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Progressive Myoclonus Epilepsy EPM1 Locus Maps to a 175-kb Interval in Distal 21q

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Summary

The EPM1 locus responsible for progressive myoclonus epilepsy of Unverricht-Lundborg type (MIM 254800) maps to a region in distal chromosome 21q where positional cloning has been hampered by the lack of physical and genetic mapping resolution. We here report the use of a recently constituted contig of cosmid, BAC, and P1 clones that allowed new polymorphic markers to be positioned. These were typed in 53 unrelated disease families from an isolated Finnish population in which a putative single ancestral EPM1 mutation has segregated for an estimated 100 generations. By thus exploiting historical recombinations in haplotype analysis, EPM1 could be assigned to the ~175-kb interval between the markers D21S2040 and D21S1259.

Introduction

Progressive myoclonus epilepsy of Unverricht-Lundborg type (MIM 254800) is an autosomal recessive disorder characterized by stimulus-sensitive myoclonus and tonic-clonic seizures with onset at age 6–15 years (Koskiniemi et al. 1974; Norio and Koskiniemi 1979; Koskiniemi 1990). It occurs at a low frequency in many populations but is, however, believed to be the most prevalent type of progressive myoclonus epilepsy in North America (Berkovic et al. 1986). The underlying biochemical defect is unknown. The gene, EPM1, has been localized by linkage analysis to an ~5-cM region between marker loci CBS and CD18 on chromosome 21q22.3 (Lehesjoki et al. 1991, 1992, 1993*a*). By linkage-disequilibrium mapping, the localization of the EPM1 gene was further narrowed to a 0.6-cM or smaller region around loci D21S25, PFKL, and D21S154 (Lehesjoki et al. 1993*b*).

In the absence of obvious candidate genes for EPM1 located in the region, positional cloning strategies appeared to be necessary in order to find the gene. Before embarking on the work-intensive isolation and characterization of candidate cDNA sequences, it was desirable to narrow as much as possible the region in which the gene must reside. The above mapping data were based mainly on poorly informative RFLP markers. Moreover, part of the EPM1 critical region (i.e., the interval between markers D21S141 and D21S25) totally lacked informative markers and was not represented by intact clones in YAC libraries (Gardiner et al. 1995). In this paper, experiments aimed at higher-resolution genetic mapping of EPM1 are described. A contiguous array of cosmid, BAC and P1 clones covering the region (Stone et al. 1996) allowed us to search for and map suitably spaced highly polymorphic genetic markers. We took advantage of the unique structure of the Finnish population (de la Chapelle 1993) to apply mathematical models of disease-gene mapping by linkage-disequilibrium and haplotype analysis (Hästbacka et al. 1992; Lehesjoki et al. 1993*b*; Terwilliger et al. 1995). We report the results of such experiments and calculations in an expanded series of 53 Finnish EPM1 families that allowed us to considerably refine the assignment of EPM1.

Families, Material, and Methods

Families

A total of 53 unrelated Finnish nuclear families were studied. Of them, 39 had only one affected child, and 14 had more than one affected child. Thirty-eight of the families were included in a previous study (Lehesjoki et al. 1993*b*). From each family, only one affected individual and both parents, if available, were considered in the analysis. In all, the series comprised 73 parents and 53 affected individuals. From each individual studied, informed consent was obtained before a blood sample was drawn. When the genealogies were traced 3–10 generations back, only three consanguineous matings

