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Case study

Severe muscle–eye–brain disease is associated with a homozygous mutation in the *POMGnT1* gene

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ABSTRACT

Muscle–eye–brain (MEB) disease is an autosomal recessive disorder characterized by a broad clinical spectrum including congenital muscular dystrophy, ocular abnormalities, and brain malformation (type-II lissencephaly). Herein, we report on two Turkish siblings with a homozygous mutation in the *POMGnT1* gene. A 6-year-old sibling has a severe form of MEB disease, which in some aspects is more suitable with the diagnosis of Walker–Warburg syndrome. However, the same mutation resulted in a less severe form of MEB in the older sibling, who is 14 years old. These two cases suggest that *POMGnT1* mutations may cause MEB disease with different phenotypes even in the same family.
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1. Introduction

Congenital muscular dystrophies (CMD) are rare autosomal ressesive diseases characterized by a brain malformation including neuronal migration defects, eye abnormalities, and muscular dystrophy. Different specific phenotypes have been described, many of them defined on molecular basis.[1,2] Abnormal glycosylation of α-dystroglycan (α-DG) in the muscle and brain is a key feature of these disorders.[3] The well-defined CMDs include Fukuyama CMD (FCMD), muscle–eye–brain (MEB) disease and Walker–Warburg syndrome (WWS).[4–6] These syndromes have been associated with mutations of at least six genes (Table 1). It has become evident that some of the underlying genes may cause a broad spectrum of phenotypes. For example, *O*-mannosyltransferase-1 (*POMT1*) gene can cause not only WWS but also type-II limb girdle muscular dystrophy.[7]

Lack of consistent ocular abnormalities in FCMD has allowed a clear clinical distinction of this syndrome, whereas the phenotypic distinction between MEB and WWS has remained diffucult.[8]

MEB disease is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities, and brain malformation (type-II lissencephaly) and is seen mainly in Finland. Ocular abnormalities include severe congenital myopia, congenital glaucoma, pallor of the optic discs, and retinal hypoplasia. Most of the infants with MEB are physically 'floppy' from birth with generalized muscle weakness that includes the facial and neck muscles. Muscle biopsies show dystrophic changes and brain magnetic resonance imaging (MRI) reveal a pachygyria-type cortical neuronal migration disorder, hydrocephalus, flat brain stem, and cerebellar hypoplasia.[9]

Herein, we report two Turkish siblings with MEB disease born to first cousin parents who have unique *O*-linked

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Table 1 – Detected mutations in the genes responsible for different congenital muscular dystrophy syndromes are depicted by + (rare) and ++ (common)

Genes	WWS	MEB	FCMD
POMT1	++		
POMGnT1	+	++	
FCMD	+		++
FKRP	+	+	
POMT2	+		
LARGE	+		

POMT1, *O*-mannosyltransferase-1; *POMGnT1*, *O*-mannose-β-1,2-*N*-acetylglucosaminyltransferase; *FKRP*, Fukutin-related protein; WWS, Walker–Warburg syndrome, MEB, muscle–eye–brain.

mannose β-1,2-*N*-acetylglucosaminyltransferase 1 (*POMGnT1*) gene mutation displaying differrent phenotypes.

2. Case reports

A 6-year-old boy living in an orphanage was admitted to our hospital with pneumonia and septic shock. He was born to first-degree cousin parents but no other information was known about the delivery or postnatal period. He had been diagnosed in a number of different hospitals with cerebral palsy, epilepsy, and malnutrition. As he had seizures and the EEG showed partial epilepsy, valproic acid therapy was initiated. He had been feed through a nasogastric tube for about a 1 year. The father was known to be healthy but the mother was reported to be bedridden and mentally retarded. He had a 14-year-old sister and 11-year-old brother. The brother was reported to be healthy but the sister was motor and mentally retarded with eye abnormalities.

