POMT1 mutation results in defective glycosylation and loss of laminin-binding activity in α-DG

D.-S. Kim, MD, PhD; Y.K. Hayashi, MD, PhD; H. Matsumoto, MD; M. Ogawa, BS; S. Noguchi, PhD; N. Murakami, MD, PhD; R. Sakuta, MD, PhD; M. Mochizuki, MD, PhD; D.E. Michele, PhD; K.P. Campbell, PhD; I. Nonaka, MD, PhD; and I. Nishino, MD, PhD

Abstract—Walker–Warburg syndrome (WWS) is a congenital muscular dystrophy associated with neuronal migration disorder and structural eye abnormalities. The mutations in the *O*-mannosyltransferase 1 gene (*POMT1*) were identified recently in 20% of patients with WWS. The authors report on a patient with WWS and a novel *POMT1* mutation. Their patient expressed α-dystroglycan (α-DG) core protein, but fully glycosylated α-DG antibody epitopes were absent, associated with the loss of laminin-binding activity.

NEUROLOGY 2004;62:1009–1011

Walker–Warburg syndrome (WWS; MIM 236670), Fukuyama-type congenital muscular dystrophy (FCMD; MIM 253800), and muscle-eye-brain disease (MEB; MIM 253280) are closely related congenital muscular dystrophies (CMDs) with cobblestone lissencephaly and eye abnormalities. Although they are known to be caused by the mutations of different genes encoding putative glycosyltransferases,[1] it now is clear that the mutations of each gene produce overlapping clinical phenotypes.[2,3] In addition, they share a similar pattern of selective loss of α-dystroglycan (α-DG) on immunohistochemical study.[1] A recent study showed hypoglycosylation of α-DG and loss of binding activity of α-DG to laminin, neurexin, and agrin in FCMD, MEB, and the mutant myodystrophy (Large^{myd}) mouse, suggesting a defect in the same post-translational modification pathway of glycosylation in α-DG.[4]

Mutations in the *O*-mannosyltransferase 1 gene (*POMT1*) were implicated recently in 20% of patients with WWS.[5] The laminin-binding site in α-DG is thought to reside in *O*-mannosyl-linked carbohydrate side chains, which may require POMT1 for synthesis.[6]

We report our experience with a Japanese boy with WWS and a novel *POMT1* mutation, who showed reduced glycosylation and loss of laminin-binding activity of α-DG in skeletal muscle.

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 March 23 issue to find the title link for this article.

Methods. *Patient.* The patient was a Japanese boy aged 3.5 years from apparently nonconsanguineous parents. No other family member was affected. Prenatal ultrasonography showed that the patient had a meningoencephalocele. He was born at gestational week 38 by Cesarean section with a body weight of 2,042 g. He was floppy with an enlarged head. He underwent surgery to remove a meningoencephalocele, and a ventriculoperitoneal shunt was added 21 days after birth. Mild microphthalmia and corneal clouding also were observed. Serum creatine kinase levels were markedly elevated to 600 to 31,000 IU/L (upper normal limit, 70 IU/L). He exhibited markedly delayed milestones. He could not control his head, roll over, or sit. He showed lack of facial expression with an inability to smile and never developed the ability to speak. Brain MRI revealed agyric frontal and temporo-occipital lobes mixed with pachygyric parietal cortex. Hypoplasia of brain stem and cerebellum also was observed (figure 1). EEG showed multifocal spikes, and the muscle biopsy showed marked increase in fatty tissue with evidence of necrosis and regeneration. The mutational analysis for fukutin and protein *O*-mannose β-1,2-N-acetylglucosaminyl-transferase gene (*POMGnT1*) did not show any abnormalities.