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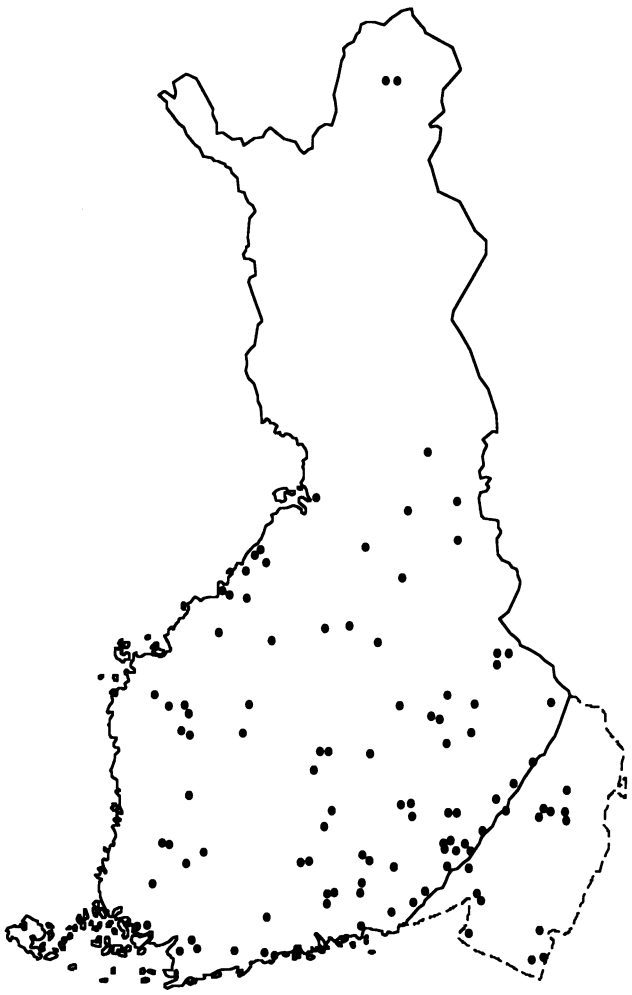

Figure 1 Map of Finland, showing the distribution of birthplaces of the parents of the 53 EPM1 families analyzed.

were detected: in one family, the parents were first cousins once removed, in another, second cousins, and, in another, third cousins. In these families only one disease chromosome was considered in the analysis. The geographical distribution of the birthplaces of the patients' parents is shown in fig. 1.

Microsatellite Repeat Polymorphisms

Five microsatellite $(CA)_n$ repeat polymorphisms were studied: a previously published polymorphism in PFKL (Polymeropoulos et al. 1991), three novel markers developed at Généthon (D21S1885, D21S1259, and D21S1912), and a new repeat described in this study. This $(CA)_n$ polymorphism was detected in cosmid 10E6 by hybridization of a $(GT)_{16}$-probe to cosmid DNA digested with *Eco*RI. The cosmid DNA was then digested with *Sau*3AI and ligated into *Bam*HI-digested pBS-SK vector (Stratagene). The subclone pools were transformed into a competent *Escherichia coli* XL1-blue strain (Stratagene). Positive white colonies were selected by the IPTG-X-gal method and sequenced (ABI, Perkin Elmer). The following primers were designed to amplify a 170-bp fragment from the positive clones: forward: 5′-CGGGCAGCACGCGCGCGCA-3′; reverse: 5′-CAGCCACTCCTCCCACTC-3′. The repeat showed three alleles with the frequencies of .41, .55, and .04, as determined in 105 unrelated chromosomes giving a heterozygosity of 53%. The sequence has been deposited in GenBank (U46013).

To determine polymorphic alleles, PCR reactions were performed as follows: genomic DNA (5–20 ng) was amplified in a 10-μl reaction mix in a buffer provided by the enzyme supplier (Finnzymes Oy), 200 μM dNTPs, 5–10 pmol of each primer end-labeled with [γ-^{32}P]dATP, and 0.1–0.5 U of DynaZyme™ (Finnzymes Oy). The cycling conditions were: 95°C 4 min; 35 cycles at 95°C 30 s, 49°C–55°C 30 s, and 72°C 30 s; 72°C 4 min. The PCR products were fractionated on 6% polyacrylamide, 50% urea sequencing gels in TBE buffer (0.089 M TRIS, 0.089 M boric acid, 0.002 M EDTA). Gels were run for 2–3 h and dried and exposed to X-ray film for 2–48 h. The alleles for D21S1885, D21S2040, D21S1259 and D21S1912 were numbered consecutively, "1" being the largest allele. The numbering of alleles in PFKL was as described by Lehesjoki et al. (1993*b*), and allele 7 is 145 bp in length. The markers used to construct the extended haplotypes are described in Lehesjoki et al. (1993*b*).

