (comparing table 5 with fig. 3), as previously noted by Kaplan et al. (1995).

We also investigated the effect of varying g on estimates of θ. We performed calculations for $g = 75$ and $g = 125$, corresponding to founding populations living 1,500 and 2,500 years ago, respectively. For $g = 75$, the maximum-likelihood estimate for d was .13. As expected, this resulted in higher estimates for θ; Luria-Delbrück formulas produced an estimate of .00335 (two-sigma range .00267–.00447), and simulations yielded an estimate of .004 (2-LOD-unit range .0002–.008). For $g = 125$, the maximum-likelihood estimate for d was .07, yielding a Luria-Delbrück estimate for θ of .00177 (two-sigma range .00140–.00236), and a Poisson-based estimate of .0024 (2-LOD-unit range .0001–.0052).

Discussion

This report represents the first attempt to assemble a panel of FMF families from several affected ethnic groups, for the purpose of refined recombinational mapping and linkage disequilibrium studies. The present data support the view that a single locus causes FMF in the Jewish, Armenian, and Arab populations. Pooling data from all 65 families, we observed recombinants that place the gene in the 4.1-cM (sex-averaged) interval between *D16S246* and *D16S523*.

Earlier studies of several consanguineous families in our panel have indicated a high rate of homozygosity

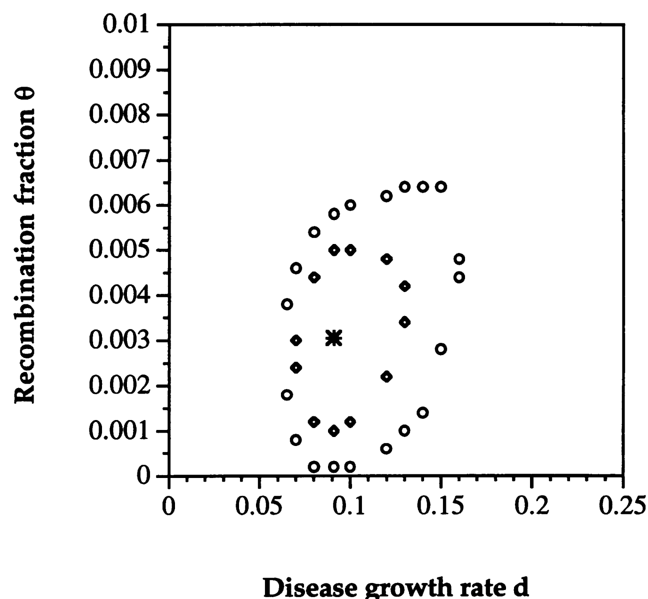

Figure 3 Plot of the best joint estimate of population growth rate, d, and recombination fraction, θ, as well as the 1-LOD-unit and 2-LOD-unit bounds, as obtained by simulations. The asterisk (*) indicates the best estimate on the d-θ plane. The diamonds mark the contour of the one-LOD dropoff, and the circles mark the contour of the two-LOD drop-off. See text for details.

for markers in this region of chromosome 16p, but the analysis of such families has been of limited value in the refined mapping of *MEF* because of computational constraints and the limited polymorphism of some of the markers in the region. The HOMOZ program, which uses a newly described algorithm for the analysis of recessive diseases in nuclear families (Kruglyak et al. 1995), allowed us to calculate multipoint LOD scores between *MEF* and eight polymorphic markers in this region. Moreover, with an increased density of markers in the region, we were able to identify important recombinants by homozygosity mapping.

Since our present panel of families includes two Jewish subpopulations as well as Armenians and Arabs, it was possible to test whether common alleles or haplotypes are seen in these different groups. For markers sufficiently close to the disease gene, one would expect this to be the case if there was one major ancient FMF mutation. We had previously observed a strong haplotype association among Moroccan Jews (Aksentijevich et al. 1993*a*), and we confirmed this observation in the present study for *D16S283-D16S94-D16S246*. This haplotype was also overrepresented, but to a lesser extent, in the non-Moroccan Jewish population, but was not associated with FMF in Armenian or Arab populations. Moreover, the closest flanking locus in this haplotype, *D16S246,* showed a highly significant association of the 2.5-kb allele among both Moroccan and non-Moroccan Jews. Different alleles were increased in frequency among the Armenians and Arabs, although not to the level of statistical significance. Such data suggest that the same mutation may be found in a large percentage of Moroccan and non-Moroccan Jewish FMF patients but that other mutations may be found in Armenians and Arabs. The higher frequency of the ancestral haplotype in present-day Moroccan Jews, relative to non-Moroccans, may be due to several factors, including consanguinity, bottlenecks, and the smaller size and genetic isolation of the Moroccan Jewish community.

