Assignment of the Muscle-Eye-Brain Disease Gene to 1p32-p34 by Linkage Analysis and Homozygosity Mapping

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Summary

Muscle-eye-brain disease (MEB) is an autosomal recessive disease of unknown etiology characterized by severe mental retardation, ocular abnormalities, congenital muscular dystrophy, and a polymicrogyria-pachygyria–type neuronal migration disorder of the brain. A similar combination of muscle and brain involvement is also seen in Walker-Warburg syndrome (WWS) and Fukuyama congenital muscular dystrophy (FCMD). Whereas the gene underlying FCMD has been mapped and cloned, the genetic location of the WWS gene is still unknown. Here we report the assignment of the MEB gene to chromosome 1p32-p34 by linkage analysis and homozygosity mapping in eight families with 12 affected individuals. After a genomewide search for linkage in four affected sib pairs had pinpointed the assignment to 1p, the MEB locus was more precisely assigned to a 9-cM interval flanked by markers D1S200 proximally and D1S211 distally. Multipoint linkage analysis gave a maximum LOD score of 6.17 at locus D1S2677. These findings provide a starting point for the positional cloning of the disease gene, which may play an important role in muscle function and brain development. It also provides an opportunity to test other congenital muscular dystrophy phenotypes, in particular WWS, for linkage to the same locus.

Introduction

Several genetic diseases occur at substantially higher frequencies in Finland than in the rest of the world because of the particular features of the Finnish population, which has expanded, in isolation, from a small number of founders ~100 generations ago (de la Chapelle 1993). Muscle-eye-brain disease (MEB; MIM 253280) is a typical example of these disorders, which constitute the so-called "Finnish disease heritage." MEB is inherited in an autosomal recessive manner and was first described in Finnish patients by Santavuori et al. (1977). Worldwide, ~30 patients with MEB have been reported, the majority from Finland, where the incidence of this disorder is estimated to be <1:50,000. Outside Finland, ~10 sporadic cases bearing a close resemblance to MEB have been described (Korinthenberg et al. 1984; Echenne et al. 1986; Mielke et al. 1986; Federico et al. 1988; Leyten et al. 1992; Topaloglu et al. 1995; van der Knaap et al. 1997).

MEB patients present with congenital hypotonia, mental retardation, and visual failure (Raitta et al. 1978; Santavuori et al. 1989; Pihko et al. 1995). The occurrence of severe progressive myopia from infancy is typical, whereas retinal degeneration with pale retina and low or isoelectric electroretinogram, as well as cataracts, develop with age. In addition, optic atrophy and delayed and giant (>50 μV) flash visual evoked potentials (VEP) are typical in MEB. This VEP finding, present in 10 of 11 Finnish patients studied, strongly supports the diagnosis of MEB (Santavuori et al. 1998). The serum creatine kinase (CK) levels are elevated, findings from electromyography (EMG) show myopathy, and muscle biopsy findings are compatible with dystrophy. Magnetic resonance imaging (MRI) studies of the CNS reveal a uniform structural pattern characterized by a nodular cortical surface combined with midline defects and a flat brainstem (Valanne et al. 1994). The cerebral and cerebellar cortices are totally disorganized, without horizontal lamination (Haltia et al. 1997).

Clinically, MEB resembles Fukuyama congenital muscular dystrophy (FCMD; MIM 253800) (Fukuyama et al. 1981) and Walker-Warburg syndrome (WWS; MIM 236670) (Dobyns et al. 1989), with confusing overlap in individual patients. All three conditions share the unusual combination of congenital muscular dystrophy

Received August 14, 1998; accepted for publication November 17, 1998; electronically published January 15, 1999.

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0002-9297/99/6401-0018$02.00

(CMD) and congenital brain malformations. In addition, ocular abnormalities are typical in WWS and in MEB. Although the clinical features of FCMD are well characterized, the delineation of WWS and MEB has remained controversial (Dubowitz 1997). It has been suggested that MEB and WWS should be considered the same clinical entity (Dobyns et al. 1989, 1990). However, two of us (P.S. and H.P.), after evaluation of 19 Finnish patients, argued for the distinctness of MEB (Santavuori et al. 1990). The clinical presentation of MEB seems to be uniform in Finland, with the exception of two patients (Valanne et al. 1994). The final resolution of the controversies between the different patient groups will depend on the mapping and characterization of the underlying gene defects. In that regard, the gene for FCMD has been localized to chromosome 9q31-q33 (Toda et al. 1993) and recently identified (Kobayashi et al. 1998). A linkage study of seven Finnish MEB families with 12 affected individuals showed that the MEB phenotype was not linked to the FCMD locus (Ranta et al. 1995). The gene for WWS has not yet been mapped.