Six of the eight marker loci used to construct the extended haplotypes have been mapped to specific *Eco*RI fragments by hybridization (Stone et al. 1996; present study), and the remaining two markers (D21S141 and D21S1885) have been assigned to the 520-kb *Not*I restriction fragment immediately centromeric to marker D21S2040 (authors' unpublished data). The order of the markers is cen–(D21S141, D21S1885)–D21S2040–D21S1259–D21S1912–D21S25–PFKL–D21S 154–tel.

Linkage-Disequilibrium Analysis

To test whether there is a single allele with a significantly higher frequency on disease-bearing chromosomes than on normal chromosomes, we examined each allele separately in search of an increase in frequency. We used a standard one-sided test (Fisher's exact test) and corrected for multiple testing by using a Bonferroni correction, under the assumption that the tests of each of the alleles detected are approximately independent.

The distances between the EPM1 locus and the five markers that showed the strongest linkage disequilibrium were estimated according to earlier assumptions about the EPM1 founder effect and Finnish population genetics, by using the Luria-Delbrück–based method (Hästbacka et al. 1992; de la Chapelle 1993; Lehesjoki

et al. 1993*b*). Our calculations were based on $g = 100$ (the number of generations since the start of expansion of the EPM1 mutation in Finland), $\mu = 7 \times 10^{-6} - 8 \times 10^{-7}$ (the mutation frequency at the EPM1 locus), and $q = .007-.008$ (the EPM1 disease-allele frequency in the Finnish population). The estimate of g is based on the occurrence of the disease in the entire region of early settlement (Norio 1981; de la Chapelle 1993) as defined by the geographical distribution of birthplaces of known carriers, that is, parents of affected individuals (fig. 1). The estimate of μ is based on the rarity of the disease. The disease-allele frequency estimate is based on actual incidence and prevalence figures in Finland (Norio and Koskiniemi 1979). These figures were used to calculate α (the proportion of disease-causing chromosomes descending from the putative common ancestor) by using the formula $\alpha = (1 - \mu g q^{-1})$. Allelic excess was calculated by the following formula: $p_{excess} = (p_{affected} - p_{normal})/(1 - p_{normal})$, where p denotes allele frequency. The theta value was calculated using the formula $p_{excess} = \alpha(1 - \theta)^g$.

A multipoint method of linkage-disequilibrium analysis was applied to the data using the likelihood-based DISMULT program, version 2.1, developed for analyzing data from multiallelic markers (Terwilliger 1995). It uses information from all the marker loci simultaneously and has a built-in location parameter. The calculations are based on the parameter λ, which is equal to the proportion of increase of allele i in the disease chromosomes, relative to its population frequency. In the multipoint method, θ is fixed between any given map position and each of the marker loci, and the likelihood is maximized, at that map position, over α (the proportion of disease-causing chromosomes descending from the common ancestor) and n (the number of generations since founding). In this analysis, an EPM1 disease-allele frequency of .007 was used, and the value of n was set between 20 and 500. Intermarker distances (in kb) were: S1185–300–S2040–176–S1259–276–S1912–143–PFKL (Stone et al. 1996; present study; authors' unpublished data) and were transformed into genetic distances under the assumption of 1 cM = 1 Mb. Most-likely haplotypes were constructed manually, assuming a minimum number of recombinations in each family.

Results

Fine Mapping of EPM1 by Linkage Disequilibrium

Alleles at five microsatellite repeat marker loci on the EPM1-bearing chromosomes of 53 Finnish EPM1 patients were compared with alleles on the normal chromosomes of 73 parents. In the three families where the parents were known to be closely related, only one disease chromosome was considered in the analysis. The results are shown in table 1. Significant linkage disequilibrium was detected for all markers studied. The frequency of the most common allele on the EPM1-bearing chromosomes versus normal chromosomes were: 93%/73% (D21S1885), 96%/58% (D21S2040), 96%/40% (D21S1259), 93%/21% (D21S1912), and 79%/14% (PFKL).

