Physical and Functional Association of Human Protein O-Mannosyltransferases 1 and 2

Received for publication, February 6, 2006, and in revised form, May 12, 2006. Published, JBC Papers in Press, May 12, 2006, DOI 10.1074/jbc.M601091200

Keiko Akasaka-Manya, Hiroshi Manya, Ai Nakajima, Masao Kawakita, and Tamao Endo

From the Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Foundation for Research on Aging and Promotion of Human Welfare, Tokyo 173-0015, Japan and Department of Applied Chemistry, Kagaku University, Tokyo 163-8677, Japan

A defect of protein O-mannosylation causes congenital muscular dystrophy with brain malformation and structural eye abnormalities, so-called Walker-Warburg syndrome. Protein O-mannosylation is catalyzed by protein O-mannosyltransferase 1 (POMT1) and its homologue, POMT2. Coexpression of POMT1 and POMT2 is required to show O-mannosylation activity. Here we have shown that POMT1 forms a complex with POMT2 and the complex possesses protein O-mannosyltransferase activity. Results indicate that POMT1 and POMT2 associate physically and functionally in vivo. Recently, three mutations were reported in the POMT1 gene of patients who showed milder phenotypes than typical Walker-Warburg syndrome. We coexpressed these mutant POMT1s with POMT2 and found that none of them had any activity. However, all POMT1 mutants, including previously identified POMT1 mutants, coprecipitated with POMT2. These results indicate that the mutant POMT1s could form heterocomplexes with POMT2 but that such complexes are insufficient for enzymatic activity.

Dystrophin-glycoprotein complex is composed of α-, β-dystroglycan (DG), dystrophin, and some other molecules. Dystrophin-glycoprotein complex is thought to act as a transmembrane linker between the extracellular matrix and intracellular cytoskeleton (1). α-DG is a central component of the dystrophin-glycoprotein complex and is heavily glycosylated, and its sugars have a role in binding to extracellular matrices such as laminin, neurexin, and agrin (2). Previously we reported that the glycans of α-DG include O-mannosyl oligosaccharides and that a sialyl O-mannosyl glycan, Siaα2–3Galβ1–4GlcNAcβ1–2Man, is a laminin binding ligand of α-DG (3). We have also found that muscle-eye-brain disease (OMIM 253280), a congenital muscular dystrophy, was caused by mutations in the gene encoding POMGnT1 (protein O-mannose β1,2-N-acetylgalcosaminyltransferase 1), which forms a GlcNAcβ1–2Man linkage of O-mannosyl glycans (4, 5).

Protein O-mannosyltransferase 1 (POMT1) and its homologue POMT2 are responsible for the catalysis of the first step in O-mannosyl glycan synthesis (6). Mutations in the POMT1 and POMT2 genes are considered to be the cause of Walker-Warburg syndrome (WWWS; OMIM 236670), an autosomal recessive developmental disorder associated with congenital muscular dystrophy, neuronal migration defects, and ocular abnormalities (7, 8). Previously, seven mutations in the POMT1 gene (G76R, Q303X, Q385X, L421del, V428D, V703fs, and G722fs) were identified in patients with WWS (7, 9). We have demonstrated that these mutations in the POMT1 gene lead to defects of POMT activity (10). This may cause a defect in α-DG glycosylation and result in failure of binding to laminin or other molecules in the extracellular matrix and interrupt normal muscular function and migration of neurons in developing brain. Recently, other mutations in the POMT1 gene were found (11–13). Among them, three mutations (G65R, A200P, and M582C) display milder pathology than typical WWS. Patients with A200P mutation are characterized by mild mental retardation and microcephaly without brain malformation (14), and patients with G65R and M582C mutations are characterized by calf hypertrophy, microcephaly, and severe mental retardation, but no eye abnormalities (15). These findings suggested that these mutations would not completely abolish enzymatic activity, which prompted us to examine POMT activities of mutated POMT1 with milder phenotypes.

