

# Worldwide distribution and broader clinical spectrum of muscle–eye–brain disease

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**Muscle–eye–brain disease (MEB), an autosomal recessive disorder prevalent in Finland, is characterized by congenital muscular dystrophy, brain malformation and ocular abnormalities. Since the MEB phenotype overlaps substantially with those of Fukuyama-type congenital muscular dystrophy (FCMD) and Walker–Warburg syndrome (WWS), these three diseases are thought to result from a similar pathomechanism. Recently, we showed that MEB is caused by mutations in the protein O-linked mannose  $\beta$ 1,2-N-acetylglucosaminyltransferase 1 (*POMGnT1*) gene. We describe here the identification of seven novel disease-causing mutations in six of not only non-Finnish Caucasian but also Japanese and Korean patients with suspected MEB, severe FCMD or WWS. Including six previously reported mutations, the 13 disease-causing mutations we have found thus far are dispersed throughout the entire *POMGnT1* gene. We also observed a slight correlation between the location of the mutation and clinical severity in the brain: patients with mutations near the 5' terminus of the *POMGnT1* coding region show relatively severe brain symptoms such as hydrocephalus, while patients with mutations near the 3' terminus have milder phenotypes. Our results indicate that MEB may exist in population groups outside of Finland, with a worldwide distribution beyond our expectations, and that the clinical spectrum of MEB is broader than recognized previously. These findings emphasize the importance of considering MEB and searching for *POMGnT1* mutations in WWS or other congenital muscular dystrophy patients worldwide.**

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## INTRODUCTION

Muscle–eye–brain disease (MEB; MIM 253280) is an autosomal recessive disorder characterized by congenital muscular dystrophy (CMD), ocular abnormalities and brain malformation (type II lissencephaly) (1). Patients with MEB show congenital muscular dystrophy, severe congenital myopia, congenital glaucoma, pallor of the optic discs, retinal hypoplasia, mental retardation, hydrocephalus, abnormal electroencephalograms and myoclonic jerks. From birth, infants with MEB are floppy with generalized muscle weakness, including facial and neck muscles. Muscle biopsies show dystrophic changes, and brain MRIs reveal pachygyria-type cortical neuronal migration disorder, flat brainstem and cerebellar hypoplasia. Walker–Warburg syndrome (WWS; MIM 236670) is another extreme of CMD, which shows the most severe brain malformation, characterized by type II lissencephaly and eye involvement. WWS is usually lethal within the first year of life (2). Fukuyama-type congenital muscular dystrophy (FCMD; MIM 253800) is a recessively inherited CMD with type II lissencephaly that occurs exclusively in Japan (3). In some cases, the clinical resemblance makes it difficult to differentiate between MEB, FCMD and WWS. These three diseases are thought to be caused by a similar pathomechanism.

Molecular genetic studies have been helpful in defining subgroups of CMD. The genes responsible for both MEB and FCMD have been identified and characterized. Through linkage analysis, the gene responsible for MEB was localized to chromosome 1p32–34 (4), and we recently showed that MEB is caused by loss of function mutations in the gene encoding protein *O*-linked mannosyl  $\beta$ 1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1) (5). *O*-mannosylation is a rare type of glycosylation in mammals, occurring in a limited number of brain, nerve and skeletal muscle glycoproteins (6). Sialyl *O*-mannosyl glycan is known to be a laminin-binding ligand of  $\alpha$ -dystroglycan (7), and POMGnT1 catalyzes the transfer of *N*-acetylglucosamine to *O*-mannose of glycoproteins.

FCMD is caused by mutations in the *fukutin* gene on chromosome 9q31, which we positionally cloned previously (8–11). The function of *fukutin* is not yet clear; however, sequence analysis predicts it to be an enzyme that modifies cell-surface glycoproteins or glycolipids (12). Immunoreactivity to the glycans of  $\alpha$ -dystroglycan has been undetectable in skeletal muscle from both MEB and FCMD patients (13–15), and the core  $\alpha$ -dystroglycan protein shows an electrophoretic mobility shift (15). These findings have suggested a common pathomechanism for MEB and FCMD, in which defects in *O*-mannosylation compromise laminin binding. Identification of the genes responsible for MEB and FCMD now enables the definition of these complicated diseases at the molecular level, since their symptoms are often similar and complicated. In particular genetic analysis of FCMD is being performed frequently and has been highly informative (16).