The proportion of disease-causing chromosomes descending from the common ancestor (α) was calculated using the formula $(1 - \mu g q^{-1})$ with different values for μ and q (see Families, Material, and Methods), and $g = 100$. This resulted in values ranging from .95 to .99. These values are concordant with the haplotype data (see below). Table 2 shows p_{excess} and the estimated distance θ for the five loci in linkage disequilibrium with EPM1 under two assumptions about α, .95 and .99. EPM1 appears to lie closest to markers D21S2040, D21S1259, and D21S1912, within 0.091, 0.058, and

Table 1

Allelic Counts in EPM1 and Normal Chromosomes in 53 Finnish Families

Marker/Allele	No. of non-EPM1 Chromosomes	No. of EPM1 Chromosomes	*P* value[a]
D21S1885:			
1	9	2	.0016
2	7	4	
3	52	94	
4	3	1	
D21S2040:			
1	29	4	1.5×10^{-9}
2	43	95	
3	2	0	
D21S1259:			
1	7	1	$\sim 10^{-16}$
2	1	0	
3	9	1	
4	27	97	
5	23	2	
6	1	0	
D21S1912:			
1	9	1	$\sim 10^{-22}$
2	6	4	
3	20	1	
4	15	94	
5	7	1	
6	5	0	
7	4	0	
8	4	0	
PFKL:			
1	0	1	$\sim 10^{-17}$
2	11	81	
3	9	1	
4	44	14	
5	5	2	
6	6	4	
7	2	0	

[a] Fisher's exact test with Bonferroni correction.

Table 2

Estimates of Genetic Distances from Marker Loci to EPM1 as a Function of the Proportion of EPM1-Bearing Chromosomes Descended from a Putative Common-Founder Chromosome

		Genetic Distance (θ) to EPM1 (cM)			
Locus	p_{excess} ± 1 SD[a]	α = .95	±1 SD[a]	α = .99	±1 SD[a]
D21S1885	.740 ± .055	.248	.176–.326	.289	.217–.367
D21S2040	.904 ± .064	.050	0–.123	.091	.023–.164
D21S1259	.934 ± .072	.017	0–.097	.058	0–.138
D21S1912	.912 ± .077	.041	0–.129	.082	.001–.171
PFKL	.751 ± .080	.235	.134–.347	.276	.175–.388

[a] Standard deviation based on sampling error for p_{excess} and corresponding theta values.

0.082 cM, respectively. Table 2 also shows the standard deviations based on sampling error for p_{excess} and theta values.

The results of the likelihood-based multipoint linkage-disequilibrium analysis are shown in figure 2. We analyzed marker loci D21S1885, D21S2040, D21S1259, D21S1912, and PFKL simultaneously. The maximum likelihood–ratio value on a lod unit scale was 62.13 and was obtained at ~0.08 cM distal to locus D21S1259. The 99% confidence interval for this estimate, calculated as Z_{max} ± 3 lod units, spans ~0.8 cM around the locus D21S1259, extending ~0.46 cM proximal and ~0.34 distal. On the physical scale, the 0.8-cM interval corresponds to a distance of 800 kb, on the assumption that 1 cM = 1 Mb.