Here we report the localization of the MEB gene to chromosome 1p32-p34. We performed a genomewide search for linkage, using a set of microsatellite markers with an average spacing of 10 cM, initially in four Finnish sib-pairs and their parents. The MEB gene was further localized to a 9-cM interval by means of a combination of linkage analysis and homozygosity mapping in four additional pedigrees.

Subjects and Methods

Subjects and Samples

Seven Finnish (F1–F7) families and one Turkish (T1) family, with a total of 12 affected individuals and 27 unaffected relatives, were studied. Four of the families had two affected children, whereas the rest had only one affected child. Two of the pedigrees, F7 and T1, were consanguineous. Genealogic studies were performed on family F7, which was traced back six generations in search of common ancestors. Several consanguinity loops were found at different levels. The pedigree structures are shown infigure 1.

The MEB diagnosis in the Finnish patients was based on the presence of the following findings: severe mental retardation, ocular changes including myopia >-6 diopters, hypotonia and weakness, myopathic/dystrophic changes in muscle biopsy specimens, myopathic EMG findings and/or elevated serum CK values, as well as the presence of cobblestone cortex, midline defects, abnormally thin brainstem, and cerebellar hypoplasia, with absent inferior vermis in MRI studies (fig. 2). The symptoms were present from birth or the first months of life. Detailed clinical data from the MEB patient in family T1 are given in Topaloglu et al. (1995), where the propositus is referred to as "case 1." The main clinical features of the Finnish and Turkish patients are summarized in table 1.

Genomic DNA was isolated by standard methods from venous blood or fibroblast or lymphoblast cell lines from all consenting individuals. All samples from Finnish families were drawn in accordance with the Helsinki declaration.

