

Clinical and genetic distinction between Walker–Warburg syndrome and muscle–eye–brain disease

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Article abstract—*Background:* Three rare autosomal recessive disorders share the combination of congenital muscular dystrophy and brain malformations including a neuronal migration defect: muscle–eye–brain disease (MEB), Walker–Warburg syndrome (WWS), and Fukuyama congenital muscular dystrophy (FCMD). In addition, ocular abnormalities are a constant feature in MEB and WWS. Lack of consistent ocular abnormalities in FCMD has allowed a clear clinical demarcation of this syndrome, whereas the phenotypic distinction between MEB and WWS has remained controversial. The MEB gene is located on chromosome 1p32–p34. *Objectives:* To establish distinguishing diagnostic criteria for MEB and WWS and to determine whether MEB and WWS are allelic disorders. *Methods:* The authors undertook clinical characterization followed by linkage analysis in 19 MEB/WWS families with 29 affected individuals. With use of clinical diagnostic criteria based on Finnish patients with MEB, each patient was categorized as having either MEB or WWS. A linkage and haplotype analysis using 10 markers spanning the MEB locus was performed on the entire family resource. *Results:* Patients in 11 families were classified as having MEB and in 8 families as WWS. Strong evidence in favor of genetic heterogeneity was obtained in the 19 families. There was evidence for linkage to 1p32–p34 in all but 1 of the 11 pedigrees segregating the MEB phenotype. In contrast, linkage to the MEB locus was excluded in seven of eight of the WWS families. *Conclusion:* These results allow the classification of MEB and WWS as distinct disorders on both clinical and genetic grounds and provide a basis for the mapping of the WWS gene(s).

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The coexistence of lissencephaly (smooth brain) and eye anomalies was first described by Walker¹ in 1942. Warburg^{2,3} subsequently reported several patients in whom hydrocephalus and retinal detachment were associated and noted this occurrence in the son of first-cousin parents, suggesting autosomal recessive inheritance. Chemke et al.⁴ and Pagon et al.⁵ reported several multiplex families with brain and ocular malformations, whereas Williams et al.⁶ described the coexistence of myopathy. Several reports suggested the designation Walker–Warburg syndrome (WWS).^{6–8} In Finland, Santavuori et al.⁹ described an apparently new disorder in which con-

genital muscular dystrophy (CMD), severe brain malformation, and abnormalities of the eyes coexisted. This autosomal recessively inherited condition was given the name muscle–eye–brain disease (MEB).^{9–11} A third autosomal recessive disorder with both CMD and a neuronal migration defect, Fukuyama congenital muscular dystrophy (FCMD), was described in Japanese patients.¹²

The clinical features of FCMD are well defined¹³ and allow distinction from both WWS and MEB in most cases. Moreover, identification of the gene responsible for FCMD on 9q31–q33¹⁴ allows this syndrome to be defined at a molecular genetic level. A

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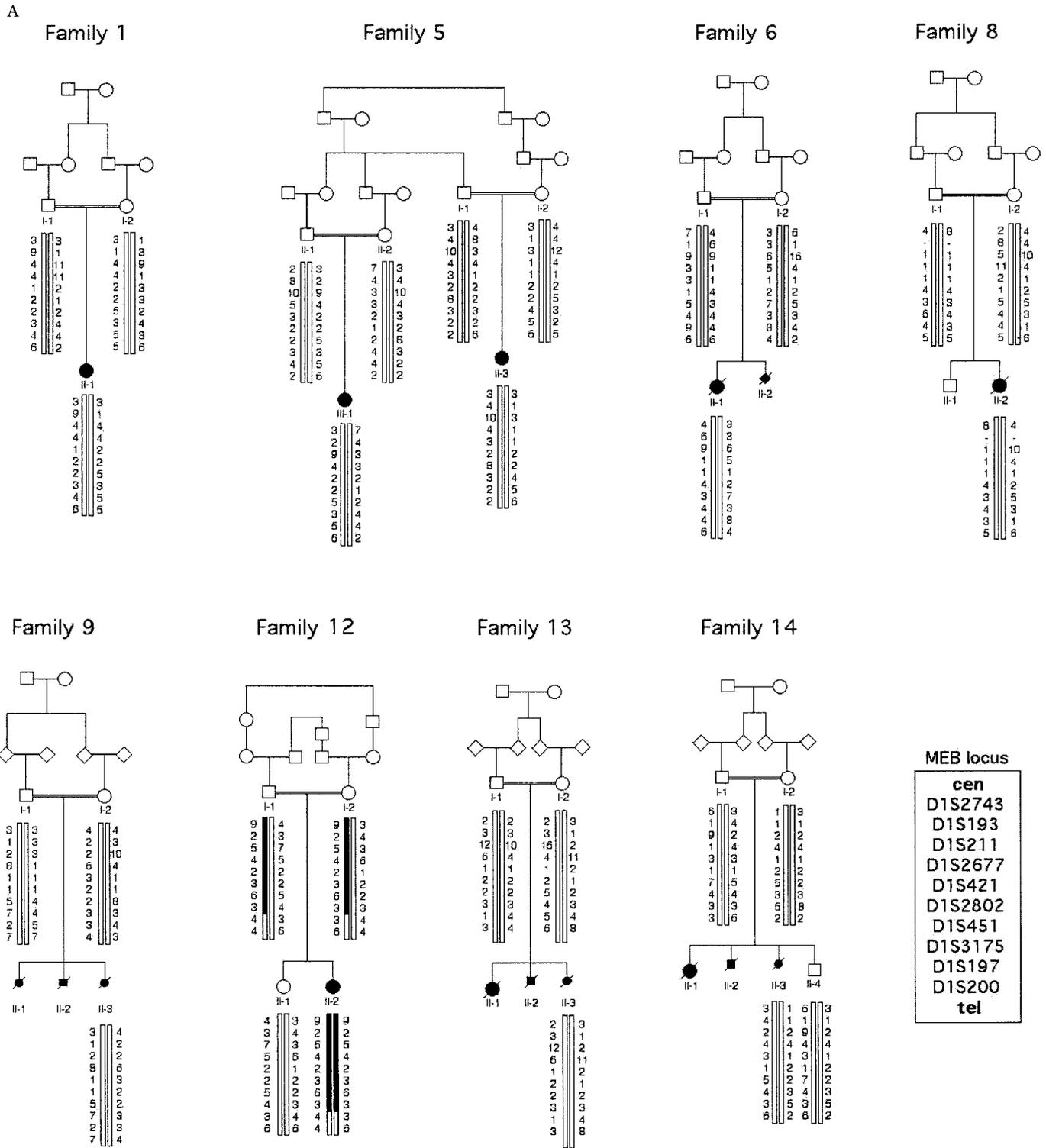