WWS has been observed in many population groups with a worldwide distribution. In contrast, both MEB and FCMD show striking founder effects. MEB was first described in Finland, where it is most prevalent, owing to a strong founder effect followed by genetic drift (17). Consequently, most MEB patients have come from a small, geographically isolated

population in Finland, with few Caucasian exceptions. Most FCMD mutations can be traced to a single ancestral founder, who carried a 3 kb retrotransposal insertion in the 3' non-coding region of the *fukutin* gene (11,18). Thus far, FCMD patients have been identified exclusively in Japan.

We describe here the identification of different MEB-causing mutations in Japanese and Korean patients as well as Caucasian patients initially diagnosed as FCMD, MEB, or WWS. Our results show that MEB is present in diverse population groups with a worldwide distribution and has a broader clinical spectrum than previously expected. Furthermore, we have shown a slight genotype–phenotype correlation in the brain among the patients.

## RESULTS

### Patients and mutation analysis

In a previous study, we showed that mutations in the *POMGnT1* gene are the primary genetic defect in MEB. Mutation analysis and characterization of the gene product has demonstrated that MEB is inherited in a loss-of-function manner (5). In this study, we extended our analysis to screen the entire coding region and exon/intron flanking sequences of the *POMGnT1* gene for mutations in 30 patients who were clinically diagnosed for WWS, severe FCMD, or MEB. To determine whether MEB patients exist in Asia, we included Japanese and Korean patients in this study.

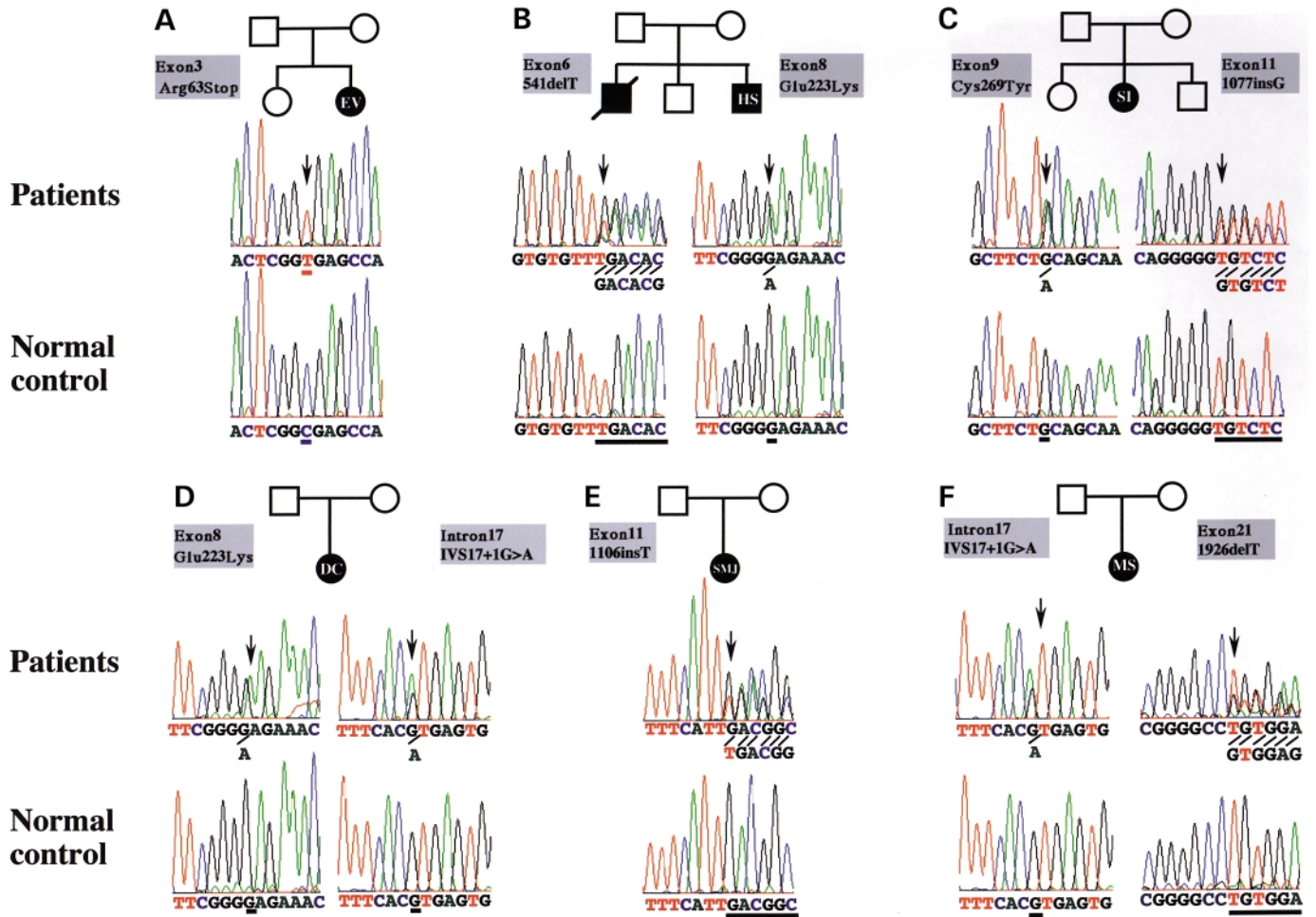
Our analysis identified seven novel mutations and one recurrent mutation in six patients (Fig. 1, Table 1). None of these individuals harbored mutations in the *fukutin* gene. Combined with our previous results, we have now identified a total of 13 different mutations in the *POMGnT1* gene.