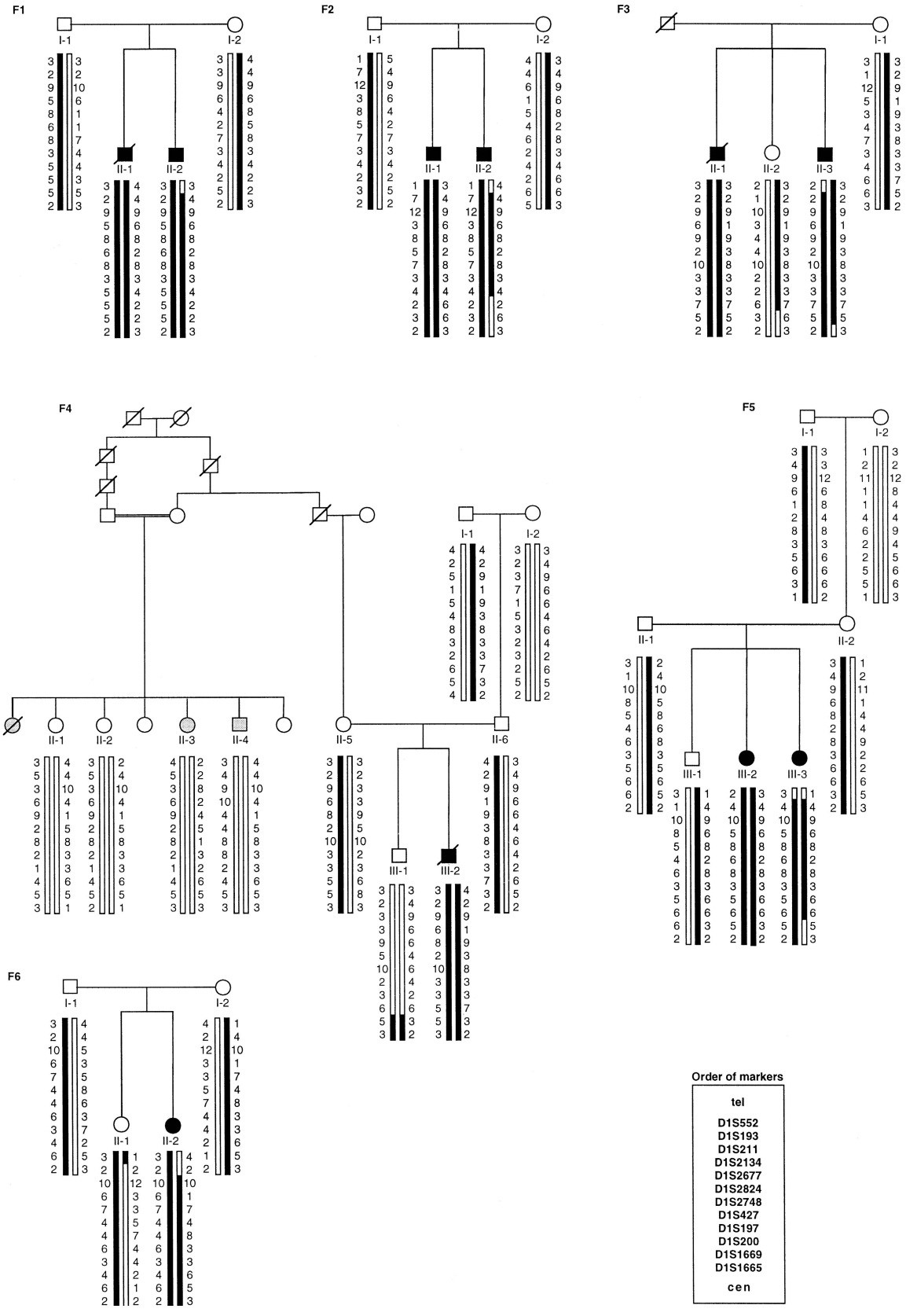
Genotyping

For the genomewide search, we analyzed DNA samples by PCR amplification using a panel of 350 highly polymorphic microsatellite markers with an average spacing of 10 cM, mainly from the Généthon collection (Dib et al. 1996), as described elsewhere (Avela et al. 1997). In the initial screening, four multiplex nuclear families were scored for the presence or absence of recombinations by familywise comparison of the alleles of affected individuals. The parents were also genotyped to assess the informativity of the markers. If both affected sibs in a family had the same genotype, the family was scored with a plus sign; if not, the family was marked with a minus sign. Markers giving four plus signs were selected for further study.

For the refinement of the candidate area on chromosome 1p, we used the tetranucleotide repeat markers D1S552, D1S2134, D1S1669, and D1S1665 from the Cooperative Human Linkage Center collection (Murray et al. 1994), as well as the dinucleotide repeat markers D1S234, D1S513, D1S255, D1S2743, D1S193, D1S211, D1S2677, D1S2824, D1S2748, D1S427, D1S197, D1S200, D1S220, D1S209, D1S438, and D1S198 from the Généthon collection (Dib et al. 1996). Primers were purchased from the MapPairs set (Research Genetics) or were synthesized with sequence data from the CEPH public database. In all cases the forward primer was modified at the 5′ end with a FAM, TET, or HEX fluorescent label. The order and distances (in centimorgans) of the markers used in the linkage analysis are depicted in figure 3. The most likely location for markers D1S552, D1S2134, D1S1669, and D1S1665 was computed by the CMAP program of the LINKAGE package (Lathrop et al. 1984) and was based on data from all the available CEPH reference families. Each of these four tetranucleotide repeat markers was run over a fixed set of markers from the Généthon map (Dib et al. 1996). The multipoint LOD scores supporting the locations of markers D1S552, D1S2134, D1S1669, and D1S1665, given in figure 3, were 36.10, 26.91, 32.65, and 39.09, respectively.

The PCR reactions were performed under the following conditions: 50 ng genomic DNA, 1′ × Perkin-Elmer PCR buffer, 130 μM dNTPs, 5 pmol of both the forward

A


F1
I-1
I-2
II-1
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F2
I-1
I-2
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F3
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II-2
II-3
F4
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II-2
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III-2
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I-1
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III-1
III-2
III-3
F6
I-1
I-2
II-1
II-2
Order of markers
tel
D1S552
D1S193
D1S211
D1S2134
D1S2677
D1S2824
D1S2748
D1S427
D1S197
D1S200
D1S1669
D1S1665
cen