Figure 1. Pedigree and haplotype analysis of the families with Walker-Warburg syndrome (A) and muscle-eye-brain disease (MEB) (B) at the MEB locus (1p32-p34). Affected individuals are denoted by filled symbols. Aborted fetuses are indicated with smaller symbols. Regions of homozygosity in the affected individuals of consanguineous matings or regions segregating with the disease phenotype in multiplex families are shown by black bars situated between the marker alleles. The names and order of the markers are depicted in the insets.

linkage study excluded the FCMD locus in seven Finnish MEB families, confirming that these disorders are distinct genetic entities.¹⁵ Similar exclusion results were found in both the MEB and the WWS families described herein (data not shown).

In contrast, the nosology of WWS and MEB has remained controversial owing to significant clinical overlap. The rarity of these syndromes has also generated difficulties in their clinical distinction. The typical clinical presentation of MEB is that of a

B

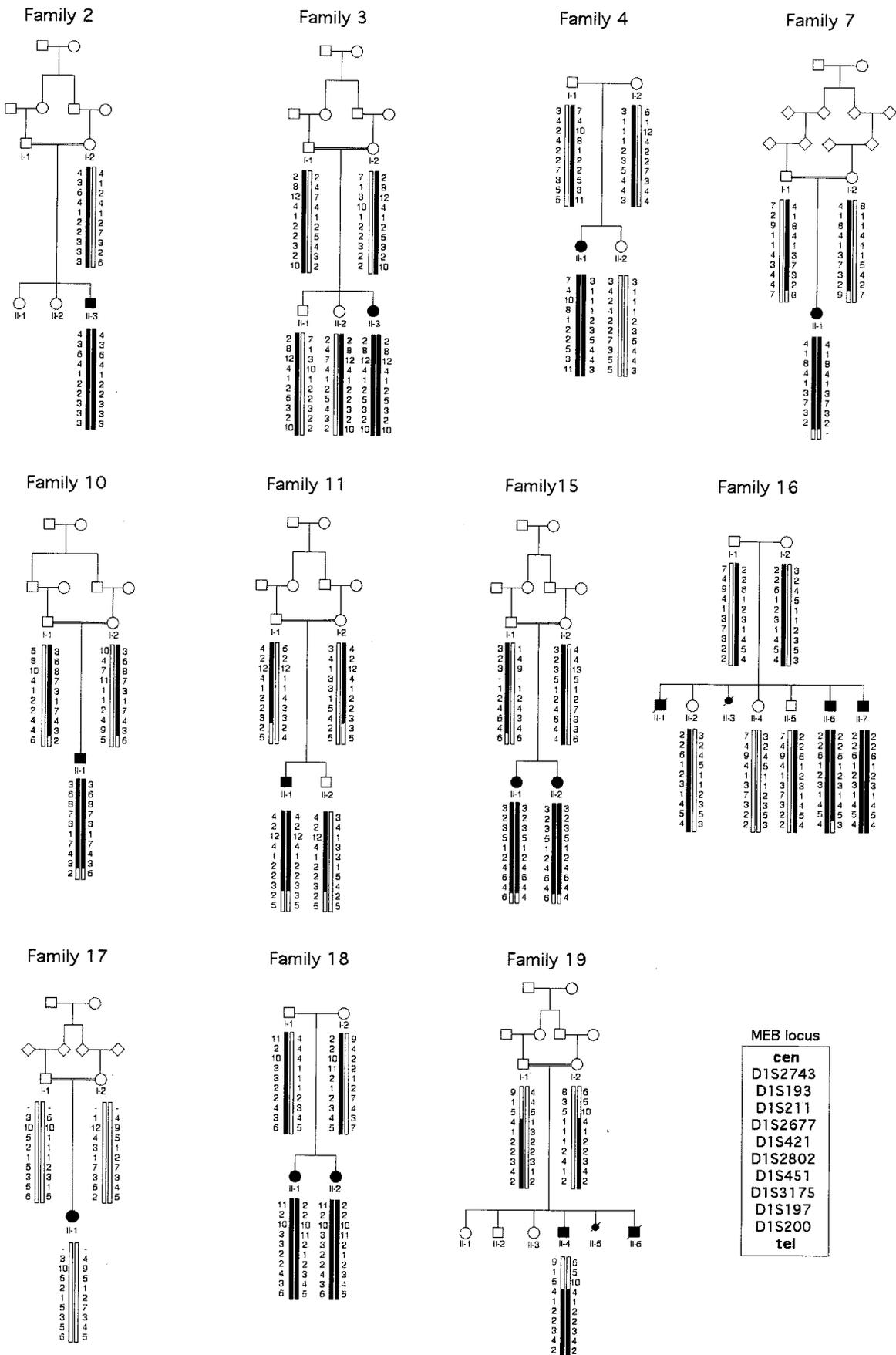


Figure 1. (Continued)

floppy, mentally retarded infant with suspected visual problems.¹¹ The patients survive past early childhood. Exceptional patients learn to say a few words and to walk before the age of 4 years, but the ability to walk is lost before age 20. The majority of the patients have very little active movement, which is further limited by spasticity and contractures that develop with age. Patients with WWS are often diagnosed prenatally because of severe hydrocephalus.¹⁶ They typically have severe eye malformations and virtually no active movements. Many die within the first months of life, with a few surviving to about age 3. In addition, patients with clinical features intermediate between these typical groups have been described. Some authors¹⁷ have emphasized consistent differences between these disorders, whereas others^{16,18} have considered the clinical features to reflect the spectrum of one disease with variable severity and no clear distinguishing features.