Patient EV carried a homozygous C281T transition in exon 3, which results in an Arg63Stop nonsense mutation (Fig. 1A). EV is a 12-year-old Italian female who was hospitalized at one year of age for a ventriculo-peritoneal shunt operation for hydrocephalus. She is unable to speak or walk (Table 2).

Patient HS is a 12-year-old Japanese male. He is a compound heterozygote who carried a 1 bp deletion at base 541 in exon 6 (frameshift and premature termination at codon 167) and a G761A transition in exon 8 (Glu223Lys) (Fig. 1B). Severe hydrocephalus was observed prenatally by an ultrasonograph, and an MR image at 6 years of age showed extreme ventricular dilatation and agenesis of the septum pellucidum (Fig. 3A). Of all the patients examined, HS showed one of the more severe phenotypes (Table 2).

SI, a 7-year-old female Japanese patient, was identified as a compound heterozygote with a G900A transition in exon 9 (Cys269Tyr) and a 1 bp insertion at base 1077 in exon 11 (frameshift and premature termination at codon 338; Fig. 1C). Dilated ventricles were observed prenatally by an ultrasonograph, and, at one year of age, hydrocephalus required a ventriculo-peritoneal shunt. SI also shows a more severe phenotype (Table 2).

Patient DC, an 8-year-old Belgian female, is compound heterozygous for a G761A transition in exon 8 (Glu223Lys) and a G-to-A substitution in intron 17, which alters the conserved GT splicing donor sequence to AT (Fig. 1D). In our



**Figure 1.** Novel point mutations in six patients with MEB. (A) Patient EV carried a homozygous C281T transition in exon 3, which results in an Arg63Stop nonsense mutation. (B) Patient HS is a compound heterozygote who carries a 1 bp deletion at base 541 in exon 6 (frameshift) and a G761A transition in exon 8 (Glu223Lys). (C) Patient SI is compound heterozygous for a G900A transition in exon 9 (Cys269Tyr) and a 1 bp insertion at base 1077 in exon 11 (frameshift). (D) Patient DC is compound heterozygous for a G761A transition in exon 8 (Glu223Lys) and a G-to-A substitution in intron 17, which causes abnormal splicing. (E) Patient SMJ was shown to be a putative compound heterozygote who carries a 1 bp insertion at base 1106 in exon 11 (frameshift). Thus far, no coding region mutation has been detected in the other allele. (F) Patient MS is compound heterozygous for a G-to-A substitution in intron 17 (abnormal splicing) and a 1 bp deletion at base 1926 in exon 21 (frameshift).