Haplotype Analysis

Haplotypes for the markers D21S1885, D21S2040, D21S1259, D21S1912, and PFKL were determined in 88 EPM1 and 62 normal chromosomes (data on normal chromosomes not shown). The different haplotypes on EPM1 chromosomes are shown in table 3. The ancestral

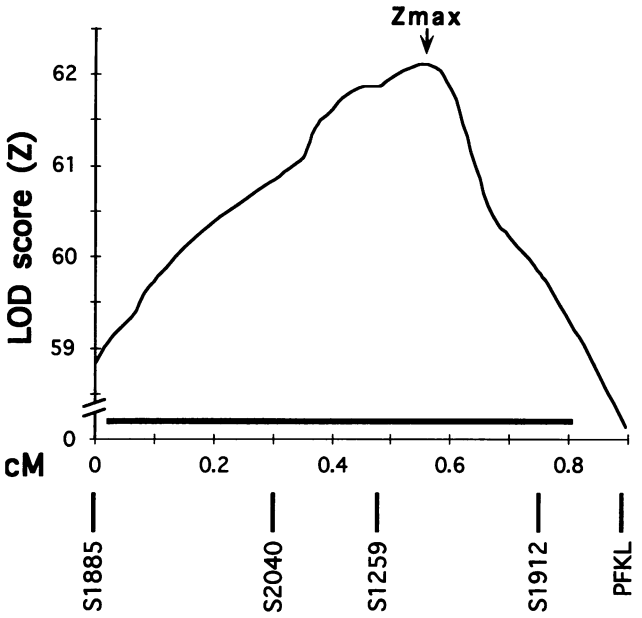

Figure 2 Results of multipoint association testing according to Terwilliger (1995). The likelihood-ratio estimate on a lod unit scale is plotted as a function of map distance from D21S1885 along the chromosome. A maximum lod score value of 62.13, showing the estimated map position of EPM1, is indicated with an arrow. The bar on the X-axis indicates the 99% confidence interval (corresponding to Z_{max} ± 3 lod units). The centromere is to the left.

Table 3

Haplotypes Associated with 88 EPM1 Chromosomes Spanning 900 kb on Chromosome 21q

Haplotype						
D21S1885	D21S2040	*EPM1*	D21S1259	D21S1912	PFKL	*N*
3	2	–	4	4	2	65
3	2	–	4	4	4	6
3	2	–	4	4	6	1
3	2	–	4	4	5	2
3	2	–	4	4	3	1
1	2	–	4	4	2	2
2	2	–	4	4	2	2
3	2	–	4	2	6	1
...	2	–	4	2	6	1
3	2	–	4	3	2	1
3	2	–	3	1	4	1
3	2	–	1	5	4	1
3	1	–	4	4	2	2
1	1	–	4	4	2	1
2	2	–	5	4	4	1

Note.—Boxes show the extent of the putative ancestral EPM1 haplotype. Dashes (–) refer to the most-likely position of EPM1. Ellipses (. . .) indicate missing data.

Table 4

Extended Haplotypes in Nine EPM1 Chromosomes with Putative Historical Recombinational Events

Haplotype No.	D21S141	D21S1885	D21S2040	EPM1	D21S1259	D21S1912	D21S25	PFKL	D21S154	N
Ancestral	2	3	2	–	4	4	2	2	2	
1	2	3	2	–	3	1	1	4	2	1
2	1	3	2	–	1	5	1	4	2	1
3	1	1	2	–	4	4	2	2	1	1
4	1	1	2	–	4	4	2	2	2	1
5	1	2	2	–	4	4	2	2	2	1
6	...	2	2	–	4	4	2	2	2	1
7	...	3	1	–	4	4	2	2	2	2
8	1	1	1	–	4	4	2	2	2	1

NOTE.—Boxes show the extent of the putative ancestral EPM1 haplotype. Dashes (–) refer to the most-likely position of EPM1. Ellipses (. . .) indicate missing data.

haplotype 3-2-4-4-2 was seen in 65 EPM1 chromosomes and in only 1 normal chromosome. Haplotypes in 22 EPM1 chromosomes deviated from the main haplotype by single changes interpretable as historical recombinational events. Two chromosomes deviated from the putative ancestral haplotype at D21S1259, D21S1912 and PFKL, placing EPM1 proximal to D21S1259. Four chromosomes had lost the ancestral allele at D21S1885, placing EPM1 distal to D21S1885. In addition, three chromosomes had lost the ancestral allele at D21S2040 while sharing the ancestral haplotype distally. Of these three chromosomes, two had the allele "3" at D21S1885, which is the ancestral allele, but is also very common in normal chromosomes (table 1), and one had lost the ancestral allele at D21S1885. These data suggest that EPM1 is distal to D21S2040. On the basis of these putative historical recombinations, we propose that the EPM1 gene lies between the markers D21S2040 and D21S1259.