Since there is good evidence that most Moroccan Jewish FMF carrier chromosomes are descended from a common ancestor, we applied Luria-Delbrück formulas and related simulations to the Moroccan linkage disequilibrium data. In assessing whether these analyses would be appropriate in the present case, we considered whether certain basic assumptions are satisfied. Our Moroccan haplotype data were consistent with the requirement that most disease-causing chromosomes be descended from a common ancestor, and the genetic isolation of the Moroccan Jewish population (Roth 1972) has favored founder effects (Navon and Proia 1991). Moreover, the estimated population age of 2,000 years is old enough for the region of strongest linkage disequilibrium to be relatively small. Finally, we have shown elsewhere that the Moroccan Jewish families in our panel represent a random sampling of the population, in that they trace their ancestry to communities encompassing most of the habitable areas of Morocco, and none of the families have any known recent common ancestry with any other family in the panel (Aksentijevich et al. 1993*a*).

Genetic distances calculated by Luria-Delbrück formulas or simulations vary inversely with the age of the population. Our estimated population age of 2,000 years is supported by historical data placing Jews in the western Mediterranean early in the Christian era and haplotype data suggesting that many Moroccan and non-Moroccan FMF carrier chromosomes are descended from a common ancestral chromosome in pre-Inquisition Spain. Unfortunately, it has not been possible to determine the relative proportions of our Moroccan Jewish families descended from the various migrations from Spain that are known to have occurred over the past 2,000 years. We therefore estimated θ for a range of possible population ages.

There are other potential sources of error. The high rate of consanguinity in the Moroccan Jewish population may increase estimates of linkage disequilibrium (and thereby decrease estimates of genetic distance) by obscuring recombination. High rates of mutation at marker loci would tend to have the opposite effect (Hästbacka et al. 1992), but we have not observed any new mutations at any of the marker loci we have examined in our 65 families, and under usual circumstances mutation at marker loci does not appreciably affect estimates of θ (Kaplan and Weir 1995). If a substantial percentage of FMF chromosomes descend from individuals who immigrated to Morocco (from countries other than Spain) during the past 2,000 years, we may have overestimated α, creating inflated estimates of genetic distance. Finally, although simulations may accurately represent evolutionary processes, this approach does not entail statistical sampling from populations, and therefore the variation in the resulting estimates may be reduced (see also Kaplan and Weir 1995).

The present analysis has avoided other potential sources of error. For many of the applications of Luria-Delbrück formulas to the Finnish population, the rate of growth d of the number of disease chromosomes has been estimated from r, the growth rate of the population. However, for Moroccan Jews there are insufficient historical data to estimate n_0, the total number of founder chromosomes, and so we have derived values of d based solely on the expansion of a single founder FMF chromosome to the present-day 38,000 Moroccan Jewish FMF chromosomes. The corresponding values of n_0 can then be calculated.

Moreover, we have used both Luria-Delbrück formulas and Poisson-branching simulations to estimate θ (be-

tween *MEF* and *D16S246*) from the Moroccan Jewish population. The estimates obtained by the two methods were very similar, although the Luria-Delbrück estimates were more sensitive to the choice of *d*. As noted elsewhere (Kaplan et al. 1995; Kaplan and Weir 1995), two-sigma Luria-Delbrück bounds are narrower than the 2-LOD-unit confidence interval identified by simulation, which more closely reflects the 95% confidence region for the gene's location.

Based on the genome-wide average of 1 cM = 1 Mb, our analysis of the Moroccan Jewish population suggests that *MEF* is within 640 kb of *D16S246*. However, it is important to emphasize that the strong linkage disequilibrium at *D16S246* in both Moroccan and non-Moroccan Jews would place *MEF* close to this marker, even in the absence of Luria-Delbrück analysis. We are now constructing a YAC/cosmid contig extending from *D16S246* into the FMF candidate region. With the eventual identification of the FMF susceptibility gene, it will be possible to test the validity of Luria-Delbrück distance estimates in the Moroccan Jewish population.

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