**Table 1.** Summary of novel mutations of the *POMGnT1* gene in MEB patients

Patients	Mutation	Location	Effect	Status
EV	281C>T	Exon3	Arg63Stop Nonsense	Homozygote
HS	541 del T	Exon6	Phe149 frameshift 167Stop	Compound heterozygote
	761G>A	Exon8	Glu223Lys Missense	
SI	900G>A	Exon9	Cys269Tyr Missense	Compound heterozygote
	1077 ins G	Exon11	Val328 frameshift 338Stop	
DC	761G>A	Exon8	Glu223Lys Missense	Compound heterozygote
	IVS17+1G>A <sup>a</sup>	Intron17	Glu514read-through 526Stop/Leu472-His513del	
SMJ	1106 ins T	Exon11	Asp338 frameshift 338Stop	Compound heterozygote
	?	Noncoding region?	?	
MS	IVS17+1G>A <sup>a</sup>	Intron17	Glu514read-through 526Stop/Leu472-His513del	Compound heterozygote
	1926 del T	Exon21	Leu611 frameshift 633Stop	

<sup>a</sup>Mutation was reported in the previous study (5).

**Table 2.** Clinical features of MEB patients

Patient	EV	HS	SI	DC	SMJ	KO <sup>a</sup>	YA <sup>a</sup>	SA <sup>a</sup>	MK <sup>a</sup>	CC <sup>a</sup>	MS	TLG <sup>a</sup>
Origin	Italy	Japan	Japan	Belgium	Korea	Turkey	Turkey	Turkey	Turkey	Turkey	USA/Japan	France
Age (years)	12	12	7	8	6	12	6	7	5	10	25	3
Clinical diagnosis	Atypical WWS	WWS or MEB	MEB or severe FCMD	FCMD or MEB	FCMD	MEB	MEB	MEB	MEB	MEB	A milder WWS	MEB
Mutation (location)	Arg63Stop (exon 3)	541delT (exon 6) Glu223Lys (exon 8)	Cys269Tyr (exon 9) 1077insG (exon 11)	Glu223Lys (exon 8) IVS17+ 1G>A (intron 17)	1106insT (exon 11) ni (noncoding?)	IVS17+ 1G>T (intron 17)	IVS17+ 1G>T (intron 17)	IVS17+ 1G>A (intron 17)	Ser535-Ser550 del (exon 19)	1813delC (exon 20)	IVS17+ 1G> (intron 17) 1926delT (exon 21)	Pro493Arg (exon 17) 1970delG (exon 21)
<i>Brain</i>												
Mental retardation	+++	+++	+++	+++	ni	+++	++	++	+++	+++	++	+++
Speech	No words	No words	No words	Single words	No words	No words	No words	ni	ni	No words	A single word	No words
IQ/DQ	IQ < 30	DQ < 10	DQ < 20	IQ < 30	ni	ni	ni	ni	ni	ni	DQ < 43	ni
Hydrocephalus	✓	✓	✓	✓	—	✓	ni	—	—	—	—	ni
Brainstem hypoplasia	+++	+++	++	+++	ni	+++	++	++	+++	+	+	+++
Septum pellucidum agenesis	—	✓	✓	—	—	✓	ni	—	—	—	—	ni
Corpus callosum hypoplasia	—	✓	✓	—	✓	✓	ni	✓	✓	✓	—	ni
White matter lucency	—	—	++	++	—	++	++	+	++	++	++	+
Type II lissencephaly	++	++	++	++	—	++	ni	+++	+++	+	++	++
Cerebellar vermis hypoplasia	✓	✓	—	—	✓	✓	✓	✓	✓	✓	✓	ni
<i>Eyes</i>												
Myopia	✓	✓	✓	✓	ni	✓	ni	ni	—	✓	✓	✓
Retinal dysplasia	✓	✓	✓	—	ni	—	ni	ni	✓	—	✓	—
Anterior chamber malformation	—	—	—	—	ni	✓	✓	ni	—	✓	—	—
Microphthalmia	—	—	✓	—	ni	—	✓	ni	—	—	—	—
High VEP	—	✓	✓	—	ni	ni	ni	—	ni	ni	✓	—
<i>Muscle</i>												
Maximum motor function (age)	Sit with support (10 years)	Head control (8 years)	No head control (7 years)	Sit with support (2 years)	ni	No Head control (12 years)	Head control (4 years)	head control (3 years)	No head control (8 years)	Sit with support (5 years)	Sit with support (3 years)	No head control (3 years)
CK(U/I) (age)	700 (10 years)	2365 (6 years)	852 (6 years)	1844 (ni)	ni	42271 (2 years)	434 (10 months)	844 (4 years)	628 (4 years)	1212 (3 years)	1339 (12 years)	4778 (1 year)