We constructed extended haplotypes of the nine chromosomes with putative ancestral recombinational events, described above, by adding data from RFLP markers D21S141, D21S25, and D21S154 (table 4). Further evidence supporting the suggestion of the localization of EPM1 between D21S2040 and D21S1259 was obtained in three chromosomes. On the distal side, the two chromosomes that had lost the ancestral alleles at D21S1259, D21S1912, and PFKL (haplotypes 1 and 2) had the nonancestral allele "1" at D21S25 that resides between D21S1912 and PFKL. This supports the previous interpretation. On the proximal side, one chromosome (haplotype 8) had lost the ancestral allele at D21S141, in addition to D21S1885 and D21S2040, again supporting the previous interpretation.

One EPM1 chromosome had a very different 2-2-5-4-4 haplotype (table 3) that does, however, share the common allele 2 at D21S2040 and allele 4 at D21S1912 with the ancestral haplotype. It is likely that this haplotype represents another EPM1 mutation. This is compatible with the calculated value of α being .95–.99.

Discussion

Linkage-disequilibrium mapping exploits recombinations that have occurred during the entire history of a population. It is a powerful tool in refining disease-gene localizations for Mendelian disorders that are relatively rare in the population (Jorde 1995). Ideally, most of the disease chromosomes in the population should descend from a single ancestral mutation, and the mutation should be old enough so that recombination has made the region of strongest linkage disequilibrium small, but not too small (Hästbacka et al. 1992). Such is the case in Finland, where the present-day population of five million is believed to originate largely from a small founder population whose expansion began 2,000–2,500 years ago (Nevanlinna 1972; Norio 1981; de la Chapelle 1993). This provides some 100 generations during which recombinations have had time to break up allelic associations that existed on the founding mutant chromosomes. As a consequence of this remarkable population history in isolation, bottlenecks, genetic drift, and other chance effects have caused many Mendelian disorders to occur with substantially higher and lower frequencies than in the rest of Europe (Nevanlinna 1972; Norio 1981; de la Chapelle 1993). Some of these disease genes have been studied by linkage disequilibrium (e.g., Hästbacka et al. 1992; Lehesjoki et al. 1993*b*; Aaltonen et al. 1994; Sulisalo et al. 1994; Männikkö et al. 1995; Nikali et al. 1995; Tahvanainen et al. 1995).

Our previous linkage-disequilibrium data (Lehesjoki et al. 1993*b*) were based mainly on relatively poorly

informative RFLP markers. Moreover, when one considers that marker D21S141 resides some 1 Mb proximal to D21S25, PFKL, and D21S154, it showed a remarkably high degree of linkage disequilibrium, but no informative markers existed in the region between D21S141 and D21S25. For lack of genetic resolution, we were reluctant to embark on gene searches across a large genomic region, so we opted to study genetic markers instead in order to refine the map. The physical map that was subsequently produced (Stone et al. 1996) allowed us both to determine physical distance and to find and map new markers. The five markers we studied span ~900 kb of DNA with intermarker spacings of 140 to 300 kb. This degree of precision could be obtained by combining the existing data from pulsed-field gel electrophoresis mapping of *Not*I fragments (Ichikawa et al. 1993) and an *Eco*RI fragment map (Stone et al. 1996). As a result, among other findings, the EPM1 locus critical region flanked by markers D21S2040 and D21S1259 could be firmly determined to comprise some 175 kb of DNA.

