Expanding the clinical spectrum of *POMT1* phenotype

Abstract—Mutations in *POMT1* have been identified in Walker–Warburg syndrome and in patients with limb-girdle muscular dystrophy and mental retardation (LGMD2K). The authors report new *POMT1* mutations in three unrelated children with severe motor impairment, leg hypertrophy, and mental retardation but without brain and ocular malformations. These patients are similar to LGMD2K but have earlier onset and more severe motor disability. The current findings expand the spectrum of *POMT1*-associated phenotypes.

NEUROLOGY 2006;66:1564–1567

A. D'Amico, MD*; A. Tessa, PhD*; C. Bruno, MD; S. Petrini, PhD; R. Biancheri, MD; M. Pane, MD; M. Pedemonte, MD; E. Ricci, MD; A. Falace, PhD; A. Rossi, MD; E. Mercuri, MD; F.M. Santorelli, MD; and E. Bertini, MD

The congenital muscular dystrophies (CMDs) with glycosylation defects are a heterogeneous group of inherited disorders often involving brain, eyes, and muscle, characterized by an abnormally glycosylated α -dystroglycan (α -DG) and mutations in proven or putative glycosyltransferases.¹ The complex heterogeneity of CMD with reduced α -DG is both genetic and clinical. Mutations in the same glycosyltransferase gene can be associated with different clinical phenotypes,² and patients with the same clinical diagnosis may harbor mutations in different glycosyltransferases.^{3–4} Overlap between established nosographic entities is also possible.⁵

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the May 23 issue to find the title link for this article.

*These authors contributed equally to this work.

From the Unit of Molecular Medicine (A.D., A.T., S.P., F.M.S., E.B.), Department of Laboratory Medicine, IRCCS-Bambino Gesù Children's Hospital, Rome, Italy; Neuromuscular Unit, Department of Neuroscience and Rehabilitation and Department of Pediatric Neuroradiology, IRCCS-G. Gaslini Institute, Genoa, Italy (C.B., R.B., M. Pedemonte, A.F., A.R.); Child of Neurology Unit, Department of Pediatrics (M. Pane, E.M.) and Department of Neuroscience (E.R.), Catholic University, Rome, Italy.

Research in the authors' laboratories is supported in part by grants from the Italian Ministry of Health (Ricerca Corrente e Finalizzata) to E.B. and C.B. and the Italian Ministry of Health 2003 on Limb Girdle Muscular Dystrophies to E.R. The authors also acknowledge the financial support of Telethon-Onlus (grant #GUP03558 to E.B. and E.M.).

Disclosure: The authors report no conflicts of interest.

Received August 1, 2005. Accepted in final form February 9, 2006.

Address correspondence and reprint requests to Dr. Filippo M. Santorelli, Molecular Medicine and Neurology, IRCCS Bambino Gesù Children's Hospital, Piazza S. Onofrio, 4, 00165 Rome, Italy; e-mail: fms3@na.flashnet.it

Mutations in *POMT1* have originally been identified in patients with Walker–Warburg syndrome (WWS, MIM 236670)—the most severe form of the genetically known CMD, characterized by cobblestone lissencephaly, neuronal migration defects and other structural brain changes, eye abnormalities, and death in early infancy.³ More recently, a subgroup of Turkish patients with limb-girdle muscle dystrophy, mental retardation, microcephaly, and normal MRI have been shown to carry a new *POMT1* mutation (p.Ala200Pro),⁶ proposing further clinical heterogeneity.

We report *POMT1* mutations in three patients with a distinct CMD phenotype characterized by early onset, severe motor disability, microcephaly, and mental retardation not associated with structural brain changes on neuroimaging.

Methods. All patients included in this study had relative leg hypertrophy and other features suggestive of CMD associated with an *O*-glycosylation defect.¹ We systematically collected data regarding age and mode of onset, muscle power, pattern and severity of contractures, functional abilities, and progression of the disease. Data on cognitive function and brain MRI findings were also collected. Griffiths mental scales were used to assess neurodevelopmental abilities.

Muscle samples, obtained for diagnostic purposes after receiving parental informed consent, were processed according to standard histologic and histochemical techniques. Immunohistochemistry used the following monoclonal antibodies: anti-mouse α -DG (VIA4-1, Upstate Biotechnology, USA), and anti-mouse merosin (80-kd fragment of the M-chain, 5H2, Chemicon Inc., Temecula, CA).

Direct sequencing of the PCR-amplified exons, and their intronic flanking sequences, of the *FKRP* and *POMT1* genes used intronic primers (oligonucleotide sequences and PCR conditions are available on request). PCR-restriction fragment length polymorphism analysis was used to confirm missense variants in *POMT1* in the patients and their relatives and to exclude their occurrence in a panel of 400 normal, ethnically matched control chromosomes.