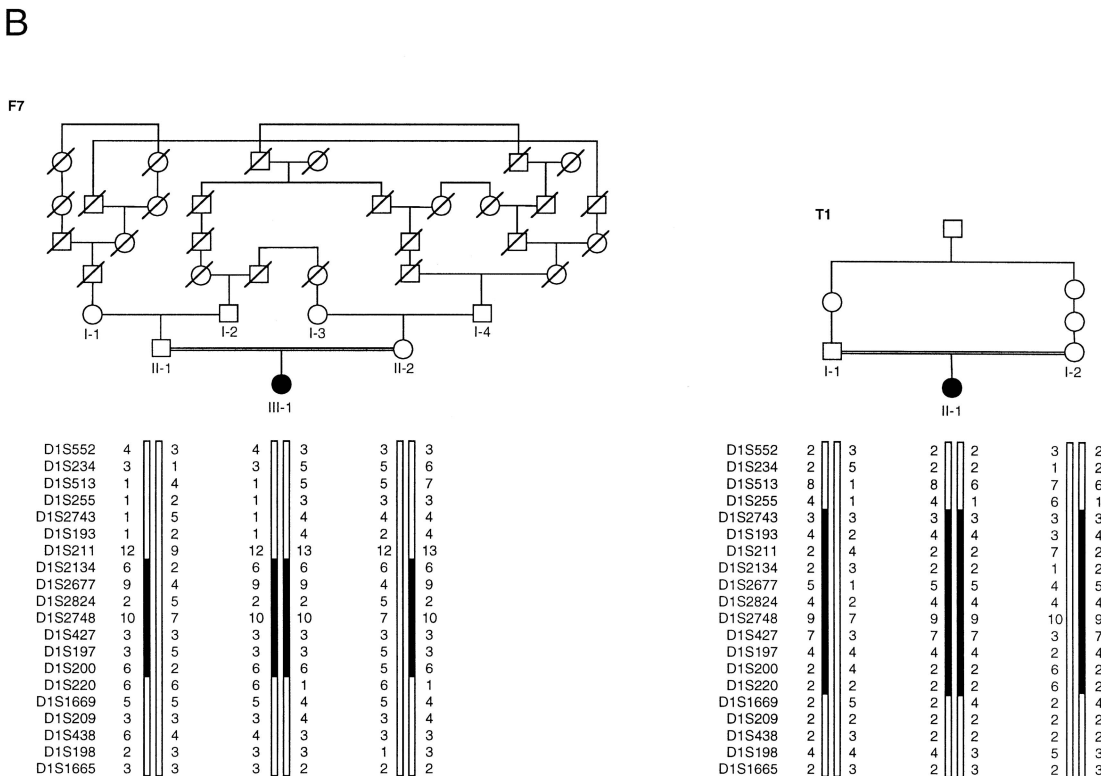

Figure 1 *A* (*opposite*), Pedigrees of the Finnish MEB families F1–F6, showing haplotypes for 12 polymorphic markers on 1p. Affected individuals are denoted by blackened symbols, deceased family members are indicated by diagonal slashes, and consanguineous matings are marked by double lines. The haplotypes segregating with the disease phenotype are shown by black bars situated between the marker alleles. Families F1, F2, F3, and F5 were used in the first stage of the genomewide screening. Individuals II-3 and II-4 from family F4 did not fulfill the diagnostic criteria for MEB and were thus excluded from the initial linkage studies. The haplotype analysis on 1p excluded linkage to the MEB locus in these two individuals (see the Results section). *B* (*above*), Pedigrees of the consanguineous MEB families F7 (Finnish) and T1 (Turkish). Affected individuals are denoted by blackened symbols. Haplotypes for 20 markers surrounding the MEB gene on 1p are shown. The regions of homozygosity are indicated by black bars. tel = telomere; cen = centromere.

and reverse primer, and 0.75 U AmpliTaq Gold polymerase (Perkin-Elmer) in a final volume of 15 μl. The amplification conditions were 94°C for 10 min for 1 cycle; 94°C for 30 s, 55°C for 1 min 15 s, and 72°C for 1 min, for 30 cycles (FAM and TET markers) or 35 cycles (HEX markers); and 72°C for 10 min. Amplified products were separated by electrophoresis on a 4.25% polyacrylamide-6 M urea gel by a 377 DNA sequencer apparatus (Applied Biosystems), and the results were processed by GENESCAN version 2.0.2 and GENOTYPER version 1.1 software.

Linkage Analysis

We used simulation programs SLINK and MSIM, version 2.51 (Weeks et al. 1990), to compute the maximum expected pairwise LOD scores in our panel of MEB families, assuming a marker heterozygosity value of 0.7 over 100 replicates. The initial panel of four families was estimated to give a maximum two-point LOD score (Z_{max}) of 2.41 at a recombination fraction (θ) of 0.00 from the disease gene and a Z_{max} of 1.72 at $\theta = 0.10$. When all the family material available was considered, the expected Z_{max} was 4.15 at $\theta = 0.00$ and 2.83 at $\theta = 0.10$.

Pairwise LOD scores were calculated by the MLINK program of the LINKAGE package, version 5.2 (Lathrop et al. 1984). An autosomal recessive model of inheritance with complete penetrance and a population frequency of 0.004 for the disease allele was assumed. The allele frequencies of the polymorphic markers were considered to be equal. Inbreeding loops were broken by the MAKEPED program, by counting twice an individual who had both parents and children included in the pedigree.

We used the GENEHUNTER program (Kruglyak et al. 1996) to calculate multipoint LOD scores for MEB against a fixed map of seven marker loci, assuming equilibrium between the marker and test loci. The parameters in the multipoint analysis were the same as those described for the two-point analysis. The 95% confidence limits for the maximum recombination fraction