Immunohistochemistry and immunoblotting studies. The following antibodies were used: monoclonal anti-α-DG (VIA4-1, Upstate Biotechnology, Lake Placid, NY), polyclonal goat anti-α-DG (GT20ADG),[4] monoclonal anti-β-DG (43DAG1/8D5, Novocastra Laboratories, Newcastle upon Tyne, UK), monoclonal anti-laminin-α2 chain (5H2, Chemicon, Temecula, CA), monoclonal antidystrophin C-terminal (Dy8/6C5, Novocastra Laboratories), and monoclonal antisarcoglycan antibodies (Novocastra Laboratories). The detailed techniques of the immunohistochemistry, immunoblotting, and laminin overlay assays have been described previously.[4,7]

Mutation analysis. Genomic DNA was extracted from frozen muscle tissue using standard method with informed consent. Primer

From the Department of Neuromuscular Research (Drs. Kim, Hayashi, Matsumoto, Noguchi, and Nishino, M. Ogawa), National Institute of Neuroscience, National Center for Neurology and Psychiatry (NCNP), Tokyo, Japan; Department of Neurology (Dr. Kim), Pusan National University Hospital, Korea; Department of Pediatrics (Drs. Murakami and Sakuta), Dokkyo Medical School, Saitama, Japan; Department of Neurology (Dr. Mochizuki), Saitama Children's Medical Center, Japan; Howard Hughes Medical Institute, Department of Physiology and Biophysics (Drs. Michele and Campbell), University of Iowa College of Medicine, Iowa City, IA; and National Center Hospital for Mental, Nervous, and Muscular Disorders (Dr. Nonaka), NCNP, Tokyo, Japan.

Supported by Grants-in-Aid for Research on Psychiatric and Neurologic Diseases and Mental Health from the Ministry of Health, Labor, and Welfare and the Ichiro Kanehara Memorial Foundation, Japan. K.P.C. is an investigator for the Howard Hughes Medical Institute.

Received May 27, 2003. Accepted in final form November 10, 2003.

Address correspondence and reprint requests to Dr. Yukiko K. Hayashi, Department of Neuromuscular Research, National Institute of Neuroscience, NCNP, 4-1-1 Ogawa-higashi, Kodaira, Tokyo 187-8502, Japan; e-mail: hayasi_y@ncnp.go.jp

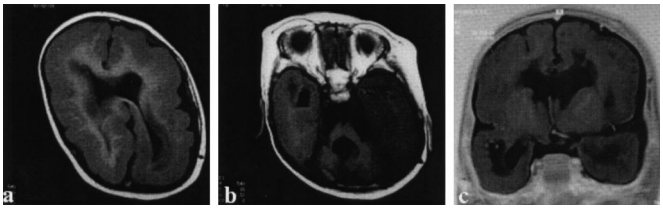

Figure 1. Brain MRI of patient at age 3 years shows agyric frontal and temporo-occipital lobes mixed with pachygyric parietal cortex, hypoplasia of brain stem and cerebellum, and defect of septum pellucidum. The periventricular white matter change (A and B, TR540/TE15; C, TR5400/TE90) also is seen.

pairs were designed to amplify all coding exons and flanking intronic sequences of *POMT1*. The amplified products were sequenced using an ABI PRISM 3100 (Applied Biosystems, Foster City, CA). For the detection and screening of L421del (1260 to 1262 delCCT) in exon 13 of *POMT1*, primers F-CAGTAGCAGCAACTCATGGG, R-ACGGTTGTGGCTGCTATAGC, and restriction enzyme *AvaI* were used. One hundred healthy Japanese individuals served as control subjects.

Results. *Immunohistochemical and immunoblotting analyses.* The immunohistochemical analysis revealed an almost complete loss of immunoreactivity with VIA4-1 anti-α-DG antibody in the patient, whereas anti-α-DG core protein GT20ADG showed membrane staining in each muscle fiber (figure 2). Immunoreaction against the laminin-α2 chain was reduced slightly, but β-DG (see figure 2), dystrophin, and sarcoglycans (not shown) were well preserved.

Immunoblotting analysis using GT20ADG showed a band with a reduced molecular mass, whereas VIA4-1 showed no detectable band for α-DG in the patient (figure 3, A and B). The molecular weight shift observed in our patient (>60 kDa) was almost identical to those reported in FCMD and MEB.[4] On laminin overlay assay, the patient's muscle showed an almost complete loss of laminin-binding activity of α-DG (figure 3C).