The cobblestone cortex,^{19,20} common to FCMD, MEB, and WWS, is characterized by multiple coarse gyri with agyric regions, a disorganized cortex of variable thickness in both the cerebral hemispheres and the cerebellum, and deficient neuronal migration. Other features associated with cobblestone cortex in these syndromes include enlarged lateral ventricles, flat brainstem, and cerebellar hypoplasia. We will later refer to the combination of these neuropathologic features as "cobblestone complex." In WWS, the convolutions of the brain may be minimal and resemble lissencephaly. The term "lissencephaly type 2" has previously been described to characterize the brain malformation associated with WWS.¹⁶ However, the disorganized cortical layers on pathologic examination clearly distinguish this malformation from classic four-layered lissencephaly. The neuropathologic distinction of the cobblestone complex in WWS and MEB is based mainly on the degree of severity, although additional distinguishing features such as occipital encephalocele, fusion of the hemispheres, and absent corpus callosum occur in some patients with WWS.

The ocular abnormalities of MEB are characterized by coarse trabecular meshwork in the anterior chamber, predisposing to glaucoma and progressive myopia, progressive retinal atrophy, and juvenile cataracts.²¹ Previously published ocular abnormalities in WWS have been congenital, including cataracts, microphthalmia, buphthalmus, persistent hyperplastic primary vitreous, and Peter anomaly.^{2,3,16}

The MEB gene was recently localized to chromosome 1p32-p34 by cosegregation analysis and homozygosity mapping in one Turkish and seven Finnish families.²² The localization of the MEB gene provides the opportunity to investigate whether or not WWS may be allelic to MEB or whether it is due to mutations in a different gene. We have undertaken a clinical and linkage study in 19 families of different ethnic origins in which the affected children have a diagnosis of either MEB or WWS. The linkage analysis at the MEB locus on 1p32-p34 was under-

taken assuming a single phenotype (MEB or WWS) to define the affected status and allowing for locus heterogeneity. The results provide strong evidence that MEB and WWS are distinct clinical and genetic disorders.

Methods. *Inclusion criteria.* MEB and WWS are rare worldwide, and thus patients in this study were referred from several centers. All had to have CMD indicated by elevated serum creatine kinase level or abnormal muscle biopsy, a malformation of neuronal migration compatible with cobblestone complex based on either neuropathologic or MR data, and ocular abnormalities. We considered the term "lissencephaly type 2"¹⁶ as a synonym for cobblestone complex. We also included fetuses diagnosed as having "lissencephaly" by the referring physician. Patients in whom the cortical structure could not be defined in the presence of hydrocephalus, but in whom other features of the cobblestone complex were present, were also included. Eye abnormalities were recorded but were not used in classification of patients to either the MEB or the WWS group.

Patients. Nineteen families including 29 individuals with a diagnosis of MEB or WWS were included in the study. The patients were diagnosed at university hospitals in Sweden, Germany, the Netherlands, the United Kingdom, Turkey, Israel, and the U.S.A. Sixteen of the families were consanguineous, the parents most often being first cousins. The subjects included 12 Turkish families (1 to 3, 16, based in Turkey; 5, 6, 8, 9, from the Netherlands; and 10, 11, 13, 14, from Germany), 2 Pakistani families (12, 19), 2 Swedish families (4, 17), 1 Palestinian (15), 1 Dutch (7), and 1 American (18) family. The pedigree structures, including the consanguinity loops, are shown in figure 1. Corresponding database numbers from the Lissencephaly Research Project are as follows: LP99-153, Family 3; LP95-146, Family 10; LP95-148, Family 11; LP97-123, Family 12; LP95-147a1-a3, Family 13; LP96-042a1-a3, Family 14; LP96-037a1 and a2, Family 15; LP96-017a1 and a2; Family 16; LP96-056a1 and a2, Family 18; and LP95-052, Family 19. At the time of the latest investigation, the subjects were between 9 months and 13 years old. Six infants had died between 49 days and 3 years of age. Seven pregnancies had been terminated because of an affected fetus with hydrocephalus. A summary of the clinical data is presented in table 1.

The clinical features of all patients and MR patterns of 19 patients were re-evaluated by two clinicians and a neuroradiologist. A diagnosis of MEB was based on the previously published findings on Finnish MEB patients.^{19,27-29} These included survival past age 3 years, cobblestone complex on MRI with mild or moderate cerebellar/vermis hypoplasia, absent or moderate ventricular dilatation, and an intact corpus callosum that was often thin if hydrocephalus was present. The diagnosis of WWS was made if the patient had died in infancy, MRI showed a thin cerebral mantle and smooth cerebral surface, the cerebellar/vermis atrophy was severe, the corpus callosum was missing, and the cerebral hemispheres were fused. Three of the WWS patients and two WWS fetuses also had an occipital encephalocele (tables 1 and 2). A diagnosis of "lissencephaly" was considered to be compatible with WWS. Extensive,

Table 1 Clinical features of 29 muscle–eye–brain (MEB)/Walker–Warburg syndrome (WWS) patients of various ethnic origins

