POMGnT1 Mutations in Congenital Muscular Dystrophy

Genotype-Phenotype Correlation and Expanded Clinical Spectrum

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Background: Muscle-eye-brain disease is a congenital muscular dystrophy with eye and brain involvement due to POMGnT1 mutations.

Objective: To describe the clinical and molecular features of 3 Italian patients with POMGnT1 mutations.

Design: Case reports.

Patients: One patient had muscle and brain abnormalities without eye involvement. Two patients had a classic muscle-eye-brain disease phenotype with different levels of clinical severity.

Results: Brain magnetic resonance imaging showed cortical malformation and posterior fossa involvement. Immunofluorescence for glycosylated α-dystroglycan performed on muscle biopsy specimens demonstrated an absent signal in 1 patient and reduced staining in 2 patients. Molecular analysis identified 5 mutations, 2 of which are novel.

Conclusion: This article adds to what is known about the genotype-phenotype correlation and expands our awareness of the clinical spectrum associated with POMGnT1 mutations.

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CASE 2 AND CASE 3

Patient 2 and patient 3 manifested ocular abnormalities and muscular hypotonia at birth and at 2 months of age, respectively. Epileptic seizures occurred only in patient 2, at age 10 months. Patient 2 at age 6 years had moderate mental retardation, and patient 3 at age 7 had severe mental retardation. Serum creatine kinase levels were elevated in both patients (1500 U/L in patient 2 and 1000 U/L in patient 3).

METHODS

MUSCLE BIOPSY SPECIMENS

After obtaining informed consent, muscle biopsy specimens were obtained for diagnostic purposes and were processed according to standard histological and immunohistochemical techniques. Immunohistochemical analysis was performed using the following monoclonal antibodies: antimouse α-dystroglycan (VIA4-1; Upstate Biotechnology, Lake Placid, NY), antilaminin α2 chain (merosin) (5H2; Chemicon, Milano, Italy), and antilaminin 2 α2 chain (4H8-2; ALEXIS Biochemicals, Lau sen, Switzerland).

MOLECULAR ANALYSIS

DNA was obtained from peripheral lymphocytes using standard techniques. The entire coding region and the exon-intron boundaries of POMGnT1 were amplified by polymerase chain reaction using a set of intronic primer pairs. Total RNA was prepared from a muscle biopsy sample of patient 2 using a kit from Qiagen (Qiagen Total RNA; Qiagen Inc, Studio City, Calif), and reverse transcriptase–polymerase chain reaction was performed using oligo-dT priming. Polymerase chain reaction products were purified and directly sequenced using a multicolor fluorescence-based DNA analysis system (ABI Prism 3100 Genetic Analyzer; Applied Biosystems, Foster City, Calif).

RESULTS

Clinical and brain magnetic resonance imaging findings are summarized in the Table and in Figure 1 and Figure 2. Muscle biopsy specimens showed mild fiber diameter variability, a slightly increased number of fibers with central nuclei, and an absence of necrosis and fibrosis in patient 1; specimens showed marked fiber

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Symptoms at Onset</th>
<th>Maximum Motor Ability*</th>
<th>Eye Involvement</th>
<th>Magnetic Resonance Imaging Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/30</td>
<td>Seizures</td>
<td>Walk unassisted</td>
<td>None</td>
<td>Frontocortical dysplasia, hypoplastic pons, abnormal vermian foliation</td>
</tr>
<tr>
<td>2/F/6</td>
<td>Ocular abnormalities, muscular hypotonia</td>
<td>Walk unassisted</td>
<td>Buphthalmus, corioretinal atrophy (right eye), congenital myopia (both eyes)</td>
<td>Frontotemporal cortical dysplasia, white matter T2-weighted hyperintensity, hypoplastic pons, cerebellar cortical dysplasia, cerebellar cysts</td>
</tr>
<tr>
<td>3/M/7</td>
<td>Ocular abnormalities, muscular hypotonia</td>
<td>Stand with support</td>
<td>Buphthalmus, megalocornea, glaucoma, and cataract (right eye)</td>
<td>Frontocortical dysplasia, hypoplastic pons, cerebellar cortical dysplasia, cerebellar cysts</td>
</tr>
</tbody>
</table>

*Maximum motor ability was achieved in all patients at age 3 years.