As expected, significant linkage disequilibrium was detected between EPM1 and all markers studied. The most significant allelic association was identified with markers D21S2040 and D21S1912, 96% of EPM1 chromosomes showing the ancestral marker allele in both cases. By applying Luria-Delbrück–derived analysis (Hästbacka et al. 1992; Lehesjoki et al. 1993*b*), we estimated the genetic distances between EPM1 and the marker loci studied. Because the birthplaces of known carriers of EPM1 are evenly distributed over the region of old settlement (Norio 1981; de la Chapelle 1993) in Finland (fig. 1), constituting a typical feature of an old mutation, we estimated the number of generations since "founding" to be 100. The proportion of the disease chromosomes that have descended from the putative single ancestral chromosome was estimated to be between 95% and 99%. This estimate correlates well with our predictions from haplotype data (table 3; see Discussion). By this analysis, EPM1 resides closest to markers D21S2040, D21S1259, and D21S1912, while the flanking markers show a considerably larger distance to EPM1. We are fully aware of the fact that these distances may be underestimates rather than overestimates (Kaplan and Weir 1995), and only cloning of the gene will reveal the true distance. As in the case of diastrophic dysplasia, similar predictions turned out to be correct; Hästbacka et al. (1992) used the Luria-Delbrück–derived analysis and estimated the distance from the closest markers to be 0.065 cM. The subsequent positional cloning of the gene showed it to lie at ~60 kb from these markers (Hästbacka et al. 1994). In the present study, multipoint association analysis of the data was performed with a likelihood-based approach developed for data from multiallelic markers. It has previously been proved accurate in predicting the known location of the cystic fibrosis gene solely on the basis of very close markers (Terwilliger 1995). The most likely suggested localization for EPM1 was distal to D21S1259, and the 99% confidence interval covered ~0.8 cM around it.

Powerful evidence regarding the localization of the EPM1 gene was obtained by constructing haplotypes for the markers in the EPM1 critical region. The analysis of the microsatellite markers D21S1885, D21S2040, D21S1259, D21S1912, and PFKL showed that most of the EPM1 chromosomes are derived from a single founder chromosome carrying the haplotype 3-2-4-4-2 (table 3). Of the 88 haplotyped chromosomes, 65 carry the ancestral haplotype, whereas we propose that 22 derive from it by historical recombinations. Clearly, haplotypes deviating from the ancestral haplotype only at one marker locus could represent mutation events at microsatellite repeat loci. However, several chromosomes had deviations at more than one locus consistent with recombinational events. Further evidence supporting this hypothesis was obtained by extending the haplotypes with RFLP markers (table 4). Among the different haplotypes, one can distinguish three key haplotypes that help define the EPM1 critical region. Two chromosomes carrying haplotypes (2-3)-2-3-1-1-4-2 and (1-3)-2-1-5-1-4-2 deviate from the putative ancestral haplotype at markers D21S1259, D21S1912, D21S25, and PFKL, which suggests that EPM1 resides proximal to these markers. The third key haplotype, (1-1)-1-4-4-2-2-2, suggests that the localization is distal to D21S2040. On the basis of these key haplotypes, we propose that EPM1 resides between loci D21S2040 and D21S1259. Only one chromosome carries a very different haplotype that could best be explained by the occurrence of an independent mutation. Other explanations would involve several historical recombinations and/or marker-allele mutations. In summary, the haplotype data suggest that the most likely position of EPM1 is between D21S2040 and D21S1259, a region that is ~175 kb in length. Our prediction on the localization of EPM1 is strengthened by data obtained from a separate study (M. Faham, K. Virtaneva, J. Warrington, N. Stone, E. Stewart, A. de la Chapelle, A.-E. Lehesjoki, R. M. Myers, and D. R. Cox, unpublished data) where we show using the mismatch repair detection method (Faham and Cox 1995) that two key haplotypes, (2-3)-2-3-1-1-4-2 and (1-1)-2-4-4-2-2-2, have arisen due to historical recombinations and define an ~200-kb EPM1 interval proximal to D21S1259. This assignment is precise enough to encourage the search for EPM1 candidate genes.

Acknowledgments

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