Family	Origin	Patient/age, y (age at death)	Encephalocele	Ocular abnormality	CK (U/L)/age at measure*
WWS†					
1	Turkish	II-1/6 mo	+	Buphthalmus, anterior chamber dysgenesis, congenital cataract, hyaloid artery, optic nerve atrophy	2716/2 mo
5	Turkish	III-1/(49 d)	+	Microphthalmia, corneal clouding, anterior chamber dysgenesis	>10000
6	Turkish	II-1/(8 mo)	+	Microphthalmia, congenital cataract, anterior chamber dysgenesis, glaucoma, myopia	2000
		II-2/fetus	ND		
8	Turkish	II-2/(59 d)	–	Glaucoma, optic nerve hypoplasia	>2000
9	Turkish	II-1/fetus (27 wkg)	–		ND
		II-2/fetus (22 wkg)	–	Retinal dysplasia	ND
		II-3/fetus (20 wkg)	–	Retinal dysplasia	ND
12	Pakistani	II-2/(3 y)	–	Buphthalmus, corneal clouding	15572/1 mo
13	Turkish	II-1/(5 mo)	–	Microphthalmia, congenital cataract	ND
		II-2/fetus (23 wkg)	+		ND
		II-3/fetus (20 wkg)	–		ND
14	Turkish	II-1/(3 y)	–	Microphthalmia, buphthalmus, megalocornea, glaucoma, pupillary membranes, retinal dysplasia	1716–4028
		II-2/fetus (20 wkg)	–		ND
		II-3/fetus (20 wkg)	+		ND
MEB†					
2	Turkish	II-3/5 y	–	Microphthalmia, coloboma, myopia, retinal dysplasia	654/3 y
3	Turkish	II-3/5 y	–	ND	798/3 y
4	Swedish	II-1/10 y	–	Myopia, retinal detachment	60
7	Dutch	II-1/10 y	–	Iris synechiae, optic nerve hypoplasia, retinal detachment and dysplasia	200–476/10 y
10	Turkish	II-1/4 y	–	Corneal opacity, myopia, retinal dysplasia	1202/2 y, 1050/3 y
11	Turkish	II-1/10 y	–	Coloboma, myopia, optic nerve atrophy, retinal dysplasia	1000–2000/>5 y
15	Palestinian	II-1/19 mo	–	Buphthalmus, anterior chamber dysgenesis, glaucoma, megalocornea, optic nerve	566/19 mo
		II-2/9 mo	–	Hypoplasia, hypopigmented retina, mild megalocornea	1890/3 mo
16	Turkish	II-6/8 y	–	Optic nerve hypoplasia, retinal dysplasia, persistent hyaloid artery	628
		II-7/5 y	–	Optic nerve atrophy, retinal dysplasia	1385/20 mo
17	Swedish	II-1/13 y	–	Myopia, optic nerve hypoplasia	18/11 y
18	USA	II-1/6 y	–	Myopia, retinopathy	735/4 y
		II-2/5 y	–	Microphthalmia, myopia, retinopathy, retinal detachment	2630/2.2 y
19	Pakistani	II-4/6 y	–	Myopia, retinopathy, high VEP	3000/2 mo, 1465/5 mo

* The normal range of CK measures was <280 U/L, except in Patients II-1 (Family 4), II-1 (Family 7), and II-1 (Family 17), in which the normal ranges were <6, <75, and <2.5, respectively. The majority of the CK values were 2–15 × the upper limit of normal, with occasional values exceeding 50×.

† Since the discovery of muscular dystrophy in WWS patients, several authors believed that the distinction between WWS and MEB was artificial. Patients reported from countries other than Finland were usually diagnosed as having WWS. In the current series, patients from Families 9,²³ 10 (Patient 4 in reference 24), 11 (Patient 3 in reference 24), 15,²⁵ and 16²⁶ were originally published as WWS.

VEP = visual evoked potential; wkg = gestation weeks; ND = not determined.

Table 2 Distinctive MRI criteria for MEB and WWS

Criteria	MEB	WWS
Cortical abnormality	Cobblestone cortex	Cobblestone cortex, thin cortical mantle, resembling lissencephaly
White matter abnormality	+, Age related	+++
Fused hemispheres	-	+
Dilated ventricles	-/++	+/+/+++
Absent septum	-/Broken	+
Corpus callosum	Normal/thin	Absent
Abnormal tectum	+	+
Flat pons	+	++
Hypoplastic vermis	++	+++
Absent vermis	-	+
Cerebellar hypoplasia	++	+++
Encephalocele	-	+

MEB = muscle-eye-brain disease; WWS = Walker-Warburg syndrome; - = absent; + = mild; ++ = moderate; +++ = severe.

confluent white matter abnormalities were considered typical of WWS or MEB in infants.

Genomic DNA isolation and genotyping. Genomic DNA was isolated from venous blood or lymphoblastoid cell lines using standard methods, after informed consent. For analysis of the MEB locus on chromosome 1p32-p34, we used nine dinucleotide repeat markers (D1S2743, D1S193, D1S211, D1S2677, D1S421, D1S2802, D1S451, D1S197, and D1S200) from the Généthon collection³⁰ and one tetranucleotide repeat marker (D1S3175) from the Cooperative Human Linkage Center set.³¹ The order of and distances between the markers are as follows: tel-D1S2743-2.8 cM-D1S193-1.5 cM-D1S211-2.3 cM-(D1S2677, D1S421, D1S2802, D1S451, D1S3175)-0.7 cM-D1S197-6.0 cM-D1S200-cen. MEB is localized within 0 cM from markers D1S2677, D1S421, D1S2802, D1S451, and D1S3175 (B. Command et al., unpublished data).

Primers were purchased from the MapPairs set (Research Genetics, Huntsville, AL) or synthesized using sequence data from the Généthon public database (<http://ftp.genethon.fr/pub/Gmap>). The forward primers were modified at the 5' end with a FAM, TET, or HEX fluorescent label. PCR reactions were performed as previously described.²² The amplified products were separated by electrophoresis on a 4.25% polyacrylamide/6 M urea gel (FMC, Rockland, ME) using a 377 DNA sequencer apparatus (Applied Biosystems, Weiterstadt, Germany). The results were processed by Genescan v2.02 and Genotyper v1.1 software (Applied Biosystems).

Linkage analysis. The simulation programs SLINK and MSIM v2.51³² were used to compute the maximum expected pairwise lod scores in the panel of MEB/WWS families. One hundred replicates were run, assuming genetic homogeneity and an average marker heterozygosity value of 0.7.

Pairwise lod scores were calculated using the MLINK program from the LINKAGE package v5.2,³³ assuming au-

tosomal recessive inheritance with 100% penetrance and a disease allele frequency of 0.005. Allele frequencies of the polymorphic markers were assumed to be equal.

The HOMOG program v3.33 was used to test the family panel for genetic heterogeneity at the MEB locus. HOMOG carries out an admixture test^{34,35} under the following hypotheses: H_0 (no linkage), H_1 (linkage and homogeneity), and H_2 (linkage and heterogeneity). In addition, HOMOG was used to calculate the conditional probability of linkage to the MEB locus for each of the 19 families.