Figure 1. Brain magnetic resonance imaging of patient 1 (A and B), patient 2 (C and D), and patient 3 (E and F) (axial fast spin-echo T2-weighted images [A, C, and E] and sagittal spin-echo T1-weighted images [B, D, and F]). A, Cortical thickening over the inferolateral aspect of both frontal lobes, with lack of digitation of the underlying subcortical white matter and incomplete opercularization of the right sylvian fissure. B, Mild hypoplastic pons, enlarged cisterna magna, and abnormal vermian foliation. C, Thickened cortex and coarse gray-white matter at the level of both frontal lobes, with marked T2-weighted hyperintensity involving the frontal and peritrigonal white matter and external capsules. D, Hypoplastic pons and vermis with poor definition of the folia and absence of normal fissuration, as well as enlarged cisterna magna. E, Diffuse cortical abnormality involving both frontal lobes, with coarse interdigitation between the thickened cortex and the underlying white matter in which the signal intensity is normal. F, Hypoplastic pons and vermis with poor definition of the folia and fissures and enlarged cisterna magna.
Figure 2. Mutational analysis of POMGnT1 in 3 patients. A, Electropherograms showing mutations in the patients. Mutation sites are indicated by arrows; nucleotide and amino acid sequences of normal and mutant alleles are shown next to the corresponding electropherogram. Reverse transcriptase–polymerase chain reaction analysis from patient 2 shows 3 different messenger RNA isoforms (see the “Results” section). B, Comparison of POMGnT1 amino acid sequences encompassing the Ser198Arg change in various species. C, Schematic representation of the POMGnT1 protein showing the location of mutations found in our patients. bp Indicates base pair; C, C terminus; IVS, intron variation sequence; N, N terminus; NC, healthy control; MA, marker numbers (70, 80, and 90); P1, patient 1; UDP-GlcNac/Mn²⁺, uridine diphosphate–N-acetylglucosamine and manganese ion; and 1, 37, 59, and 660, amino acids.
diameter variability and signs of necrosis and fibrosis in patient 2 and patient 3. Immunofluorescence for glycosylated α-dystroglycan showed an absent signal in patient 2, markedly reduced staining in patient 1, and reduced staining in patient 3. Merosin deficiency was observed in all patients, probably a secondary manifestation as previously reported. 

Five mutations, 2 of which are novel, were identified. Patient 1 carried a homozygous glutamine to arginine transition (c932G>A) in exon 10, with a predicted effect of pArg311Gln. Patient 2 was compound heterozygous for a novel cytosine to thymine transition (c931C>T) in exon 10, changing an arginine to a stop (pArg311Stop) and for an arginine to glutamine transition at the splice acceptor site of intron 15 (c1285-2A>G). To verify its pathogenic role, we amplified the complementary DNA comprising the region. This mutation produces 3 different messenger RNA (mRNA) isoforms, the first retaining the entire intron 15 with an in-frame insertion of 81 nucleotides, the second skipping exon 16, and the third resulting in residual correct splicing as observed in healthy subjects. In patient 3, we identified a novel cytosine to thymine transversion in exon 7 (c594C>G, pSer198Arg) and a glutamine to arginine transition (c1469G>A) in exon 17 (c1469G>A, pCys490Tyr) (Figure 2). POMT1 and POMT2 gene sequencing in patient 1 revealed no alterations. The identified novel mutations met accepted consensus guidelines for pathogenic variants, including a high degree of conservation of the affected residue among different species, and were absent from a large group of control chromosomes (from 192 white healthy control subjects). 

More than 30 subjects with mutations in POMGnTI have been identified worldwide, and the clinical spectrum is broad, ranging from the classic MEB phenotype to a more severe clinical picture, with features consistent with Walker-Warburg syndrome or Fukuyama CMD. To our knowledge, no mutational hot spot has been identified, and missense and nonsense mutations are distributed along the entire gene. Although the genotype-phenotype correlation remains unclear, mutations near the 5’ terminus are associated with a more severe clinical picture than those near the 3’ terminus. 

We identified 5 POMGnTI mutations in 3 Italian patients, 1 patient with an unusual phenotype characterized by CMD and brain involvement only (MEB-minus) and 2 patients with MEB phenotypes of different levels of severity. All 3 patients showed peculiar findings on magnetic resonance imaging of α-dystroglycanopathies, characterized by cerebral neuronal migration disorder and posterior lossa involvement, although their clinical presentation varied. The phenotype of patient 1 is unusual because of the absence of ocular abnormalities, only slightly elevated serum creatine kinase levels, and the prominent brain involvement, characterized on magnetic resonance imaging by cortical dysplasia and hypoplasia of the brainstem. The clinical pattern is also atypical for MEB because the patient is still able to walk in the third decade of life, and her prominent symptoms are mental deficiency and epilepsy, without muscle weakness. She carries a homozygous mutation (pArg311Gln), which has been detected in 2 heterozygous siblings with classic MEB showing hydrocephalus and early-onset glaucoma. 

The mild MEB phenotype in patient 2 is the result of 2 severe mutations (a null allele caused by a new nonsense variant in exon 10 [pArg311Stop] and a previously reported alteration in the conserved acceptor splice site of intron 15). The residual presence of normal mRNA encoding a normal protein could explain the lesser severity of the phenotype in this patient. The identification of mutations involving Arg311 in patient 1 and patient 2, as well as in 2 other patients described in the literature, suggests the presence of a mutational hot spot. The residue Arg311 is located in the highly conserved N-acetylgalactosaminyltransferase catalytic domain of POMGnTI and is critical for the binding of uridine diphosphate–N-acetylgalactosamine and the manganese ion. 

Patient 3, who had severe MEB, carried 2 apparently mild missense mutations, a novel pSer198Arg in the stem domain and a previously reported pCys490Tyr. The pSer198 mutation is evolutionarily conserved and results in a change from an uncharged polar to a positively charged amino acid. Unlike many other glycosyltransferases, the loss of the stem domain or single amino acid substitutions in the stem domain of POMGnTI have been shown to abolish the activity of the membrane-bound form of POMGnTI. Missense mutations in the stem domain may diminish retention of the protein in the Golgi apparatus or may trigger a conformational change that inhibits the accessibility of the acceptor or donor substrate in the catalytic domain.

This study increases our knowledge of the genotype-phenotype correlation and expands our awareness of the clinical spectrum associated with POMGnTI mutations. Furthermore, magnetic resonance imaging findings appear to be crucial in addressing appropriate genetic studies in CMDs.

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