On clinical examination, he had severe mental retardation with no oral communication. He had truncal hypotonia and contractures of the lower extremities. He could neither maintain head control nor could sit without support. He could not turn from one side to other side in bed. Deep tendon reflexes were absent. There was no musle atrophy or hypertrophy. Extrapyramidal signs were absent. He was also clinically blind with bilateral corneal clouding and microphthalmia on the right side.

Brain MRI showed lissencephaly, hypoplastic brainstem and cerebellar vermis, severe hydrocephalus, absent septum pellucidum, and thin corpus callosum. The white matter signal was increased especially in frontal areas, bilaterally. Also, persistant hyalinoid tissue was seen on the retrolentar area of the left eye (Fig. 1, Table 2). Serum creatine kinase level was 572 UI/l (nv < 172 UI/l). The electromyography (EMG) showed myopathic features. The peripheral blood chromosome analysis was normal. Musle biopsy could not be done as a written consent could not be obtained from his parents.

After therapy for his pneumonia and septic shock, he was discharged from the hospital as his blood gas analysis returned to normal. We were informed that he died at the orphan house with acute respiratuvary insufficiency a few months later. His breathing had been assisted by the use of a respiratory machine.

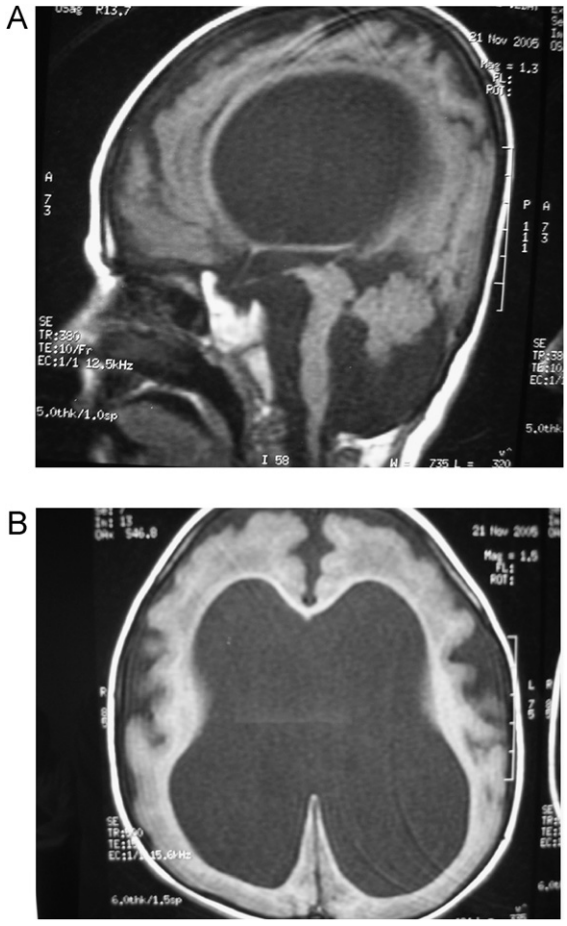

Fig. 1 – (A) Sagittal T1-weighed MR image demonstrating hypoplastic brainstem and cerebellar vermis, severe hydrocephalus and thin corpus callosum in the younger sibling. (B) Axial T1-weighed MR image showing lissencephaly and enlarged lateral ventricles in the younger sibling.

His 14-year-old sister is still living in the orphan house. In contrast to her brother, she had moderate mental retardation and could speak single words. Her neurologic examination revealed truncal hypotonia. She had good head control and was able to sit with support. Her mobilization was attained by wheel chair. Deep tendon reflexes were hypoactive. Ocular examination revealed bilateral cataracts and retinal dysplasia.

A brain MRI showed thickening of the cerebral cortex with shallow sulci, indicative of lissencephaly. Periventricular hyperintense areas on T2-weighed images and a decreased cerebral hemispheric volume were also noted. The MRI clearly showed the hydrocephaly and pontocerebellar hypoplasia (Fig. 2). Serum creatine kinase level was 1200 UI/l and EMG showed myopathic changes.