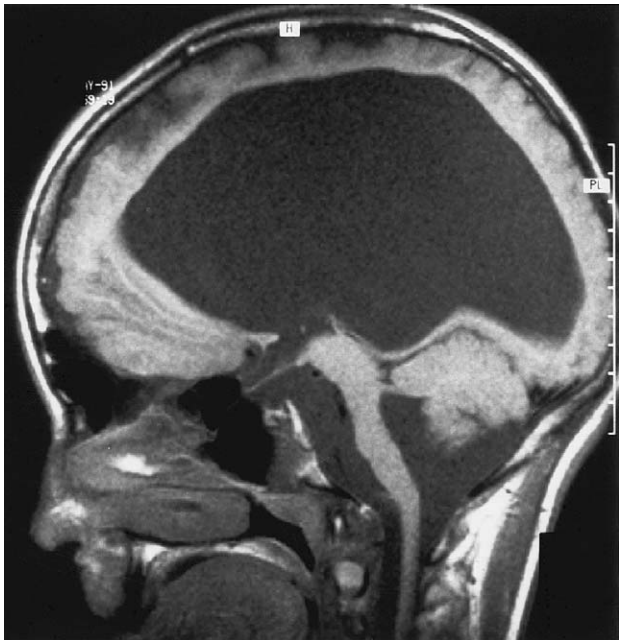

Figure 2 MRI features of the brain of an MEB patient. Sagittal view showing an enlarged lateral ventricle, thin corpus callosum, absent pons, abnormal tectum, and cerebellar atrophy with absent vermis. The patient also has a pachygyria-type cortical migration defect.

(θ_{max}) at the Z_{max} both in the multipoint and in the two-point analyses were calculated by the 1-LOD-down method (Ott 1992).

Linkage Disequilibrium and Haplotype Analysis

We evaluated the presence of linkage disequilibrium between the disease and marker loci, using the program DISLAMB version 2.1, which applies a likelihood-ratio test for linkage disequilibrium with only 1 df, irrespective of the number of alleles at a given marker (Terwilliger 1995). Sixty chromosomes from healthy Finnish individuals were genotyped for all the markers and used as controls in the calculations.

Haplotypes for 12 microsatellite markers over the MEB region were constructed in families F1 to F6 (fig. 1*A*). We performed homozygosity mapping (Lander and Botstein 1987) in the two consanguineous families (F7 and T1,fig. 1*B*), using extended haplotypes for 20 markers. The haplotypes were constructed manually by assuming the minimum number of recombinations, and the phase was determined by genotyping all the available family members.

Results

Initial Assignment of the MEB Gene

The screening to locate the MEB gene was performed in two stages. In the first stage, a genomewide scan in four Finnish families (F1, F2, F3, and F5;fig. 1*A*) was performed to identify genomic regions showing allele sharing within sibships (see the Subjects and Methods section for details). This method yielded only four loci on chromosomes 1p, 5p, 8q, and 12q that showed allele sharing in all four families. In the second stage of the screening, we studied the four candidate regions further, using all available families (fig. 1). The locations on chromosome 5p, 8q, and 12q were excluded (data not shown), whereas the marker D1S197 on 1p continued to provide evidence for linkage, with $Z_{max} = 2.81$ at $\theta = 0.00$ from the disease gene.

Refinement of the Critical Region by Linkage Analysis in an Extended Panel of Families

The analysis of additional markers closely linked to D1S197 and additional families confirmed the location of the MEB gene on 1p. The results of the two-point linkage calculations between MEB and 12 markers on chromosome 1p are shown in table 2, and the linkage map for these markers is depicted in figure 3. Four markers (D1S2134, D1S2677, D1S2824, and D1S2748) provided statistically significant pairwise LOD scores ($Z_{max} > 3.0$) without observed recombination events.

Table 1

Clinical Features of the MEB Patients Included in this Study

Family and Patient	Present Age (years)	MRI Findings[a]	VEP[b] (μV)	Myopia (D)	Serum CK[c]
F1:					
II-1	17[d]	Typical	>50	−17	2.2–71
II-2	15	Typical	Deformed[e]	−26	1.8–5.4
F2:					
II-1	27	Typical	>50	−27	2.1–8.8
II-2	22	Typical	>50	−10	3.1–5.9
F3:					
II-1	35[d]	Typical	>50	NA[f]	1.2–6.1
II-2	41	Typical	>50	−10	0.2–2.9
F4:					
III-2	22[d]	Typical	>50	−9	2.3–40.2
F5:					
III-2	14	Typical	>50	−25	5.0–5.4
III-3	9	Typical	30	−18	3.2–3.8
F6:					
II-2	20	Typical	>50	−15	1.0–28
F7:					
III-1	21	Typical	>50	−25	2.0–18.2
T1:					
II-1	11	Typical	NA[g]	−13	25–38

[a] Typical = flat brainstem, midline defects, nodular cortical surface, and cerebellar hypoplasia with absent inferior vermis.
[b] Normal 10–15 μV (Santavuori et al. 1998).
[c] Times the upper limit of normal.
[d] Age at death.
[e] Extreme hydrocephalus.
[f] Refraction could not be measured because of cataract at the age of 34 years.
[g] Not available.