PMT, protein O-mannosyltransferase, is evolutionarily conserved from prokaryotes, such as Mycobacterium tuberculosis, to eukaryotes, such as yeast, Drosophila, mouse, and human (16–19). In yeast Saccharomyces cerevisiae, O-mannosylation is required for the stability, correct localization, and/or function of proteins. Yeast O-mannosylation is initiated in the lumen of the endoplasmic reticulum (ER) by a family of PMTs that catalyze the transfer of a mannose residue from dolichol phosphate mannose to Ser/Thr residues of proteins (16). S. cerevisiae has seven PMT homologues (Pmt1p-7p) that share almost identical hydropathy profiles. The hydropathy profiles predict that PMTs are integral membrane proteins with multiple transmembrane domains (16, 20–22). The PMT family is
Association of POMT1 and POMT2
classified phylogenetically into the PMT1, PMT2, and PMT4 subfamilies. Members of the PMT1 subfamily (Pmt1p and Pmt5p) interact heterophilically with those of the PMT2 subfamily (Pmt2p and Pmt3p), whereas the single member of the PMT4 subfamily (Pmt4p) acts as a homophilic complex (17, 23). Although Pmt1p-4p and Pmt6p have O-mannosyltransferase activity by themselves (23), complex formation is essential for maximal transferase activity of yeast PMT family members (17, 24).

In human, transferase activity may also require formation of a heterocomplex of POMT1 and POMT2, because cotransfection of POMT1 and POMT2 up-regulates POMT activity in human embryonic kidney (HEK) 293T cells whereas expression of only one of these proteins does not (6). However, no direct evidence for a physical interaction between POMT1 and POMT2 proteins has been obtained so far. Here, we have demonstrated that POMT1 and POMT2 form a functional complex in vivo using immunoprecipitating techniques. Furthermore, we showed that the mutations of POMT1 protein found in WWS patients do not prevent complex formation with POMT2 but they do abolish activity of the complex.

EXPERIMENTAL PROCEDURES

Vector Construction of POMT1 Mutants and POMT2—Human POMT1 cDNA was used for site-directed mutagenesis and was cloned into pcDNA 3.1 (Invitrogen) as described previously (6). For each of the three mutations (G66R, A200P, M582C) examined in this study, the POMT1 gene was modified with a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions as described previously (10). The three mutants were generated with the following primer pairs: G66R, 5′-CTTCTTGGATGACAGTGGCCGCGCATTGGCCGCCC-3′ and 5′-GGCCAAATGGCGGCCTACTGTCATCCAAGAG-3′; A200P, 5′-GTCGGTGGTCTCCTGTCGATTGGCATTCAAG-3′ and 5′-GTCGGTGGTCTCCTGTCGATTGGCATTCAAG-3′; and M582C, 5′-CAAATTGGGCTTCAACTGTCCTGCACCCCCAGGAC-3′ and 5′-GTCCTGGGGTGCAGGCCTGCACCCCCAGGAC-3′. All mutant clones were sequenced to confirm the presence of the mutations. Other mutants (G76R, A421del, V428D) and human POMT1 or anti-POMT2 polyclonal antibody (6) or anti-Myc (9E10), and anti-calreticulin (Santa Cruz Biotechnology, Santa Cruz, CA) for overnight at 4 °C. Next, cells were washed with PBS and incubated with Alexa Fluor 488-conjugated anti-mouse IgG or Alexa Fluor 546-conjugated anti-goat IgG (Molecular Probes, Eugene, OR) for 1 h at room temperature. After a final wash with PBS, cells were observed, using fluorescence microscopy. We also used the following fluorescent primary antibodies: fluorescein isothiocyanate-conjugated anti-FLAG (Sigma) and TRITC-conjugated anti-Myc (Santa Cruz Biotechnology).

Immunoprecipitation—Microsomal fractions were lysed with assay buffer (20 mM Tris-HCl, pH 8.0, 2 mM 2-mercaptoethanol, 10 mM EDTA, 0.5% n-octyl-β-D-thioglucoside) in a final concentration of 2 mg/ml for 5 h at 4 °C. After solubilization, proteins were subjected to centrifugation at 10,000 × g for 30 min and precleared with CL-6B-Sepharose (Sigma). Pre-cleared supernatants were mixed with anti-Myc (9E10)-agarose conjugate (Santa Cruz Biotechnology) and incubated overnight. After three washes with the assay buffer, the agarose beads were suspended in sample buffer. Samples were subjected to Western blot analysis. To assay POMT activity, the precipitated beads were suspended in 60 μl of assay buffer and used as the enzyme source.