<sup>a</sup>Patients whose mutations were reported in the previous study (5). +++, severe; ++, moderate; +, mild; ✓, observed; —, not observed; ni, no information was obtained in these patients.

previous study, we found that the intron 17 mutation caused both read-through of intronic sequences, resulting in introduction of a premature termination codon, and skipping of the upstream exon 17, resulting in the deletion of 42 amino acids (5). In DC, severe myopia was found upon ophthalmological examination in the first months of life, although retinal dysplasia was not observed. Although she was able to sit with support and her speech was limited to three single words at 2 years of age, by 7 years of age she was severely hypotonic and mentally retarded. DC shows a relatively mild phenotype compared with the other patients examined (Table 2).

A 6-year-old female Korean patient, SMJ is a putative compound heterozygote who carried a 1 bp insertion at base 1106 in exon 11, causing a frameshift and premature termination at codon 338 (Fig. 1E). We were unable to detect a mutation in the other *POMGnT1* allele. It is possible that the second mutation may lie outside the coding sequence, perhaps in the promoter or a regulatory region of an intron.

Patient MS is the 25-year-old female child of a Japanese mother and an American father of Scandinavian origin (19). The *POMGnT1* allele inherited from her father harbors a G-to-A substitution in intron 17, which alters the GT splicing sequence. From her mother, MS inherited a 1 bp deletion at base 1926 in exon 21, which results in a frameshift and premature termination at codon 633 (Fig. 1F). MS was previously diagnosed with a milder form of WWS because her symptoms included relatively severe eye abnormalities and specific features such as severe hypoplasia of the cerebellar vermis and cataracts, which are common in WWS. However her mental retardation is relatively mild for MEB and she can indicate 'yes' or 'no' with gestures (Table 2).

In each case, mutations cosegregated within the pedigree (families HS, SI, SMJ, and MS). We screened at least 92 normal individuals for two missense changes (Cys269Tyr and Glu223Lys), excluding the possibility of polymorphism.

In addition, patient MK is one of the subjects examined in our previous report (5). MK is a 5-year-old Turkish male who does not show MEB-specific eye symptoms such as myopia. MK carried a homozygous G1743A transition in the final base of exon 19, which was previously reported as a missense mutation (Ser550Asn) (5). However, subsequent RT-PCR analysis of skeletal muscle from this patient has shown that this mutation causes skipping of exon 19, resulting in the deletion of 15 amino acids (data not shown).

### Genotype-phenotype correlation

We found that patients with MEB showed a broad range of severity of symptoms. In addition, we found that these patients possessed mutations that were scattered throughout the *POMGnT1* gene. To assess whether there is a genotype-phenotype correlation, we investigated the clinical features of the patients with regard to brain, eye and muscle, relative to the distribution of mutations throughout the *POMGnT1* gene (Table 2, Fig. 2). This analysis revealed a wider clinical spectrum of MEB than recognized previously. Taking into account each patient's clinical features, correlations between the location of the mutation and clinical severity seemed difficult to assess. However, a slight correlation of clinical severity in the brain was observed. Patients with mutations near

the 5' terminus of the *POMGnT1* coding region showed relatively severe brain symptoms, while patients with mutations near the 3' terminus had milder phenotypes. (Table 2). Hydrocephalus showed a particular correlation with mutations near the 5' terminus (Table 2, Fig. 3). For example, patient HS, who carried a 1 bp deletion in exon 6 and a missense mutation in exon 8, near the 5' terminus, was diagnosed as WWS or MEB and showed relatively severe phenotypes such as hydrocephalus (Table 2, Fig. 3A). On the other hand, patient CC carried a homozygous 1 bp deletion in exon 20, near the 3' terminus of the *POMGnT1* coding region. CC had a relatively mild phenotype without hydrocephalus (Table 2, Fig. 3B). These analyses suggest that the location of a mutation influences the severity of the MEB phenotype.