Commentary, see page 1461

Table Clinical and genetic data of patients with *POMT1* mutations

Pt	Age/sex	Onset	Motor ability	CK, UI/L	Contractures	Respiratory/ cardiac involvement	Mental retardation	Microcephaly	MRI changes	Genetic data
1	2 y/F	6 mo	Stand with orthoses	3,000	Ankles	No	Moderate	Yes	Normal	p.Gly65Arg, p.Arg541Stop
2	20 mo/M	2 mo	Sit alone	2,500	Ankles	No	Moderate	Yes	Normal	p.Gly65Arg, p.Gln590His
3	14 y/M	Birth	Sit alone	6,000	Ankles, elbows, knees	No	Severe	Yes	Normal	p.Ala669Thr, IVS12+1g>a (PTC)

Pt = patient; CK = creatine kinase.

Results. The table summarizes clinical and neuroimaging findings in our patients. They all had early onset of hypotonia in the neonatal period or in the first months of life. Motor milestones were grossly delayed, and none of the children has so far acquired the ability to stand unsupported (follow-up range 20 months to 14 years). On examination, they all had microcephaly, facial weakness with tendency to keep the mouth open, and a mild degree of macroglossia. They all also had relative hypertrophy and stiffness in the lower limbs, both at thigh and calf level, with some wasting in the proximal upper limbs (see figure E-1 [available on the *Neurology* Web Site at www.neurology.org]). All had antigravity movements in the lower limbs but only partial antigravity movements in the proximal upper limbs. Contractures were found in all of the cases, and they were more diffuse in the oldest patient (Patient

3), who also had scoliosis and rigid spine. Serum creatine kinase levels were grossly increased (15 to 30 times normal). Results of ophthalmologic examination, including ERG and fundus oculi, were normal. All patients had mental retardation ranging from moderate to severe; Patient 3 had some autistic tracts. All three patients had normal MRI for age (figure 1).

Muscle biopsies showed dystrophic changes with marked variation in fiber size, central nuclei, few necrotic and regenerating fibers, and interstitial fibrosis in all. Immunohistochemistry revealed severe defect of α -DG and variable reduction in immunolabeling of merosin (figure 2). Five novel *POMT1* mutations were detected (see table), and a single mutation (p.Gly65Arg) was identified in two unrelated patients. The remaining novel variants occurred only once. Mutations affected moderately (Gly65) or highly

Figure 1. Brain MRI of Patients 1 (A through C), 2 (D through F), and 3 (G through I) did not reveal any cortical or cerebellar abnormalities. A, D, and G are T1-weighted sagittal images; B, E, and H are coronal T2-weighted images; C corresponds to a T1 axial image; F and I are axial T2-weighted images.

Figure 2. Muscle sections from a control (A and E) and Patients 1 (B and F), 2 (C and G), and 3 (D and H). Immunolabeling for α -dystroglycan and merosin is shown in A through D and E through H. α -Dystroglycan was markedly reduced in all patients. Merosin was variably reduced, respect to control in all patients. Bar: 25 μ m.

(Arg541, Gln590, Ala669) conserved residues in POMT1 orthologous protein sequences, were heterozygous in healthy parents, and they were not detected in a large set of control chromosomes. The *FKRP* gene was normal.

Discussion. *POMT1* mutations were originally identified in children with a severe phenotype characterized by complete lissencephaly, eye malformations, and death in early infancy.³ A milder phenotype, consisting of a limb-girdle muscular dystrophy associated with mental retardation and microcephaly, but without eye and brain abnormalities

(LGMD2K, MIM 609308), has been related to a common p.Ala200Pro mutation.⁶

Although our cases have a substantial clinical overlap with Turkish LGMD2K patients, they all had earlier onset and more severe motor functional impairment. The absence of structural brain and ocular abnormalities makes the clinical picture milder than WWS.

These findings confirm previous suggestions that different allelic mutations in *POMT1* may result in different phenotypic severity.^{3,6} WWS and LGMD2K probably correspond to the extreme ends of the spectrum, and the phenotype reported in our patients likely represents an intermediate form. This condition mirrors what has already been observed in association with *FKRP* mutations⁷⁻⁹ proposing that other, as yet unknown factors may modify clinical features in patients with glycosylation defects.

The mutations in *POMT1* identified in this study were all novel, and a single mutation (p.Gly65Arg) occurred in two children. Most mutations in patients with WWS result in amino acid substitution or deletion located in the highly conserved protein mannosyl transferase (PMT) and mannosyl-IP3R-RyR (MIR) domains,³ whereas the mild phenotype in Turkish patients is located in a cytoplasmic oriented domain of the protein.⁶ No straightforward correlations were found between the site of the mutation and the clinical phenotype in our cases. Nonetheless, we noticed that Patient 3—who was severely retarded and presented a marked motor impairment—harbored a splice site mutation, expected to produce exon skipping, in conjunction with a p.Ala669Thr variant, which is located within a transmembrane domain. In contrast, one of the two changes found in Patients 1 and 2 (p.Gly65Arg) is not as highly conserved as many other amino acids located in the PMT domain. The other allele in Patient 1 harbors a likely null allele. The relatively milder phenotype in Patient 2 as compared with Patient 3 is probably the result of the amino acid substitution (p.Gln590His), which does not disrupt any known domain.

