Letter to the Editor
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The First Korean Case of *MAN1B1*-Congenital Disorder of Glycosylation Diagnosed Using Whole-Exome Sequencing and Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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Dear Editor,

Congenital disorders of glycosylation (CDG) is a group of metabolic disorders associated with abnormal protein or lipid glycosylation. They are associated with a broad spectrum of glycosylation pathway alterations caused by variants in various genes; therefore, CDG have heterogeneous clinical presentations, including facial dysmorphism, musculoskeletal abnormalities, growth and developmental delay, and/or intellectual disability (ID) [1]. CDG are classified based on the genes involved. Early diagnosis and intervention are important for genetic metabolic disorders; however, because of the diverse clinical features, it is difficult to accurately diagnose CDG in patients with ID. Given the recent advances in massive parallel sequencing, such as whole-exome sequencing (WES), reports and studies on CDG are markedly increasing in number [1-5]. Here, we present the first Korean case of a patient with MAN1B1-CDG diagnosed using WES. This study was approved by the Institutional Review Board (IRB) of Keimyung University, Dongsan Hospital, Daegu, Korea (IRB No. DSMC 2023-04-036). Informed consent for reporting the cases was waived since the study involved a retrospective review of medical records and the use of leftover samples.

In March 2018, an 8-yr-old girl visited the rehabilitation department because of worsening hallux valgus, developmental delay, and ID. At that time, a family history of neurofibromatosis type 1 (NF1) was identified, which affected both her mother and younger sister. They had been diagnosed based on a genetic study requested by GC Labs (Yongin-Si, Gyeonggi-do, Korea) and conducted at the Seoul St. Mary's Hospital (Seoul, Korea). However, our patient did not carry pathogenic variants in *NF1*. She was born at 39 weeks of intrauterine pregnancy via cesarean section because of premature membrane rupture combined with oligohydramnios, and her birth weight was 2,600 g. She showed motor developmental delay and was diagnosed as having attention deficit hyperactivity disorder accompanied by delayed speech and early-onset cognitive impairment. Her face

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showed some dysmorphic features, such as downward-slanting palpebral fissures and hypertelorism. She had thoracolumbar scoliosis and severe plano-valgus deformities of the foot. During follow-up, ID and delayed language development were persistent. WES was performed to identify potential causative genetic factors. We found two heterozygous nonsense variants in *MAN1B1* (NM_016219.4), c.244C>T (p.Gln82*; rs1830422749) and c.1423C>T (p.Gln475*; novel variant, absent from the gnomAD v4.1.0 database), which are related to ID, autosomal recessive 15 (MRT15, Rafiq syndrome, *MAN1B1*-CDG). Parental genetic analysis revealed that her father had c.244C>T, and her mother had c.1423C>T, confirming compound heterozygosity. The c.244C>T variant has been reported as pathogenic in one study [4], and the novel variant, c.1423C>T, was located in the enzymatic functional domain-coding region corresponding to a hotspot where numerous pathogenic variants in *MAN1B1* have been reported (Fig. 1).

MAN1B1 encodes endoplasmic reticulum (ER) mannosyl-oligosaccharide 1,2-alpha-mannosidase (α1,2-mannosidase), a major glycosylation enzyme. In May 2023 , to determine the pathogenicity of the compound heterozygous variants, including novel variant c.1423C>T, we evaluated the functionality of the patient's ER α1,2-mannosidase through *N*-glycan profiling (with peptide-N-glycosidase F treatment without permethylation) and matrix-assisted laser desorption ionization-time-of-flight-mass spectrometry (MALDI-ToF MS) [5] of sera from the patient, her family members, and healthy controls. The findings revealed an increase in mannose-rich glycans, a decrease in *N*-acetylglucosamine-linked glycans, and the presence of hybrid-type *N*-glycan in the patient's serum [4]. These results suggest a loss of ER mannosidase activity. We found no abnormalities in the sera from her family members or the healthy controls (Fig. 2).