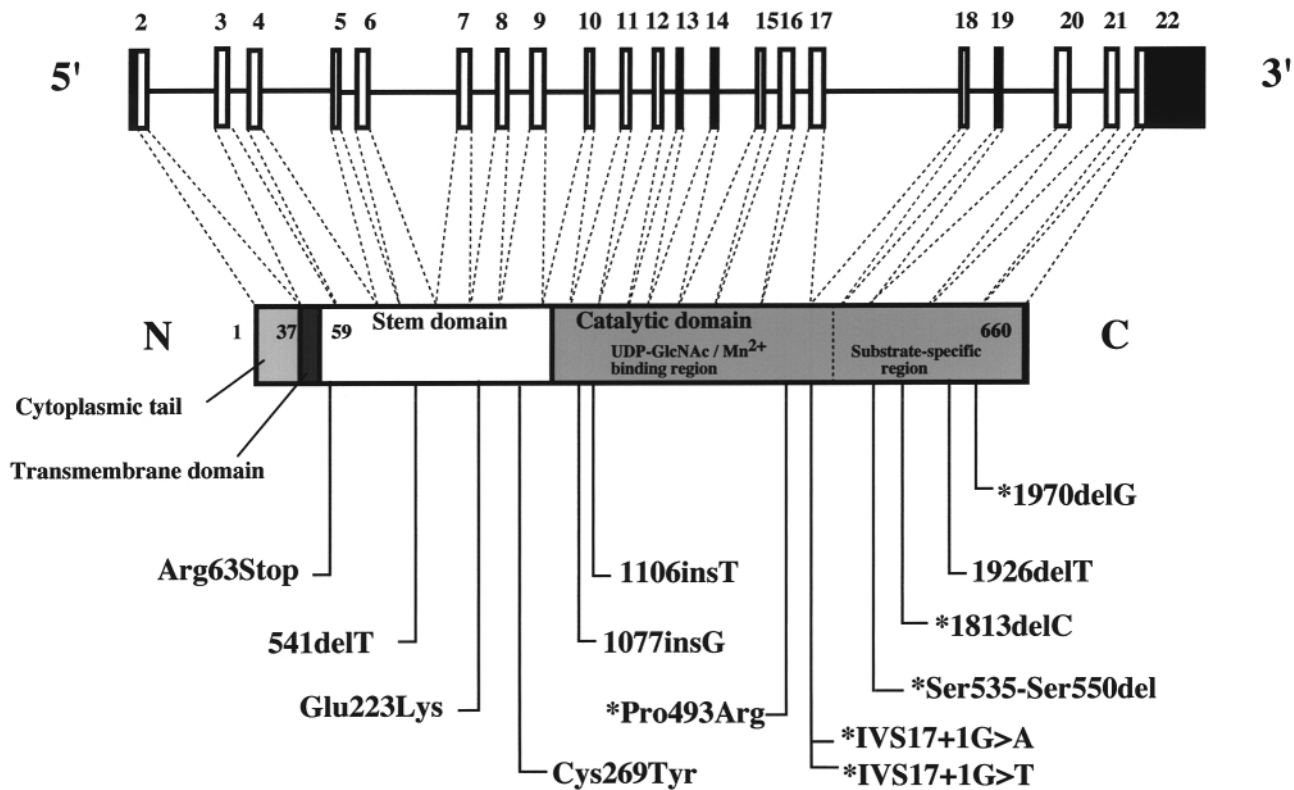
In addition, we examined the skeletal muscle tissue from both patient SI who carried mutations near the 5' terminus (Fig. 3C-E) and patient TLG who carried mutations near the 3' terminus (Fig. 3F-H) and found normal immunoreactivity for  $\beta$ -dystroglycan and laminin $\alpha$ 2 chain but greatly reduced staining for  $\alpha$ -dystroglycan. No obvious differences could be observed in the skeletal muscle of the two patients.