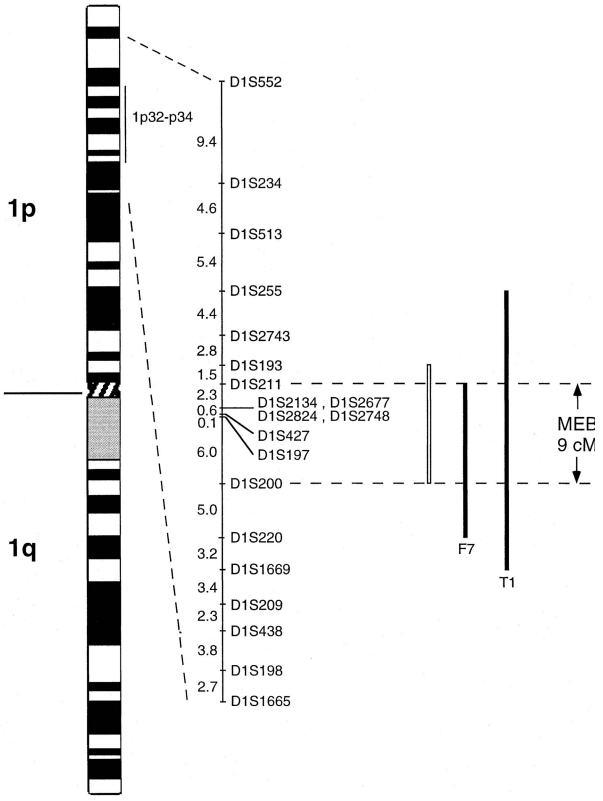

Figure 3 Ideogram of chromosome 1, showing the location and the relative order of and distances (in centimorgans) between the markers analyzed in this study. The MEB locus interval as defined by recombination events in affected individuals is indicated by a white bar. Blackened bars indicate regions of homozygosity in the affected sibs in the consanguineous families F7 and T1. Taken together, the data suggest that the MEB gene lies in a 9-cM interval between markers D1S211 and D1S200.

The highest LOD score was obtained with D1S2677 ($Z_{max} = 4.46$ at $\theta = 0.00$). The MEB locus region was bordered by obligatory recombinations, observed with marker D1S193 at the telomeric side (family F6) and D1S200 toward the centromere (family F2). These flanking markers are separated by 10.5 cM and are physically located on chromosome 1p32-p34 (Hellsten et al. 1995; Collins et al. 1996; Speleman et al. 1996). Multipoint linkage analysis, calculated for a subset of seven markers, resulted in a maximum LOD score of 6.17 at D1S2677, with a −1 LOD unit support interval of 2.07 cM distal and 5.5 cM proximal to it.

In family F4 (fig. 1*A*), individuals II-3 and II-4, who are sibs from a consanguineous marriage, presented with a milder "MEB-like" phenotype. They are still alive at 67 and 62 years and show mild mental retardation, cataracts, and muscular dystrophy. Moreover, findings from the MRI scans are normal and very different from the uniform malformation pattern present in the "typical" MEB patients, including individual III-2 from the same family (Santavuori et al. 1989; Valanne et al. 1994). Because no homozygosity or cosegregation with the disease was seen for the marker alleles in the MEB region in II-3 and II-4, and because they did not share a haplotype with III-2, the 1p32-p34 MEB locus was excluded as the one responsible for this phenotype.

Homozygosity Mapping in Two Inbred Pedigrees

To further refine the location of the MEB gene, we attempted homozygosity mapping. Extended haplotypes were constructed in two consanguineous kindreds (F7 and T1) with 20 microsatellite markers. The results are shown infigure 1*B*, and the distances between the markers are shown in figure 3. As expected on the basis of homozygosity by descent, regions of allele homozygosity were observed in the MEB locus region in the two affected individuals. The affected sib from family T1 is homozygous for the alleles of all informative markers proximal to D1S255 and distal to D1S1669, spanning a region of ~26 cM on 1p. The maximum region of homozygosity in individual III-1 from family F7 spans 14 cM from D1S211 to D1S220. These data further restrict the telomeric boundary of the MEB critical region to marker D1S211. The combined homozygosity and recombinational mapping data give the MEB gene a most likely location in an ~9-cM interval between D1S211 and D1S200 (fig. 3).

Linkage Disequilibrium and Haplotype Analysis

All the markers within the MEB critical region spanning D1S211 and D1S200 were analyzed for linkage disequilibrium by comparing our set of 13 apparently unrelated Finnish disease chromosomes with a panel of 60 Finnish healthy chromosomes. The results were not significant in any case (data not shown). No major common haplotype was observed in the Finnish MEB chromosomes.