Western Blot Analysis—The microsomal fractions (20 μg) or immunoprecipitated samples were separated by SDS-PAGE (7.5% gel), and proteins were transferred to a polyvinylidene difluoride membrane. The membrane was blocked in PBS containing 5% skim milk and 0.05% Tween 20, incubated with anti-POMT1 or anti-POMT2 polyclonal antibody (6) or anti-Myc (A-14) antibody (Santa Cruz Biotechnology), and treated with anti-rabbit IgG conjugated with horseradish peroxidase or anti-mouse IgG conjugated with horseradish peroxidase (GE Healthcare). Proteins that bound to the antibody were visualized with an ECL kit (GE Healthcare). As reported previously (6), anti-POMT1 and anti-POMT2 polyclonal antibodies did not detect endogenous POMT1 and POMT2, respectively.
Each antibody is specific for the respective recombinant protein; that is, they do not cross-react with each other.

**Assay for POMT Activity**—POMT activity was based on the amount of mannose transferred from dolichol phosphate mannosyl to a glutathione S-transferase fusion α-DG (GST-αDG) as described previously (6) with a slight modification. Briefly, assays were carried out in a 20-μl reaction volume containing 20 mM Tris-HCl, pH 8.0, 100 nM 3H-labeled dolichol phosphate mannose (Dol-P-[3H]Man, 125,000 dpm/pmol; American Radiolabeled Chemicals, St. Louis, MO), 2 mM 2-mercaptoethanol, 10 mM EDTA, 0.5% n-octyl-β-D-thioglucoside, 10 μg of GST-α-DG, and 80 μg of microsomal membrane fraction. Microsomal fractions were solubilized with buffer containing 20 mM Tris-HCl, pH 8.0, 2 mM 2-mercaptoethanol, 10 mM EDTA, 0.5% n-octyl-β-D-thioglucoside for 1 h, and the reaction was initiated by adding Dol-P-[3H]Man.

After a 1-h incubation at 25 °C, the reaction was stopped by adding 150 μl of PBS containing 1% Triton X-100, and the reaction mixture was centrifuged at 10,000 × g for 10 min. The supernatant was removed, mixed with 400 μl of PBS containing 1% Triton X-100 and 10 μl of glutathione-Sepharose 4B beads (GE Healthcare), rotated at 4 °C for 1 h, and washed three times with 20 ml Tris-HCl, pH 7.4, containing 0.5% Triton X-100. The radioactivity adsorbed to the beads was measured with a liquid scintillation counter.

**RESULTS**

**Colocalization of POMT1 and POMT2 in the ER**—To determine the subcellular localization of POMT1 and POMT2, HEK293T cells were transfected with expression constructs encoding each protein. Anti-POMT1 and anti-POMT2 antibodies did not stain untransfected HEK293T cells (data not shown), suggesting that HEK293T cells express little POMT1 and POMT2. Myc-tagged POMT1 colocalized precisely with anti-calreticulin (ER marker). Calreticulin staining localized around the nuclei and overlapped with POMT1-Myc staining (Fig. 1A). On the other hand, FLAG-tagged POMT2 also colocalized with calreticulin (Fig. 1B), in agreement with a previous finding that POMT2 localized to the ER membrane (25). Double staining of HEK293T cells by both POMT1 and POMT2 demonstrated their colocalization (Fig. 1C). Thus, we concluded that POMT1 and POMT2 reside in the ER.

**Solubilization of POMT Activity**—Colocalization of POMT1 and POMT2 in the ER and the requirement of coexpression of both components for protein O-mannosylation (6) suggest that POMT1-POMT2 complex formation is necessary for POMT activity. To obtain physical evidence for complex formation between POMT1 and POMT2, we solubilized membrane proteins with various detergents and attempted to detect a complex by immunoprecipitation. In yeast, members of the PMT1 family were found to form heteromeric complexes with members of the PMT2 subfamily in vivo by coimmunoprecipitation experiments after solubilization of membrane proteins with 0.35% sodium deoxycholate and 0.5% Triton X-100 (17). Human POMT activity could not be detected when Triton X-100 was used as a detergent, but it could be detected when n-octyl-β-D-thioglucoside was used as the detergent (6). Therefore, at first we examined the effect of various detergents on POMT activity of HEK293T cells, in addition to Triton X-100 and n-octyl-β-D-thioglucoside. n-Octyl-β-D-thioglucoside at a concentration of 0.5% was found to be most effective under our assay conditions (Fig. 2). Then we tried to solubilize POMT activity under various incubation temperatures, incubation times, and detergent concentrations. We found that the optimal conditions for solubilizing POMT activity from the microsomal membrane were 0.5% n-octyl-β-D-thioglucoside at 4 °C for 5 h.