### DISCUSSION

The six *POMGnT1* mutations identified in the previous study were all simple point mutations (5), while most FCMD patients carry a quite rare insertion mutation in the *fukutin* gene and are found exclusively in Japan. These findings led us to hypothesize that MEB mutations might have a broader distribution outside Finland. A recent linkage study reported the occurrence of MEB in some Caucasians and classified MEB and WWS as distinct disorders (20). To test our hypothesis, we examined 30 patients from various countries, including Japan and Korea, who were diagnosed as WWS, severe FCMD or MEB. In addition to the six previously described mutations, we identified seven new mutations in this study. Therefore, MEB patients may exist with a broader distribution and more varied phenotypes than previously expected.

The 13 known mutations in the *POMGnT1* gene are dispersed throughout the entire coding region (Fig. 2), with no accumulation in any particular domain. The clinical features of MEB vary among patients, and evaluation of clinical severity in each individual patient is difficult. However, we observed a slight correlation between genotype and brain phenotype: patients with mutations in the vicinity of the 5' terminus of the *POMGnT1* gene show relatively severe WWS-like symptoms. All of the patients with *POMGnT1* mutations are still alive; hence, lifespan may be one of the differences between MEB and typical WWS, in which almost patients die before one year of age.

The amino acid sequence of POMGnT1 is homologous to  $\alpha$ -3-D-mannoside  $\beta$ -1,2-N-acetylglucosaminyltransferase I (GnT-I), which is a Golgi-resident enzyme involved in the N-linked oligosaccharide biosynthetic pathway. While the crystal structure of GnT-I has been determined (21), the structure of POMGnT1 itself has not been analyzed in detail. Computer analysis predicts that the 660-amino-acid POMGnT1 protein is divided into four domains: a cytoplasmic tail (Met1-Arg37), a transmembrane domain (Phe38-Ile58), a stem domain (Leu59-Leu300), the catalytic domain consisting of the UDP-GlcNAc



**Figure 2.** Schematic representation of the *POMGnT1* gene and the corresponding protein, showing the location of mutations found in MEB. Exons are represented by boxes, and introns are represented by lines. *POMGnT1* protein is divided into four domains. Mutations detected in this study and in the previous study are shown below the protein. The asterisk represents mutations reported in the previous study (5).

and  $Mn^{2+}$  binding regions (around Asn301–Leu530), and the substrate-specific region (around Arg531–Thr660) (5). Nonsense or frameshift mutations near the 5' terminus shorten the *POMGnT1* protein significantly, probably resulting in loss of function. Missense mutations in the stem domain may diminish retention of *POMGnT1* in the Golgi apparatus (22). Mutations in the 3' region of the gene may retain some ability to transfer sugars, since the catalytic domain of the protein is preserved to some extent. Measurement of the enzymatic activity of mutant *POMGnT1* proteins will be necessary to explain possible mechanisms for the genotype–phenotype correlation seen in this study.

MEB, FCMD and WWS are clinically similar, and the nosological classification of these disorders has been controversial. In MEB and FCMD patients, the lack of full *O*-mannosylation of  $\alpha$ -dystroglycan significantly disrupts the interactions of  $\alpha$ -dystroglycan with extracellular matrix ligands (15). This result suggests that post-translational disruption of dystroglycan–ligand interactions may be a common mechanism for muscular dystrophy with brain abnormalities. The structure of laminin-binding *O*-mannosyl glycan in dystroglycan is Sia $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man-Ser/Thr (7), where *POMGnT1* catalyzes the GlcNAc $\beta$ 1-2Man linkage (5). Since the clinical presentations of MEB and WWS significantly overlap, and the most severe brain malformation and shortest life span are striking features of WWS, we postulated that the gene product responsible for WWS may be a glycosyltransferase that