The GENEHUNTER program v1.1³⁶ was used to compute multipoint lod scores over a fixed map of markers (D1S193-1.5 cM-D1S211-2.3 cM-D1S451, MEB-0.7 cM-D1S197-6.0 cM-D1S200), assuming either homogeneity or heterogeneity. An α value (proportion of linked families) of 0.55, as calculated by the HOMOG program, was used.

Haplotypes were constructed using GENEHUNTER, assuming the minimum number of recombinations.

Results. Clinical study of patients with MEB and WWS.

The 19 families were divided into two phenotypic groups after re-evaluation of clinical features and MR scans. Of the 19 families included in the study, patients in 11 were diagnosed as having MEB and in 8 with WWS (table 1). Four families originally published as having WWS (10, 11, 15, and 16; see table 1) were reclassified as MEB. In the remaining families, the original clinical diagnosis did not change after our re-evaluation of the clinical and MR data. The clinical findings are presented below and in table 1. Cranial MR patterns characteristic of MEB and WWS are shown in figure 2, and the distinctive MR criteria are summarized in table 2.

Patients with MEB. Fourteen patients from 11 families (2 to 4, 7, 10, 11, 15 to 19) were classified as having MEB (table 1). All were alive and their ages varied from 9 months to 13 years. All had MR changes compatible with the MEB pattern cobblestone complex seen in Finnish MEB patients (figure 2).^{27,28} Ocular changes consisted of microphthalmia, colobomas, buphthalmus, anterior chamber dysgenesis, glaucoma, optic nerve hypoplasia, and retinal dysplasia. None of the patients had congenital cataracts. Visual evoked potentials were recorded in one patient (II-1, Family 15) and showed grossly increased amplitude as seen in the Finnish patients.^{21,27} Serum creatine kinase level was elevated in all patients. Muscle biopsies, when available, showed changes similar to those reported in Finnish patients, consisting of excessive amount of regenerating fibers in early biopsies and dystrophic changes with increased fat and connective tissue in biopsies taken after the first years of life. The clinical features and MR abnormalities corresponded to those in the Finnish MEB patients.

Patients with WWS. Seven patients and eight fetuses from eight families (1, 5, 6, 8, 9, 12 to 14) had a more severe clinical presentation and were classified as having WWS (table 1). Family 9 had three fetuses with hydrocephalus, severe cobblestone complex, and retinal dysplasia that were aborted at 20 to 27 weeks of gestation. The other five affected fetuses came from families 6, 13, and 14. All of the liveborn probands (Families 1, 5, 6, 12 to 14) had WWS pattern cobblestone complex. In the patient from Family 8, the presence of a "cobblestone cortex" could not be evaluated owing to severe hydrocephalus. However,