**Coimmunoprecipitation of POMT1 and POMT2**—To determine whether POMT1 and POMT2 form a heterocomplex, POMT1-Myc and POMT2 were cotransfected into HEK293T cells (Fig. 3, A–C). Microsomal membrane fractions of these cells were lysed with 0.5% n-octyl-β-D-thioglucoside at 4 °C for 5 h and immunoprecipitated with anti-Myc (9E10) antibody-conjugated agarose. A Western blot analysis of precipitates revealed that POMT1-Myc and POMT2 were coimmunoprecipitated (Fig. 3, D and E, lanes 1), indicating that POMT1 and POMT2 form a complex. We then confirmed that POMT2 does not bind to anti-Myc-agarose nonspecifically. Cells that were transfected with only POMT2 were solubilized by n-octyl-β-D-thioglucoside and subjected to immunoprecipitation with anti-Myc-agarose. In this case, POMT2 was not detected in the precipitates, indicating POMT2 did not bind to anti-Myc-agarose (Fig. 3, D and E, lanes 3). On the other hand, when POMT1-Myc and POMT2 were separately expressed in different cells and then solubilized, mixed, and immunoprecipitated with anti-Myc-agarose, no complex formation between POMT1-Myc and POMT2 was detected (Fig. 3, D and E, lanes 5). These results indicated that the POMT1 and POMT2 proteins could not associate with each other when they are expressed separately and suggest that assembly of POMT1 and POMT2 occurs in the ER membrane.

Next, we examined whether the coprecipitated POMT1-POMT2 complex has POMT activity. Distinct activity was detected in the precipitates from POMT1-Myc-POMT2

![FIGURE 1. Subcellular localization of POMT1 and POMT2.](image-url)
were found to abolish POMT activity in both the present and previous studies (10). We examined six of these mutant POMT1s (all but the latter two) to determine whether they prevented complex formation with POMT2 (Table 1). The mutated POMT1-mycs were cotransfected into HEK293T cells with POMT2. POMT2 was found to precipitate with each of the POMT1 mutants (Fig. 5, A and B), indicating that these mutations did not affect complex formation with POMT2.

**DISCUSSION**

Protein O-mannosylation is an essential post-translational modification (19). In yeast and fungi, protein O-mannosylation is indispensable for cell wall integrity and normal cellular morphogenesis (16). In Drosophila, a defect of O-mannosylation causes a rotation of the abdomen due to abnormal muscle development (26, 27). In mouse, targeted disruption of the Pomt1 gene is embryonically lethal (28). In human, impairment of α-DG O-mannosylation leads to congenital muscular dystrophy and neuronal migration disorders, WWS (7, 10). Protein O-mannosylation requires at least two components, POMT1 and POMT2 (6). The present results have shown that POMT1 and POMT2 form a heterocomplex in vivo. The immunoprecipitated complex of POMT1 and POMT2 possessed POMT activity. In addition, we demonstrated that mutant POMT1s that have been found in WWS patients have the ability to form a complex with POMT2, although they lost POMT activities. In yeast, the members of the PMT1 subfamily interact heterophilically with those of the PMT2 subfamily, whereas the single member of the PMT4 subfamily acts as a homophilic complex (17, 24). On the other hand, in human the single member of the PMT2 subfamily (POMT2) interacts with a member of the PMT4 subfamily (POMT1). These results suggest that the combination of interacting molecules has changed during evolution. It is noteworthy that only a single PMT member has been found in M. tuberculosis and that it has POMT activity (18). It is unclear whether it forms a homophilic complex in vivo.

**Yeasts PMTs and human POMTs are predicted to be integral membrane proteins with multiple transmembrane domains (25, 29). However, human and yeast PMT proteins showed differences in detergent sensitivity. Triton X-100 appeared to abolish human POMT activity (6) but did not inhibit yeast PMT activity (30). This difference may be due to the lipid compositions in human and yeast, which would affect the efficiency of

---

cotransfected cells (Fig. 3F, lane 1). The precipitates from cell membranes expressing only POMT1-Myc had slight POMT activity (Fig. 3F, lane 2). This weak activity may be due to complex formation between transfected POMT1-Myc and endogenous POMT2. The POMT activity in a mixture of individually expressed POMT1-Myc and POMT2 was similar to the background level (Fig. 3F, lane 5). Based on these results, we concluded that POMT1 and POMT2 associate physically in vivo and that this state becomes functional.