Discussion

CMDs comprise a heterogeneous group of inherited neuromuscular disorders characterized by early onset of hypotonia and weakness, joint contractures, muscular dystrophy, and a slowly progressive course. Several forms of CMD show brain involvement in addition to the muscle features. The *LAMA2* gene, encoding the laminin $\alpha 2$ chain or merosin, located on chromosome 6q22, has been identified as the cause of one of these disorders (Helbling-Leclerc et al. 1995). Mutations in this gene are responsible for ~50% of CMD cases in the white population (Voit 1998). CMD patients with a merosin deficiency form a clinically homogeneous group characterized by cerebral involvement, in the form of white-matter changes without mental retardation. By contrast, merosin-positive CMD is clinically more var-

Table 2

Two-Point LOD Scores for Linkage Between the MEB Locus and 12 Chromosome 1p Markers

Locus	LOD Score at θ =							Z_{max}	θ_{max}	95% Confidence Interval [a]
	.00	.01	.05	.10	.20	.30	.40			
D1S552	−∞	−4.17	−1.62	−.74	−.16	−.01	.01	.01	.390	…
D1S193	−∞	1.43	1.89	1.77	1.19	.61	.20	1.89	.056	…
D1S211	2.83	2.75	2.39	1.97	1.20	.59	.18	2.83	.000	…
D1S2134	3.91	3.78	3.26	2.65	1.58	.75	.23	3.91	.000	$.000 < \theta < .079$
D1S2677	4.46	4.31	3.72	3.01	1.78	.85	.26	4.46	.000	$.000 < \theta < .068$
D1S2824	4.31	4.16	3.59	2.92	1.74	.84	.25	4.31	.000	$.000 < \theta < .071$
D1S2748	3.12	3.05	2.70	2.22	1.33	.65	.21	3.12	.000	$.000 < \theta < .110$
D1S427	2.34	2.26	1.94	1.56	.91	.42	.11	2.34	.000	…
D1S197	2.81	2.71	2.29	1.81	1.03	.49	.15	2.81	.000	…
D1S200	−∞	1.24	1.53	1.35	.85	.43	.15	1.53	.043	…
D1S1669	−∞	−.70	.40	.69	.64	.37	.11	.73	.133	…
D1S1665	−∞	−2.92	−1.14	−.54	−.15	−.04	−.01	.00	.500	…

[a] Confidence intervals for θ were calculated for the statistically significant Z_{max} values by means of the 1-LOD-down method (Ott 1992).

iable, which suggests that it may comprise a genetically heterogeneous group of disorders. Two genes responsible for merosin-positive CMD have been mapped so far: the Fukuyama-type CMD (or FCMD), on 9q31-q33 (Toda et al. 1993), and CMD with early rigidity of the spine (or CMD-RSS), on 1p35-p36 (Moghadaszadeh et al. 1998). The FCMD gene was identified recently (Kobayashi et al. 1998). It encodes a novel protein, termed "fukutin," that is secreted outside the cell and thus could have a role as a constituent of the extracellular matrix complex of muscle membranes (Kobayashi et al. 1998).

Here we report the localization of a third gene for a merosin-positive CMD, MEB, to chromosome 1p32-p34. The data presented confirm the recessive mode of inheritance suggested by previous pedigree studies (Santavuori et al. 1989). Interestingly, both the CMD-RSS and the MEB loci map to the distal short arm of chromosome 1. However, since the regions of homozygosity found in the three consanguineous CMD-RSS families reported by Moghadaszadeh et al. (1998) do not overlap with the MEB critical region reported in the present article, the possibility that these two disorders are allelic can be excluded.

Three conditions—FCMD, WWS, and MEB—share the combination of CMD and brain malformations. The nosological classification of these disorders has been controversial (Dubowitz 1997). Clinically, the distinguishing factor between MEB, WWS, and FCMD is the extent of ocular involvement. Severe malformations, including malformation of the anterior chamber and persistent primary vitreous, are typical of WWS. Simple myopia without structural changes occurs in some FCMD patients. The characteristic ocular malformation of MEB is that of progressive myopia and retinal degeneration associated with giant visual evoked potentials. The typical brain malformation in WWS is called "lissencephaly II," or a brain with smooth surface. The nodular cortical migration defect (Takada et al. 1984; Haltia et al. 1997) is similar in MEB and FCMD, but the brain stem, which is characteristically flat in MEB, is normal in FCMD. The clinical course of WWS is very severe, and many patients die in early infancy, which is not usually the case in MEB. The clinical course of FCMD is similar to that of MEB. Although the clinical data suggest that MEB is an independent nosological entity, only the resolution of the molecular genetic background of these three disorders will eventually clarify the existing controversy. In this regard, the FCMD locus was previously excluded as the cause for MEB in Finnish families (Ranta et al. 1995). The genetic localization of the MEB gene now provides an opportunity to test the hypothesis of WWS and MEB being allelic disorders.