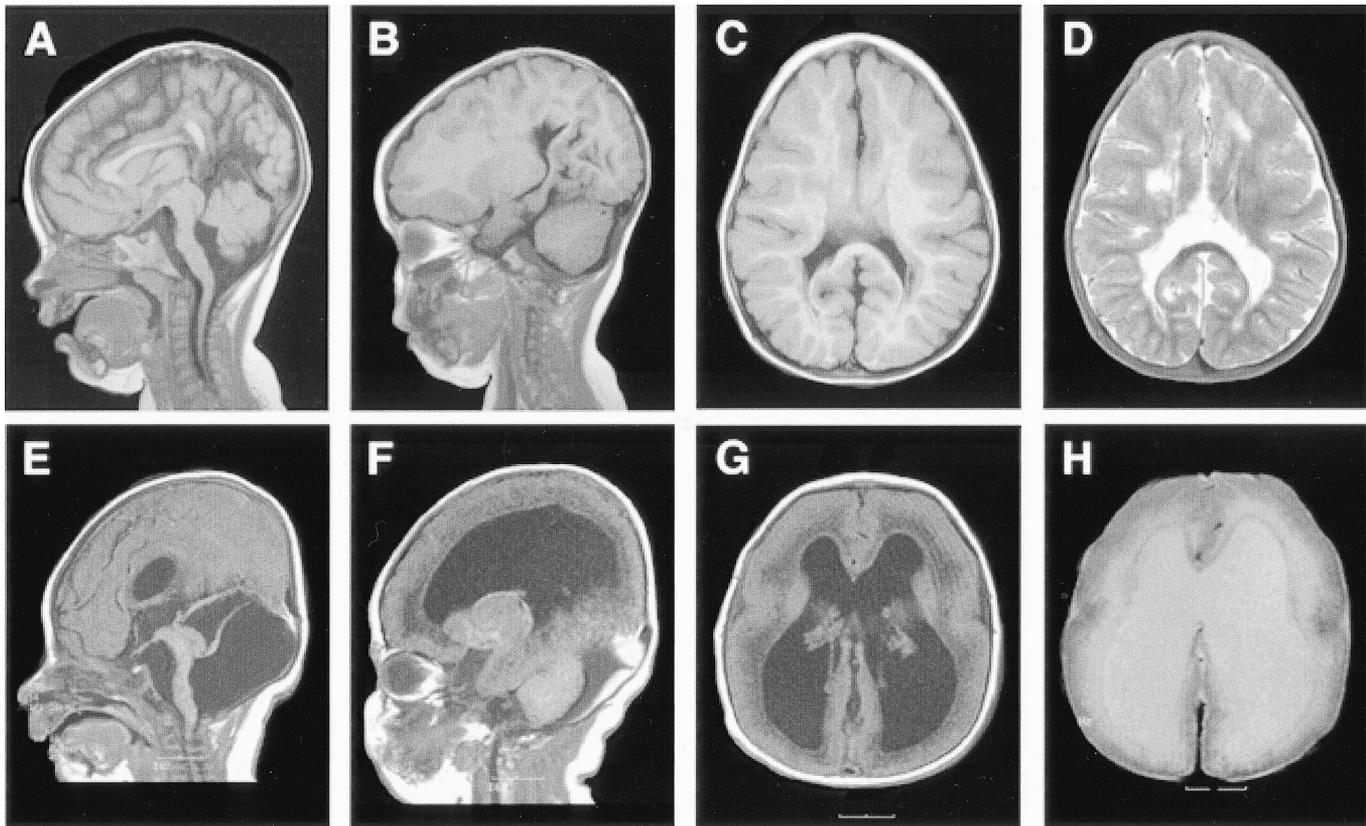


Figure 2. Comparison of MR abnormalities in muscle-eye-brain disease (MEB) (A to D) and Walker-Warburg syndrome (WWS) (E to H). Cranial MR images of patient II-1 (family 18) at age 3 years and patient II-1 (family 12) at age 4 days demonstrate typical MEB and WWS patterns of cobblestone complex. Midline sagittal images (A, E) show an intact although deformed corpus callosum, flat brainstem, and mild cerebellar vermis hypoplasia in MEB (A) compared with a severely hypoplastic corpus callosum, flat brainstem, and severe pancerebellar hypoplasia in WWS (E). Parasagittal images (B, F) show an irregular surface and atypical pachygyria in MEB (B) compared with a pebbled surface resembling lissencephaly in WWS (F). Axial T1-weighted images (C, G) show the same anteroposterior gradient with intermediate (5 to 8 mm) increased thickness of the cortex in MEB (G) compared with an almost smooth surface with no definite gradient, slightly thicker (7 to 10 mm) cortex, and absent or disrupted septum pellucidum in WWS (G). Finally, axial T2-weighted images show patchy increased signal of the white matter in MEB (D) compared with diffuse abnormal signal in WWS (H), although the child with WWS is much younger. The irregular cortical surface and cortical-white matter interface are well seen in both figures, although better in WWS (H).

other MR findings including vermis hypoplasia, flat pons, cerebellar hypoplasia, and a Dandy-Walker malformation together with death in early infancy and a high creatine kinase value were considered enough for a WWS diagnosis. Three patients and two fetuses from families 1, 5, 6, 13, and 14 had encephaloceles. Of the liveborn children with WWS, one was living at age 6 months (Family 1), two died at 3 years (Families 12 and 14), and four died before the age of 9 months. Ocular changes included microphthalmia, buphthalmus, congenital glaucoma, congenital cataract, corneal clouding, anterior chamber dysgenesis, and retinal dysplasia. Serum creatine kinase was elevated in all infants studied. Muscle biopsies, available in three infants with WWS, showed myopathic changes. Muscle studies were not performed in the first patient of Family 13, who died without a specific diagnosis. The diagnosis of WWS in this family was established in subsequent pregnancies. Muscular dystrophy could not be assessed in fetuses, as dystrophic changes are masked by rapid growth.

Linkage analysis of MEB locus: distinct loci for MEB and WWS. We analyzed the 19 MEB/WWS families as a

single nosological group. With use of the simulation program SLINK, this panel was estimated to give a maximum two-point lod score (Z_{\max}) of ≈ 14 at zero recombination fraction (θ), given genetic homogeneity. We calculated pairwise lod scores between the MEB/WWS phenotype and D1S451, the most informative of the markers showing no recombination with the MEB locus in Finnish families (B. Cormand et al., unpublished data). The results are shown in table 3. A combined lod score of -5.75 at $\theta = 0.00$ was obtained, assuming genetic homogeneity. However, 12 of the 19 pedigrees had positive lod scores (individual conditional probabilities of linkage between 0.66 and 0.97), suggesting genetic heterogeneity within the family panel, compatible with the haplotype data (see below).

We then evaluated the statistical validity of the hypothesis of genetic heterogeneity by using the HOMOG program to test the three likelihood ratios with the hypotheses H_0 , H_1 , and H_2 (see Methods) with marker D1S451. The evidence strongly favors linkage with heterogeneity. A joint test of linkage and heterogeneity (H_2 vs H_0) resulted in a likelihood ratio of 6,024 in favor of H_2 (χ^2

Table 3 Pairwise lod scores for linkage between the disease phenotype and marker D1S451 on chromosome 1p32-p34, and conditional probability of linkage (CPL) for each of the 19 MEB/WWS pedigrees

Family	Phenotype	lod score ($\theta = 0.00$)	CPL
1	WWS	-1.35	0.05
5	WWS	-5.88	0.00
6	WWS	-1.28	0.05
8	WWS	-1.31	0.05
9	WWS	-1.31	0.05
12*	WWS	+1.51	0.97
13†	WWS	0.29	0.66
14	WWS	-1.33	0.05
2	MEB	0.30	0.66
3	MEB	0.83	0.87
4	MEB	0.12	0.57
7	MEB	0.99	0.91
10	MEB	0.88	0.88
11	MEB	0.48	0.75
15	MEB	1.14	0.93
16	MEB	0.98	0.91
17*	MEB	-1.31	0.05
18	MEB	0.30	0.67
19	MEB	0.20	0.61
Total		-5.75	

* Families with discordant linkage data.

† In Family 13, the lod score value for D1S451 was slightly positive due to lack of informativity in the father. Linkage analysis using the neighboring markers provided negative lod score results and a CPL value close to 0.

WWS = Walker-Warburg syndrome; MEB = muscle-eye-brain disease.

= 17.41, $df = 2$, $p < 0.001$), whereas the test for heterogeneity given linkage against homogeneity (H_2 versus H_1) gave a p value of < 0.05 ($\chi^2 = 4.21$, $df = 1$, likelihood ratio = 8.20). Maximum likelihood for linkage to D1S451 was obtained with an α value of 0.55, indicating that 10 or 11 families from the set are consistent with linkage to 1p32-p34.

We used the GENEHUNTER program to compute multipoint lod scores over the MEB region under two different assumptions: homogeneity and heterogeneity using an α value of 0.55 (figure 3). The multipoint lod score under homogeneity was -3.92 at marker D1S451, but it increased to a significant positive lod score of $+7.02$ under the hypothesis of heterogeneity.