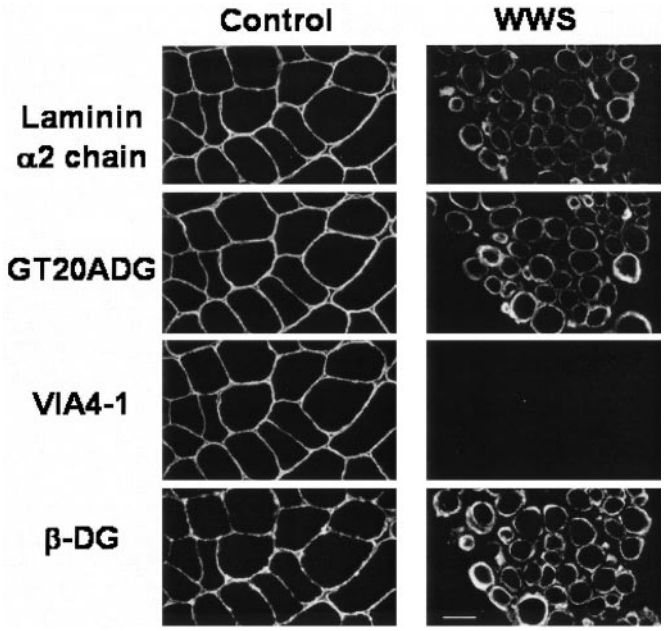

Figure 2. In the patient (with Walker–Warburg syndrome [WWS]), a complete loss of immunoreactivity is observed with the monoclonal antibody VIA4-1 against α-dystroglycan (α-DG), whereas it appears normal around muscle fibers when the polyclonal antibody GT20ADG against α-DG was used. β-DG is well preserved, but the laminin-α2 chain shows mild reduction; bar = 20 μm.

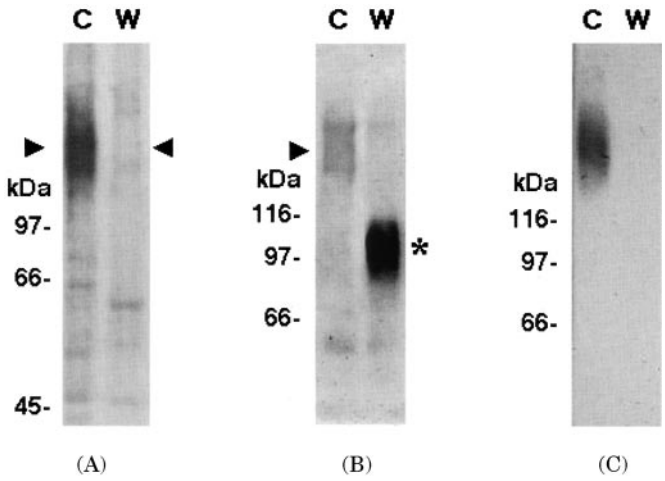

Figure 3. (A) The immunoblotting study using the antibody VIA4-1 showed a broad band around 156 kDa (arrowheads) in control skeletal muscle (C) that is undetectable in the patient (W). (B) The immunoblotting study using the antibody GT20ADG showed a band with a reduced molecular mass (≅90 kDa, asterisk) in the patient, whereas the normal band of α-dystroglycan (α-DG) at 156 kDa was detected in the control. (C) The laminin overlay assay showed loss of band in the patient, suggesting there is an almost complete loss of laminin-binding activity in α-DG from the patient's muscle. M = molecular mass.

Mutation analysis. We found a homozygous deletion of three base pairs (1260 to 1262 delCCT) in *POMT1*, which is expected to delete single amino acid leucine at position 421 (see figure E-1A on the *Neurology* Web site). No identical mutation was present in 100 normal Japanese control subjects (see figure E-1B on the *Neurology* Web site). The amino acid sequence alignment showed that the deleted amino acid leucine and surrounding primary sequence are highly conserved among different species (see figure E-1C on the *Neurology* Web site).

Discussion. In this study, we identified a deletion of the single amino acid leucine at position 421 of *POMT1* from the patient's DNA. This is considered to be a causative mutation for several reasons. First, the same change was not found among 100 Japanese control subjects. Next, the deleted amino acid leucine is located within a highly conserved region of the gene and is conserved among different species. A previously reported V428D mutation also is only seven amino acids downstream to ours.[5] These findings suggest that this conserved region plays an important role in the proper function of the protein.