3. Mutation analysis

Informed consent was obtained from their legal guardians in accordance with protocols approved by the Institutional

Table 2 – Clinical features of patients compared with cobblestone lissencephalies

Symptom	MEB	WWS	Patient 1	Patient 2
Cortical abnormality	Pachygyria/ polymicrogyria	Agyria thin mantle	Agyria/pachygyria	Agyria/pachygyria
Hydrocephalus	++	+++	+++	++
White matter abnormality	+	+++	++	++
Corpus callosum	Normal/thin	Absent	Thin	Normal
Fused hemispheres	–	+	–	–
Absent septum	–	+	+	–
Cerebellar hypoplasia	++	+++	+++	++
Cerebellar vermis	Hypoplastic	Absent	Hypoplastic	Hypoplastic
Cerebellar cysts	++	Absent	Absent	Absent
Muscular dystrophy	+++	+++	+++	+++
Micropthalmia	+	++	+	Absent
Retinal defects	++	+++	++	++
Anterior chamber abnormalities	++	+++	+	+
High CK	++	+++	572 UI/l (nv < 172 UI/l)	1200 UI/l
Life expectancy	10–30 years	< 3 years	6 years (exitus)	14 years old
Motor function			No head control, bedridden, unable to roll from one side to another side on the bed	Good head control sit with support
Mental function			Severe mental retardation, no words	Moderate mental retardation can speak a few words

WWS, Walker–Warburg syndrome; MEB, muscle–eye–brain.

Review Board of Beth Israel Deaconess Medical Center and local institutions. Genomic DNA was extracted from peripheral blood lymphocytes using standard techniques. The SNP-based genome scan was performed at the Broad Institute (Cambridge, MA). Five hundred nanograms of genomic DNA were labeled and hybridized to Affymetrix GeneChip 250K StyI Arrays according to the manufacturer's instructions. Genotyping results were analyzed using dChip software.[10] Regions of homozygosity were visualized using the LOH function in dChip. For *POMGnT1* sequencing, primers were designed for each exon including at least 50 base pairs of flanking intronic sequences. Primers and amplification conditions are available upon request. Amplicons were purified using Agencourt AMPure PCR purification solution (Agencourt Bioscience Corp., Beverly, MA). Capillary sequencing was outsourced to SeqWright (Houston, TX). Genome-wide screening was performed by hybridizing genomic DNA from the two affected children on Affymetrix 250K SNP chips. Since the patients were born from a consanguineous marriage, we first analyzed regions of homozygosity at known MEB loci. Large overlapping stretches of homozygosity, 29.7 cM for the affected boy and 19.7 cM for the affected girl, were found in the region containing the *POMGnT1* gene on chromosome 1p34.1. For *POMGnT1* sequencing, primers were designed for amplification of all exons and flanking intron sequences. Mutation analysis was performed by direct sequencing of the resulting amplicons. A transition (G > A) was identified at the splice donor site of intron 17 (ca. 1539+1 G > A) in both siblings. Removal of this splice donor site results in skipping of exon 17 and causes an in-frame deletion of 42aa.[11]

4. Discussion

MEB disease is caused by mutations in the *POMGnT1* gene and has a broad clinical spectrum. In the present case report, we have shown that mutations in *POMGnT1* gene can cause a very severe phenotype like WWS.

It was difficult to make the initial diagnosis between MEB and WWS in the 6-year-old patient, because he had very severe motor and mental impairment and had eye abnormalities including cataract, microphthalmia which are more common in WWS. Further, he had extreme hydrocephalus, thin corpus callosum and absent septum pellucidum with lissencephaly which are more commonly attributed to WWS.[12] He died at the age of 6, thus the life span was short when compared to that seen in the classical presentation of MEB. His 14-year-old sister, who had the same mutation, has a milder clinical presentation and MRI findings (Table 2). Diesen et al. reported that both Finnish and non-Finnish patients with homozygous for the *POMGnT1* mutation showed wide variation in their phenotypic spectrum, with the mildest clinical manifestations and the severe end of the clinical spectrum.[11] However, our cases clearly show that *POMGnT1* mutations may be associated with a very different phenotypical spectrum even in the same family.