Our findings further expand the spectrum of phenotypes associated with *POMT1* mutations and confirm previous suggestions that a full screening of all the known genes responsible for “glycosylated alpha dystroglycanopathies” should be performed in subjects with phenotypes suggestive of these forms, regardless of their severity.

References

1. Muntoni F, Brockington M, Torelli S, Brown SC. Defective glycosylation in congenital muscular dystrophies. *Curr Opin Neurol* 2004;17:205–209.
2. Mercuri E, Brockington M, Straub V, et al. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. *Ann Neurol* 2003;53:537–542.
3. Beltran-Valero de Bernabe D, Currier S, Steinbrecher A, et al. Mutations in the O-mannosyl-transferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. *Am J Hum Genet* 2002;71:1033–1043.
4. van Rееuwijk J, Janssen M, van den Elzen C, et al. POMT2 mutations cause alpha-dystroglycan hypoglycosylation and Walker-Warburg syndrome. *J Med Genet* 2005;42:907–912. E-pub ahead of print May 13, 2005.

5. Muntoni F. Walker–Warburg syndrome and limb girdle muscular dystrophy; two sides of the same coin. *Neuromuscul Disord* 2005;15:269–270.
6. Balci B, Uyanik G, Dincer P, et al. An autosomal recessive limb girdle muscular dystrophy (LGMD2) with mild mental retardation is allelic to Walker–Warburg syndrome (WWS) caused by a mutation in the POMT1 gene. *Neuromuscul Disord* 2005;15:271–275.
7. Brockington M, Yuva Y, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. *Hum Molec Genet* 2001;10:2851–2859.
8. Beltran-Valero de Bernabe D, Voit T, Longman C, et al. Mutations in the FKRP gene can cause muscle–eye–brain disease and Walker–Warburg syndrome. *J Med Genet* 2004;41:e61.
9. de Paula F, Vieira N, Starling A, et al. Asymptomatic carriers for homozygous novel mutations in the FKRP gene: the other end of the spectrum. *Eur J Hum Genet* 2003;11:923–923.

Lumbar radiculopathy due to chondrocalcinosis

G.R. Eichhorn, MD; B. Berka, MD; D. Garnhaft, MD; and T. Capellmann, MD, Lexington, KY; Mistelbach, Horn, and Vienna, Austria

A 74-year-old right-handed white man presented with acute right-sided leg pain attributable to the third lumbar root. Additionally, there were cutaneous calcifications and arthropathy of the hands. By history, he had recurrent bursitis of the right elbow.

MRI revealed a space-occupying lesion at the level of the third lumbar vertebra consistent with an articular cyst (figure). Surgery was performed, followed by histologic workup. Microscopically, calciumpyrophosphate crystals were detected and van-Cossa-stain was positive.

In rheumatological conditions, such as pseudogout, with radicular symptoms, the differential diagnosis should include non-disk related causes, such as arthropathy.^{1,2}

Copyright © 2006 by AAN Enterprises, Inc.

1. Fye KH, Weinstein PR, Donald F. Compressive cervical myelopathy due to calcium pyrophosphate dihydrate deposition disease: report of a case and review of the literature. *Archives of Internal Medicine* 1999;159:189–193.
2. Fujishiro T, Nabeshima Y, Yasui S, Fujita I, Yoshiya S, Fujii H. Pseudo-gout attack of the lumbar facet joint: a case report. *Spine* 2002;27:396–398.

Disclosure: The authors report no conflicts of interest.

Address correspondence and reprint requests to Dr. Gerald R. Eichhorn, Neurology Section, Lexington Clinic, 1401 Harrodsburg Road, Suite A-500, Lexington, KY 40504; e-mail: eichhornus@yahoo.com

Figure. Sagittal T2-weighted magnetic resonance image shows a hypointense lesion with intraspinal extradural location of approximately 1.8 cm in diameter at the level of L3 with distinct encroachment of the spinal canal, likely originating from a facet joint and compatible with a partially calcified articular cyst.

Neurology®

Lumbar radiculopathy due to chondrocalcinosis

G. R. Eichhorn, B. Berka, D. Garnhaft, et al.

Neurology 2006;66;1567

DOI 10.1212/01.wnl.0000208486.53923.07

This information is current as of May 22, 2006

Updated Information & Services

including high resolution figures, can be found at:
<http://n.neurology.org/content/66/10/1567.full>

References

This article cites 2 articles, 0 of which you can access for free at:
<http://n.neurology.org/content/66/10/1567.full#ref-list-1>

Permissions & Licensing

Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://www.neurology.org/about/about_the_journal#permissions

Reprints

Information about ordering reprints can be found online:
<http://n.neurology.org/subscribers/advertise>

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright . All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.