Our patient showed exceptionally long survival for WWS because most patients with WWS die during infancy and rarely survive beyond age 3

years. Because complete agyria is common in patients with WWS, the pattern of the cortical dysplasia in our patient—agyria mixed with parietal pachygyria in MRI—could be considered milder than typical WWS. Thus, our patient showed intermediate phenotype between WWS and MEB in terms of clinical severity and MRI finding. However, the diagnosis of WWS seems more accurate than MEB or FCMD in our patient because he had a meningoencephalocele, which is almost exclusively seen in WWS.[8] There are some recent reports documenting the remarkable clinical variability originating from the mutation of the same genes causing CMDs, and thus, it is possible for *POMT1* mutations to produce a more benign WWS phenotype like that seen in our patient.[2,3,9]

Although the immunoreactivity against the antibody VIA4-1 was lost completely in our patient, the reaction against the antibody GT20ADG was well preserved. Because the antibody GT20ADG recognizes the core protein of α-DG, our results indicate that α-DG localizes at the surface membrane of skeletal muscle but that the epitope for VIA4-1 antibody was specifically disrupted or masked.[4] Because the antibody VIA4-1 is thought to recognize, at least in part, the carbohydrate epitope of α-DG, the glycosylation status of α-DG is likely to be altered in our patient.[4] The results of immunoblotting and laminin overlay assays further support this speculation. The α-DG from normal skeletal muscle is a heavily glycosylated protein with a molecular weight of 156 kDa. Thus, the reduction of molecular weight, seen only by GT20ADG, is likely to be related to the loss of glycoconjugates from α-DG. Accordingly, the loss of laminin-binding activity shown in the laminin overlay assay most likely is caused by the loss of glycoconjugate, which is thought to be a laminin-binding ligand of α-DG.[6] A brain-selective deletion of dystroglycan in mice was shown recently to cause CMD-like brain malformations and defective laminin binding, giving strong evidence that abnormalities of dystroglycan underlie the neuronal migration disorder seen in this group of disorders.[10] Because similar pattern of glycosylation-deficient disruption of dystroglycan function has been observed in FCMD, MEB, and Large^{myd} mice,[4] it is likely that WWS shares a similar pathomechanism with them. In addition, the complete loss of the laminin-binding activity of α-DG in our patient with WWS is almost identical to that observed in FCMD.[4]

Our study proves that WWS caused by the mutation of *POMT1* coexists with other types of CMDs in the Japanese population. We also demonstrated that WWS is a member of the group of CMDs associated with defective glycosylation of α-DG that results in the loss of function of α-DG as a matrix receptor.

References

1. Muntoni F, Brockington M, Blake DJ, Torelli S, Brown SC. Defective glycosylation in muscular dystrophy. Lancet 2002;360:1419–1421.
2. Taniguchi K, Kobayashi K, Saito K, et al. Worldwide distribution and broader clinical spectrum of muscle-eye-brain disease. Hum Mol Genet 2003;12:527–534.
3. Silan F, Yoshioka M, Kobayashi K, et al. A new mutation of the fukutin gene in a non-Japanese patient. Ann Neurol 2003;53:392–396.
4. Michele DE, Barresi R, Kanagawa M, et al. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. Nature 2002;418:417–422.
5. Beltrán-Valero de Bernabé D, Currier S, Steinbrecher A, et al. Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. Am J Hum Genet 2002;71:1033–1043.
6. Chiba A, Matsumura K, Yamada H, et al. Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve α-dystroglycan. J Biol Chem 1997;272:2156–2162.
7. Hayashi YK, Ogawa M, Tagawa K, et al. Selective deficiency of alpha-dystroglycan in Fukuyama-type congenital muscular dystrophy. Neurology 2001;57:115–121.
8. Cormand B, Pihko H, Bayes M, et al. Clinical and genetic distinction between Walker-Warburg syndrome and muscle-eye-brain disease. Neurology 2001;56:1059–1069.
9. Topaloglu H, Brockington M, Yuva Y, et al. FKRP gene mutations cause congenital muscular dystrophy, mental retardation, and cerebellar cysts. Neurology 2003;60:988–992.
10. Moore SA, Saito F, Chen J, et al. Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature 2002;418:422–425.