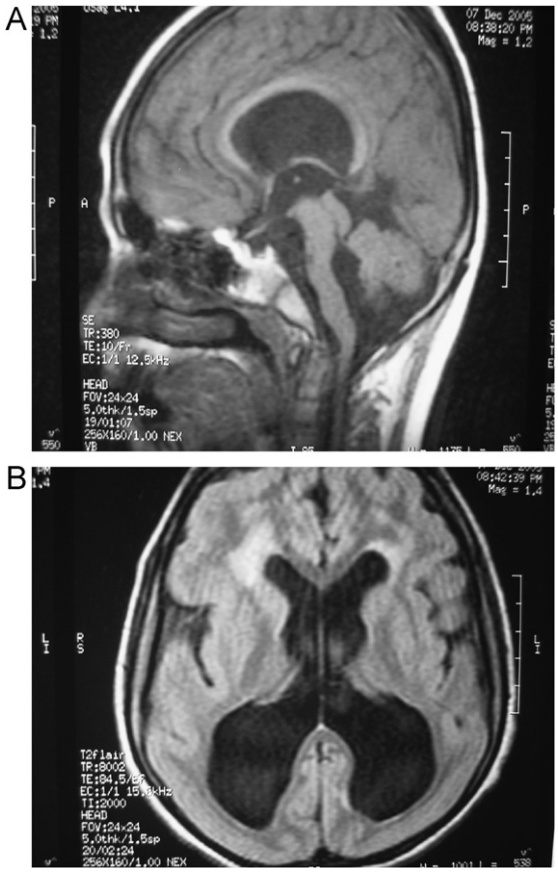

Fig. 2 – (A) Sagittal T1-weighed MR image demonstrating hypoplastic brainstem and cerebellar vermis. (B) Coranal flair T2-weighed image demonstrating lissencephaly and enlarged lateral ventricles with periventricular hyperintense areas.

The lack of *O*-linked glycosylation of α-DG is the key defect in cobblestone lissencephalies. *POMT1* is predicted to catalyze the first step in the *O*-linked protein mannosylation, the transfer of a mannosyl residue from dolichyl phosphate mannose to a serine or threonine residue in the target protein. *POMGnT1* takes care of the second step by adding an *N*-acetylglucosamine residue to a pre-existing protein O-linked mannose. Disrupted *O*-mannosylation of α-DG is seen in WWS and MEB patients with mutations in the *POMT1* and *POMGnT1* genes, respectively.[7] The phenotypical spectrum of congenital glycosylation disorders linked to different *POMT1* mutations have been considered much broader than initially anticipated like *POMGnT1* mutations. Balci et al. described five patients with a distinct clinical phenotype of limb girdle muscular dystrophy, mild microcephaly and mental retardation without any structural malformations of brain. These cases were found to be homozygous for *POMT1* mutation which were associated with a very severe clinical phenotype of WWS.[13]

Taniguchi et al.[14] reported 12 patients with MEB outside of Finland who had a wider clinical spectrum including atypical or mild WWS phenotypes. In their report, there were two cases with atypical WWS phenotypes who had severe mental retardation head control and ocular manifastations including myopia and retinal dysplasia. They also showed that 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. Two patients with atypical WWS had mutations near the 5′ terminus. Our 6-year-old case had more severe clinical findings compared to these two patients including the inability to control their heads or to speak and severe ocular impairment. It is interesting that a mutation in *POMGnT1* gene in our patient was near the 3′ terminus.

We conclude that the *POMGnT1* mutations can express themselves in severe MEB phenotype similar to WWS, so it might be better to classify CMD based on genetic rather than clinical criteria.

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