**Effect of Mutations on POMT Activity**—Recently, three mutations (G65R, A200P, and W582C) in the POMT1 gene were found (Table 1) (11–13). Because these patients have milder phenotypes than typical WWS patients, we expected the mutated POMT1s to have some POMT activity. To test this hypothesis, these mutations were introduced into POMT1-myc cDNA and cotransfected into HEK293T cells with POMT2 (Fig. 4, A–C). However, none of the POMT1 mutants, like the other mutants (10), showed any POMT activity (Fig. 4D).

**Immunoprecipitation of Mutant POMT1 and POMT2**—Eight mutations in the POMT1 gene of patients with WWS (G76R, L421del, V428D, G65R, A200P, W582C, V703fs, and G722fs) were found to abolish POMT activity in both the present and
protein solubilization by detergents and the stability of proteins after the removal of lipids by detergents.

Yeast Pmt1p has been proposed to consist of seven transmembrane helices (31). The Pmt1p N terminus and loops 2, 4, and 6 are located in the cytoplasm, and the C terminus and

### FIGURE 3.
Physical and functional association of POMT1 and POMT2. A–C, expression of POMT-Myc and POMT2 in HEK293T microsomal membrane fractions was determined by anti-POMT1 antibody (A), anti-Myc antibody (A-14) (B), and anti-POMT2 antibody (C). D and E, POMT1-POMT2 complex formation in vivo. POMT1-Myc and POMT2 were transfected into HEK293T cells and immunoprecipitated by anti-Myc (9E10) antibody-conjugated agarose. The resulting precipitates were analyzed by immunoblotting with anti-Myc antibody (A-14) (D) and anti-POMT2 antibody (E). F, POMT activity of immunoprecipitates. Lane 1, POMT1-Myc and POMT2 were transfected into cells; lane 2, cells were transfected with POMT1-Myc alone; lane 3, cells were transfected with POMT2 alone; lane 4, mock transfectant; lane 5, a mixture of individually expressed POMT1-Myc and POMT2. Asterisks indicate positions of corresponding molecules. Molecular weight standards are shown on the left. POMT activity was based on the amount of mannose transferred to a GST-αDG. Average values of three independent experiments are shown.

### TABLE 1
Summary of mutations in the *POMT1* gene of WWS patients

| Mutations | Effects          |
|-----------|-----------------|
| G193A     | Gly65 → Arg missense (G65R) |
| G598C     | Ala300 → Pro missense (A200P) |
| G1746C    | Trp582 → Cys missense (W582C) |
| C226A     | Gly78 → Arg missense (G76R) |
| 1260 to 1262 del CCT | Leu221 deletion (L221del) |
| T1283A    | Val428 → Asp missense (V428D) |

### FIGURE 4.
Enzymatic activity mutated POMT1-Myc with POMT2. A–C, Western blot analyses of POMT1-Myc and POMT2 proteins detected by anti-POMT1 antibody (A), anti-Myc antibody (A-14) (B), and anti-POMT2 antibody (C). D, POMT activities of the POMT1-Myc mutants coexpressed with POMT2. Lane 1, mock; lane 2, POMT1-Myc and POMT2; lane 3, G65R and POMT2; lane 4, A200P and POMT2; lane 5, W582C and POMT2; lane 6, G76R and POMT2; lane 7, L221del and POMT2; lane 8, V428D and POMT2. Asterisks indicate the migration positions of each POMT1-Myc protein (A, B) and POMT2 protein (C). Molecular weight standards are shown on the left. POMT activity was based on the amount of mannose transferred to a GST-αDG. Average values of three independent experiments are shown.
loops 1, 3, and 5 are located in the ER lumen. A large hydrophilic region (loop 5) and loop 1 are important for enzymatic activity (17, 31, 32). Based on this model, seven of ten POMT1 mutations identified in WWS patients appear to be located in loops 1 and 5 (7, 9, 11–13), and three of four POMT2 mutations identified in WWS patients are located in loop 5 (8). Taken together, these results indicate that loops 1 and 5 are important for catalysis, as they are in yeast PMTs. Furthermore, deletion of loop 5 in yeast Pmt1 eliminates enzymatic activity, but not Pmt1- Pmt2 interactions (32). Similarly, mutant POMT1 has the ability to form a complex with POMT2, although the complex does not have POMT activity. Further studies are needed to clarify the role of each domain in POMT activity and complex formation. Additionally, it has been shown that an Arg residue in the transmembrane domain is necessary for complex formation in yeast PMTs and a Glu residue in loop 1 is necessary for enzymatic activity (32). These amino acid residues are conserved in human POMT1 (33), and so it will be interesting to see whether they also are necessary for complex formation and activity. It is also of interest to screen uncharacterized WWS patients for the Arg and Glu mutations.