As several families were consanguineous, we constructed haplotypes for alleles of 10 markers in the MEB region to gain further data for or against linkage using homozygosity criteria. The haplotypes for the 19 families are shown in figure 1. Eight of the 16 pedigrees with known consanguinity (2, 3, 7, 10, 11, 12, 15, and 19) were homozygous at markers encompassing the MEB locus. Homozygosity was also seen in Family 16, but in this case no

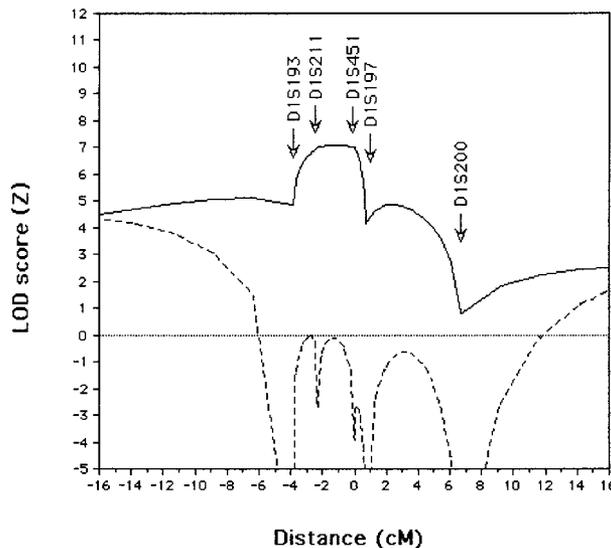


Figure 3. Multipoint linkage analysis between the disease phenotype and five markers on chromosome 1p32-p34, performed with GENEHUNTER. The marker names are indicated on the graph. Locus D1S451 was selected as a starting point for the map. The calculations were run under the hypotheses of homogeneity (dotted line) and heterogeneity with an α value (proportion of linked families) of 0.55 (solid line).

consanguinity was recognized, although both parents were born in a small isolated village in southeastern Turkey. In contrast, the MEB locus was excluded by lack of homozygosity in pedigrees 1, 5, 6, 8, 9, 13, 14, and 17. In the remaining two nonconsanguineous families (4 and 18), the disease phenotype cosegregated with markers on chromosome 1p. The haplotype data thus were consistent with 11 pedigrees being linked to the MEB locus and 8 being unlinked.

Genotype-phenotype correlation. We subsequently evaluated the correlation between the clinical phenotype and linkage to 1p32-p34 in the family resource. These data as well as clinical data on a group of previously studied Finnish patients linked to the MEB locus are summarized in table 4. The affected children from 11 families (2 to 4, 7, 10, 11, 15 to 19) fulfilled criteria for MEB, whereas the affected children from 8 families (1, 5, 6, 8, 9, 12 to 14) had the more severe WWS. Ten of the 11 families with MEB probands had findings consistent with linkage to 1p32-p34. Only one of the eight families with WWS probands (Family 12) was consistent with linkage to the MEB locus.

Discussion. Substantial efforts have been made in the recent years to establish a coherent classification of syndromes with CMD³⁷ and severe brain malformation, including lissencephaly.³⁸ Our study focuses on two of three syndromes characterized by the coexistence of CMD and a complex brain malformation, which includes both defects of organogenesis and neuronal migration: FCMD,¹³ MEB,²⁹ and WWS.¹⁶ Although advances in brain imaging and muscle biochemistry have allowed a more detailed phenotypic characterization of this group of disorders, the overlapping clinical spectrum has made diagnosis in indi-

Table 4 Correlation between clinical signs and linkage to 1p32-p34 in MEB and WWS families

Characteristics	WWS phenotype		MEB phenotype		
	1p32 Excluded*	1p32 Not excluded*	1p32 Excluded*	1p32 Linked*	Finnish patients†
Number of families‡	7 (1, 5, 6, 8, 9, 13, 14)	1 (12)	1 (17)	10 (2-4, 7, 10, 11, 15, 16, 18, 19)	18
Number of patients	6 Patients, 8 fetuses	1	1	13	21
Alive	1	0	1	13	13
Age, y (age at death)	(0.1-3) (mean 0.8)	(3)	13	0.7-10 (mean 5.9)	7-42 (mean 22)
Brain malformation§	WWS pattern encephalocele	WWS pattern	MEB pattern	MEB pattern	MEB pattern

* Muscle-eye-brain disease (MEB) and Walker-Warburg syndrome (WWS) patients reported in this manuscript.

† MEB patients reported elsewhere.^{11,21,27,28}

‡ In parentheses, the family identification number in this manuscript.

§ For description of the different patterns, see table 2.

vidual patients difficult. It is increasingly clear that historical, eponymous classifications may represent an unreliable guide to the complex overlap of phenotypes generated by locus and allele heterogeneity. Therefore, their ultimate categorization must await resolution of their underlying molecular genetic basis. Although this goal is still far from completion, significant progress has been made during the last few years with the identification of the FCMD gene¹⁴ and the assignment of the MEB locus.²² Whereas the clinical features and genetic basis of FCMD are well characterized,^{13,14,39} the nosological relationship between WWS and MEB has remained controversial.^{16-18,29,38}

The clinical delineation of MEB was originally based on a group of Finnish patients.⁹⁻¹¹ All were retarded, floppy infants with suspected blindness and elevated creatine kinase values. Neuropathologic studies¹⁹ and characteristic MR changes,²⁷ present in all but one of the clinically typical patients (unpublished data), further delineated this syndrome and allowed localization of the causative gene to chromosome 1p32-p34 by linkage analysis.²²

WWS was originally characterized as lissencephaly, severe eye malformations, and early death.^{1,2,3,40} The term "lissencephaly type 2" was introduced to describe the malformation, which included irregularly convoluted brain surface, flat brainstem, and cerebellar hypoplasia, and to distinguish it from classic lissencephaly.⁸ With subsequent recognition of CMD, the diagnostic criteria were broadened and approached those of MEB, and the distinction between WWS and MEB was considered artificial by some authors.¹⁶ During the last few years, cases with the combination of cortical dysplasia, eye anomalies, and CMD have been described as either MEB or WWS with no consensus on the diagnostic criteria. Both cerebral white matter changes and secondary laminin changes in the muscle membrane have been offered as possible differentiating abnormalities.^{18,24,26,41,42} However, these changes have not been

consistent enough to allow distinction in individual patients.