The genes encoding a number of muscle basement membrane proteins such as laminins and integrins may be considered good candidates for MEB, for several reasons. First, some of them are mutated in other muscle disorders: laminin $\alpha 2$ in merosin-negative CMD (Helbling-Leclerc et al. 1995) and integrin $\alpha 7$ in a mild form of congenital myopathy (Hayashi et al. 1998). Second, these proteins have been shown to interact with proteins which, when deficient, cause muscular dystrophy (Campbell 1995). Finally, several laminins are expressed both in muscle and in CNS, and they have been associated with several neuronal processes, including stimulation of the migration of neuronal cells (Calof and Lander 1991; Engvall et al. 1992). Laminins are a family of extracellular matrix proteins that are composed of three different but homologous chains named α, β, and γ (Hagg et al. 1997), and integrins are a family of heterodimeric adhesion molecules composed of an α and a β subunit (Hynes 1992). Members of both families are involved in the formation of a link between the extra-

cellular matrix and the subsarcolemmal cytoskeleton, providing functional integrity to muscle cells.

In MEB, immunohistochemical studies have revealed a consistently weak staining for laminin $\alpha 2$ in muscle (Haltia et al. 1997). This feature, as well as a weak staining for other laminin subunits, is also seen in FCMD (Hayashi et al. 1993). Given that the location of the MEB gene on chromosome 1p32-p34 excludes all the already mapped laminin and integrin genes as the ones underlying the disease, the observed changes in the membrane protein composition in MEB patients are most probably secondary. It is thus tempting to speculate that a primary deficiency of another interacting basement membrane protein may cause the destabilization of the membrane complex. Similar "combined deficiencies" have been reported in other muscular disorders such as the limb-girdle-type muscular dystrophy (Bönnemann et al. 1995) and Duchenne muscular dystrophy (Campbell 1995).

On-line searches for genes mapped to 1p32-p34 in the Genome Database resulted in a listing of >100 genes. At least three of them—the collagen VIII α-2 polypeptide gene (*COL8A2*), the vascular cell adhesion molecule-1 gene (*VCAM1*), and the protein tyrosine phosphatase, receptor type, f polypeptide gene (*PTPRF*)—are known to encode basement membrane proteins and therefore constitute functional candidates for MEB. Moreover, the Human Gene Map at the National Center for Biotechnology Information lists 129 expressed sequence tags situated within the MEB critical interval between markers D1S211 and D1S200 (Schuler et al. 1996). Thirteen of these are derived from muscle, brain, and eye cDNA libraries, and they may be considered as potential candidates for MEB. There are no known mouse mutations specific for MEB, although the syntenic region on mouse chromosome 4 contains the neuromuscular phenotype *mea* (meander tail), characterized by ataxia, structural cerebellar abnormalities, muscle weakness, and myopathy (Dysmorphic Human-Mouse Homology Database).

Our current aim is to refine the localization of the MEB gene by analyzing additional markers for linkage disequilibrium in an extended panel of Finnish families, ultimately to facilitate the positional cloning of the disease gene. Interestingly, no significant linkage disequilibrium was detected for any of the markers we studied. This may suggest a relatively old origin of the putative founding mutation within the Finnish population. The existence of several different mutations is a less likely explanation. The extension of the panel of polymorphic markers that will be analyzed in the critical region will help to elucidate this point.

As MEB is both a CMD and a neuronal migration disorder, the eventual identification and characterization of the primary defect may help us to better understand the pathophysiology of muscular dystrophies and may provide new insights into normal and abnormal development of the brain.

Acknowledgments

We thank the members of the MEB families for their cooperation in this study. We thank Sinikka Lindh for collecting samples and for the genealogical studies, Pertti Sistonen for his valuable advice in the linkage calculations, and Paula Kristo and Anitta Hottinen for excellent laboratory technical assistance. We also thank Mònica Bayés for helpful discussions and continuous encouragement. This study was supported by grants from the Ulla Hjelt fund of the Finnish Foundation for Paediatric Research, the Academy of Finland, and the Rinnekoti Research Foundation. H.T. has been supported by the Association Française contre les Myopathies (AFM), France.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Centre d'Etude du Polymorphisme Humain (CEPH), http://www.cephb.fr/bio/cephdb (for sequence data used to synthesize primers)

Cooperative Human Linkage Center, http://www.chlc.org (for tetranucleotide repeat markers)

Dysmorphic Human-Mouse Homology Database, http://www.hgmp.mrc.ac.uk/DHMHD/dysmorph.html (for mouse neuromuscular phenotype *mea* [meander tail])

Genome Database (GDB), http://gdbwww.gdb.org (for genes mapped to 1p32-p34)

Human Gene Map (NCBI), http://www.ncbi.nlm.nih.gov/SCIENCE96 (for expressed sequence tags within the MEB critical interval between markers D1S211 and D1S200)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MEB [MIM 253280], FCMD [MIM 253800], and WWS [MIM 236670])

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