There is growing evidence that the glycans of glycoproteins play several roles in cellular differentiation and developmental events as well as in disease processes (34). Glycosylation is basically controlled by the combined action of many glycosyltransferases. The level and the strict substrate specificity of glycosyltransferases cooperate to synthesize specific sugar sequences and sugar linkages found in glycoproteins. Glycosyltransferase activities are regulated by other factors or by complex formation. For example, human core 1 β3-galactosyltransferase activity requires the expression of Cosmc (35). Cosmc is a molecular chaperone that specifically assists the folding/stability of core 1 β3-galactosyltransferase and is required for a glycosyltransferase expression. Mutations of COSMC were recently found in patients with Tn syndrome who could not produce core 1 structure (Galβ1–3GalNAc) (36). Another glycosyltransferase with complex regulation is human chondroitin synthase, which cannot polymerize chondroitin sulfate in vitro; rather, its activity requires the coexpression of chondroitin-polymerizing factor (37). As a third example, the bifunctional glycosyltransferases EXT1 and EXT2, which polymerize heparan sulfate, need to form a hetero-oligomeric complex to exert their optimal catalytic activities and to exist in the appropriate intracellular location (38, 39). In the present study, we observed that protein O-mannosylation can be initiated by direct complex formation of POMT1 and POMT2, but not by either enzyme by itself. POMT1 or POMT2 are thus different from EXT1 and EXT2 because the latter enzymes are active by themselves. One possibility is that formation of the POMT1-POMT2 complex creates a new catalytic domain. Further studies are needed to elucidate the mechanism of complex formation between POMT1 and POMT2 and the regulation of POMT activity. Our results, together with previous studies of glycosyltransferases, indicate that glycosylation is regulated in a complicated fashion.

Our findings that POMT1 and POMT2 associated physically and functionally in vivo and that POMT1 and POMT2 could not associate when they are expressed individually and then mixed suggest that the assembly of POMT1 and POMT2 requires specific conditions in the ER membrane. However, a heterocomplex POMT2 and mutated POMT1s suggest that single amino acid substitutions and deletion in POMT1 found in WWS patients do not affect assembly of POMT1 and POMT2. These mutations would abolish dolichyl phosphate mannose or acceptor (α-DG) binding. In the POMT assay, cells transfected with mutated POMT1 and wild-type POMT2 (Fig. 4D, lanes 3–8) had decreased enzymatic activity compared with the mock transfectant (Fig. 4D, lane 1). The decrease of endogenous enzymatic activity may be caused by the disturbance of endogenous POMT1-POMT2 formation.

WWS patients carrying three mutations (G65R, A200P, and M582C) showed milder phenotypes than typical WWS (14, 15), which led us to expect that these mutations would not completely abolish activity. Our finding that these mutant proteins did not have any enzymatic activity is thus puzzling. One possibility is that the levels of LARGE expression may be greater in these patients than in previous reported severe WWS patients. Barresi et al. (40) report that overproduction of LARGE caused hyperglycosylation of α-DG and improvement of function, e.g. laminin binding, in WWS fibroblasts. However, the change of the LARGE expression level in WWS patients may be rare because these patients had hypoglycosylated α-DG. Measurement of POMT activities of tissues from these patients may help to explain their mild symptoms. If each patient showed some POMT activities, other factor(s) that regulate POMT activity should be considered. In fact, some WWS patients have no mutations in POMT1 or POMT2 (7, 11). It is possible that mutations of such factors may cause unidentified WWS. Further studies are needed to test this hypothesis.

REFERENCES
1. Michele, D. E., and Campbell, K. P. (2003) J. Biol. Chem. 278, 15457–15460
2. Michele, D. E., Barresi, R., Kanagawa, M., Saito, F., Cohn, R. D., Satz, J. S., Dollar, J., Nishino, I., Kelley, R. I., Somer, H., Straub, V., Mathews, K. D., Moore, S. A., and Campbell, K. P. (2002) Nature 418, 417–422
3. Chiba, A., Matsumura, K., Yamada, H., Inazu, T., Shimizu, T., Kusunoki, S., Kanazawa, I., Kobata, A., and Endo, T. (1997) J. Biol. Chem. 272, 2156–2162

![Figure 5](image-url)