We undertook a clinical and linkage analysis to investigate whether or not WWS and MEB are allelic disorders. Because both WWS and MEB are rare, the families were collected from many countries. This geographic factor and the early death of WWS patients made it impossible to personally evaluate all patients clinically and achieve strict consensus criteria. Our clinical classification of the patients relied heavily on the MR scans, which were available for re-evaluation. Fourteen patients in 11 families were categorized as having MEB and 15 patients/fetuses in 8 families as WWS. The original clinical diagnosis changed from WWS to MEB in six patients from four families when the current clinical criteria were used. The genetic localization of the MEB gene on 1p32-p34 allowed us then to test linkage to this locus in the entire family resource. Both the haplotype results and a formal statistical analysis provided evidence for genetic heterogeneity in the 19 MEB and WWS families studied.

Inconsistency between the clinical phenotype and linkage to the MEB locus was observed in two families. The phenotype of all but one of the patients consistent with linkage to the MEB locus compared well with the phenotype of the Finnish patients. The one exception (Patient II-2, Family 12) was severely affected and survived until 3 years of age. Her MRI showed extreme ventricular dilatation with severe cerebellar hypoplasia and thus fulfilled our current criteria for WWS. There are two possible explanations for the observation of apparent linkage to the MEB locus in one of the WWS families. First, as the parents in Family 12 were second cousins from the grandmothers' side or first cousins once removed from the grandfathers' side, the child would be expected to be homozygous by descent at ≈ 1.5 to 3% of all loci in the genome.⁴³ Thus, this family might not be linked to 1p, with the true locus elsewhere. Second, the child could be homozygous for a mutation of the

MEB gene that results in a more severe phenotype. A variation of the phenotype due to allelic heterogeneity has been described in FCMD, where three patients with severe, clinically WWS-like FCMD who were heterozygous for the FCMD Japanese founder mutation and a nonsense mutation have been reported.^{20,44} Of the 11 families with a less severe phenotype, compatible with the definition of MEB as established in Finnish patients,²⁹ linkage to the MEB locus was supported in all but 1 (Family 17). The proband of this consanguineous Swedish family was a 13-year-old girl with typical MEB pattern MR findings, myopia, and an elevated creatine kinase level, thus fulfilling our criteria for diagnosis of MEB. However, linkage to the MEB locus was excluded by lack of homozygosity. So far, this is the only patient studied whose phenotype is compatible with the current diagnostic criteria for MEB that is not linked to the 1p locus. Either this patient is a phenocopy, or genetic heterogeneity exists even within the MEB phenotype, as defined in this study.

Owing to a founder effect, the Finnish patients with MEB are likely to be genetically homogeneous, which would be expected to reduce clinical variability as well. Accordingly, all the Finnish patients fulfilling the clinical and neuroradiologic criteria for MEB were found to be genetically homogeneous (B. Cormand et al., unpublished data). The data on non-Finnish patients in the current study did not alter the previously established clinical criteria for MEB. In particular, data on Finnish MEB patients have revealed survival past early childhood: One-third of the patients had died during a follow-up period extending 20 years, the youngest ones at the age of 6 years.²⁹ Accordingly, all patients with MEB linked to 1p32-p34 in the current series were alive, their ages ranging from 9 months to 10 years. Conversely, WWS has been considered lethal in infancy.^{1,6} Of the WWS patients in this series, excluded from 1p32-p34, one survived until 3 years of age, four died before 1 year of age, and one was a stillborn fetus.

The MR features previously used in differentiating WWS from MEB also remain valid.^{27,38} Only one patient (Family 12), whose WWS diagnosis was based on the severity of MR findings, had findings compatible with linkage to the MEB locus. All five patients with occipital encephalocele were excluded from the MEB locus. Although hydrocephalus occurs in both groups, it was more severe in WWS. White matter changes have been considered typical of WWS but rare in MEB.²⁸ In the present series, however, diffuse white matter changes were seen in MEB patients studied before the age of 1.5 years and patchy changes were visible until at least the age of 3 years and often later. Thus, the current study shows that white matter changes also occur in MEB and are highly age dependent as in FCMD.⁴⁵

The ocular changes of the current patients with MEB were more variable than in the Finnish MEB patients and overlapped to a large extent with those seen in WWS. Therefore, the ocular changes cannot

be used as differential diagnostic criteria between MEB and WWS. Microphthalmia, corneal clouding, glaucoma, retinal dystrophy, and optic atrophy occurred in both groups. None of the MEB patients had congenital cataracts. The ocular changes in WWS have been considered to be more of a congenital malformation type with Peter anomaly, anterior chamber dysgenesis, and microphthalmia. However, short survival of WWS patients prevents proof of progression of the eye abnormalities. The longer life span of MEB patients, however, has enabled a recording of the progressive nature of the ocular findings during follow-up. The current theory is that the coarse trabecular meshwork leads to an increased intraocular pressure with early glaucoma and progressive myopia, leading to atrophy of the retina.^{19,21} Retinal detachment, microphthalmia, and juvenile cataracts are late manifestations. The data available made it impossible to reconstruct the sequence, if any, of these events in the MEB patients in the present series.

Although our results show a correlation between the milder phenotype and linkage to the MEB locus, one should be cautious when studying individual families, as nuclear pedigrees are usually not big enough to provide significant linkage results. When consanguinity is present, lack of homozygosity at markers encompassing the region of the disease locus can be considered as an indication of nonlinkage even in families with only one affected individual. Only the identification of the MEB gene and the subsequent potential to detect mutations will eventually prove or exclude the involvement of this locus in each individual pedigree.

MEB was first described in Finland where it is more prevalent than elsewhere owing to a strong founder effect followed by genetic drift.^{46,47} Only a few patients have been tentatively diagnosed with MEB outside Finland.^{48,49} In contrast, WWS has been reported to have a worldwide distribution.³⁸ The results obtained in our current study demonstrate that MEB occurs in patients of variable ethnic origins.

Our results are a starting point for genetic mapping of the WWS gene. This is likely to be a more difficult challenge than mapping of the FCMD and MEB genes owing in part to the early death of most WWS patients, which hinders the collection of DNA samples. Also, WWS may be genetically heterogeneous. The ascertainment of families with a homogeneous population background (as in the cases of FCMD and MEB) or the use of large consanguineous pedigrees that can provide sufficient linkage strength by themselves may help to circumvent the potential problem of heterogeneity.

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