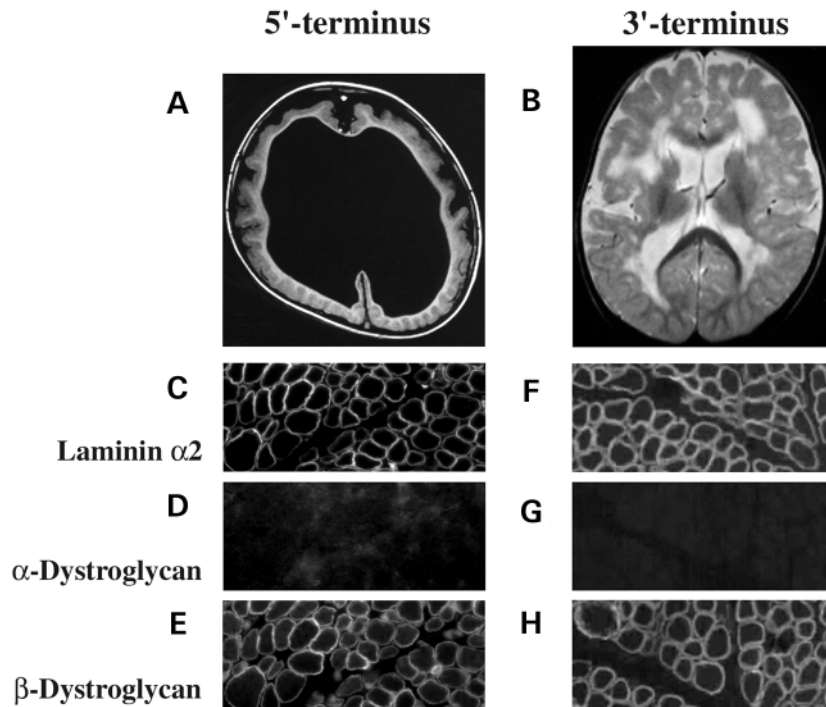
catalyzes the Man-Ser/Thr linkage in *O*-mannosyl glycans. Quite recently 20% of WWS patients have been found to have mutations in *POMT1*, a putative human counterpart of a yeast *O*-mannosyltransferase (23). In FCMD, compound heterozygotes for the FCMD founder mutation in the *fukutin* gene show severe phenotypes like WWS, and no patients have been identified with non-founder (point) mutations on both alleles, suggesting that such patients are embryonic lethal (16,24). Unlike FCMD, MEB patients with point mutations on both alleles can survive. We suppose that *fukutin* may perform a more essential role in early development than *POMGnT1*.

Further molecular genetic study will open new avenues for understanding the pathophysiological mechanisms underlying these complex disorders. It may be necessary and possible to re-classify muscular dystrophies based on genetic rather than clinical criteria. This study emphasizes the importance of considering MEB and searching for *POMGnT1* mutations in WWS or other CMD patients worldwide.

## MATERIALS AND METHODS

### Patients

We analyzed genomic DNA from 30 patients with CMD, brain malformation and ocular abnormalities. Information about the



**Figure 3.** Comparison of cranial MR images and immunohistochemical analysis in MEB patients. (A) An axial  $T_1$ -weighted image of patient HS (at 6 years of age) shows extreme ventricular dilatation with a slightly smooth cortical surface. (B) An axial  $T_2$ -weighted image of patient CC (at 13 months of age) shows pachygyria, slightly enlarged ventricles, and white matter abnormality. The mutation in patient CC has been reported previously (5). Consecutive frozen sections of skeletal muscle from patient SI (C–E) and patient TLG (F–H) immunostained with anti-laminin $\alpha_2$  chain (C, F),  $\alpha$ -dystroglycan (D, G), and  $\beta$ -dystroglycan antibodies (E, H).

six patients whose mutations were identified in this study (EV, HS, SI, DC, SMJ and MS) is briefly described in the Results section. All parents of these patients are not consanguineous. The six patients KO, YA, SA, MK, CC and TLG were described in the previous study (5). All phenotypes are summarized in Table 2.

#### Mutation analysis

Primers used for mutation analysis have been described previously (5). PCR products from patient genomic DNA were excised from gels, and direct sequencing was performed using Bigdye terminators (Applied Biosystems). Fragments were electrophoresed on an ABI Prism 3100 sequencer (Applied Biosystems).

#### Immunohistochemistry

Immunodetection was performed using a mouse monoclonal anti- $\alpha$ -dystroglycan antibody for patient SI (clone VIA4-1, Upstate Biotechnology), affinity-purified sheep antiserum directed against a 20-amino-acid C-terminal sequence of chick  $\alpha$ -dystroglycan (25) for patient TLG, a monoclonal anti- $\beta$ -dystroglycan (clone 8D5, Novocastra), a polyclonal anti- $\beta$ -dystroglycan (26) and a monoclonal anti-laminin  $\alpha_2$  chain antibody (clone 5H2, GibcoBRL, Chemicon). Skeletal muscle staining was performed as described previously (13).

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