Glycosylation is the attachment of a sugar chain to a nascent protein or lipoprotein in the cytosolic ER and Golgi. ER α1,2-mannosidase is a key enzyme in the late stage of ER glycosylation, which plays an important role in protein folding quality control and post-translational modification. Therefore, dysfunction or loss of function of this enzyme results in a global disruption of protein production, localization, and signaling pathways in several tissue types [6, 7].

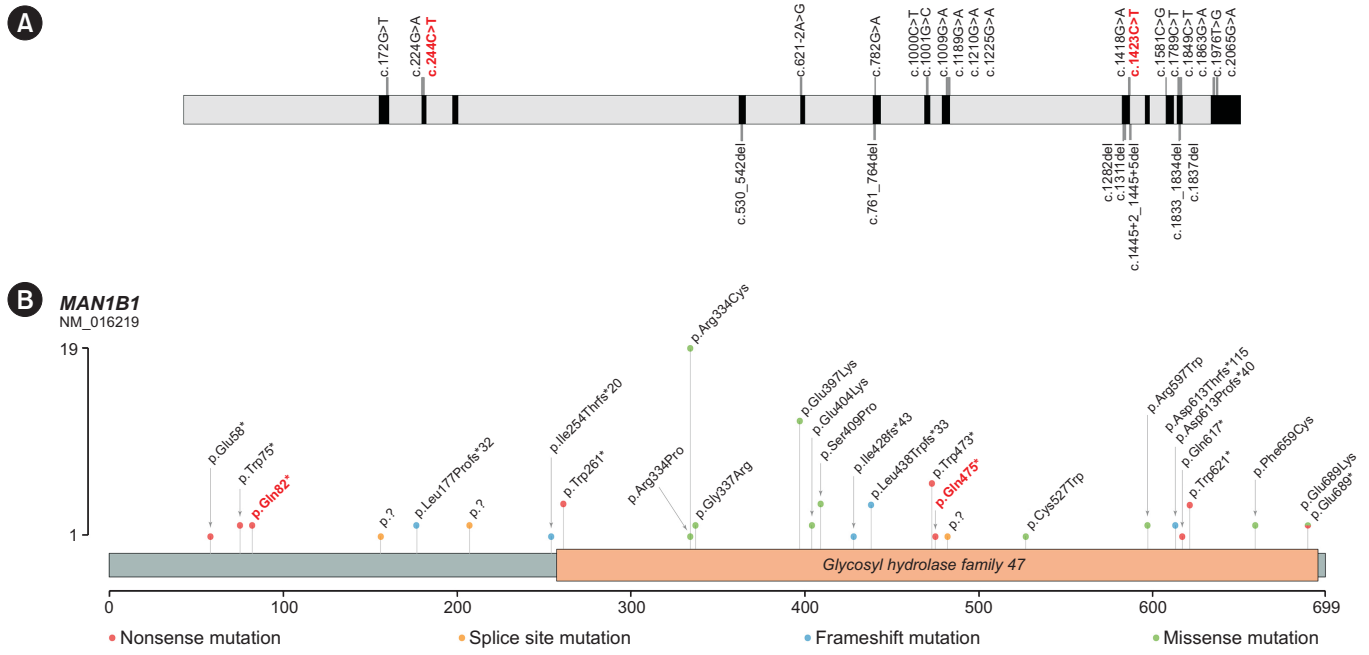

Fig. 1. Distribution of *MAN1B1* variants reported in the literatures and this paper. (A) Genomic locations of variants associated with *MAN1B1*-congenital disorder of glycosylation. The black rectangles indicate the 13 exons, and the gray rectangles indicate the 5′-untranslated region and the 12 introns. The upward-pointing text indicates single-nucleotide substitutions, and the downward-facing text indicates frameshifting deletions, except for one large deletion variant (c.465+1460_620+527del). The two variants found in our patient are highlighted in bold red. (B) Alignment of the mutations to the functional domains of endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase (NP_057303.2) encoded by *MAN1B1* (NM_016219.5). The left vertical bar represents the number of times the mutation has been reported, and the horizontal bar below indicates the location of amino acids. The corresponding amino acid changes for the two variants reported in this paper are highlighted in bold red.

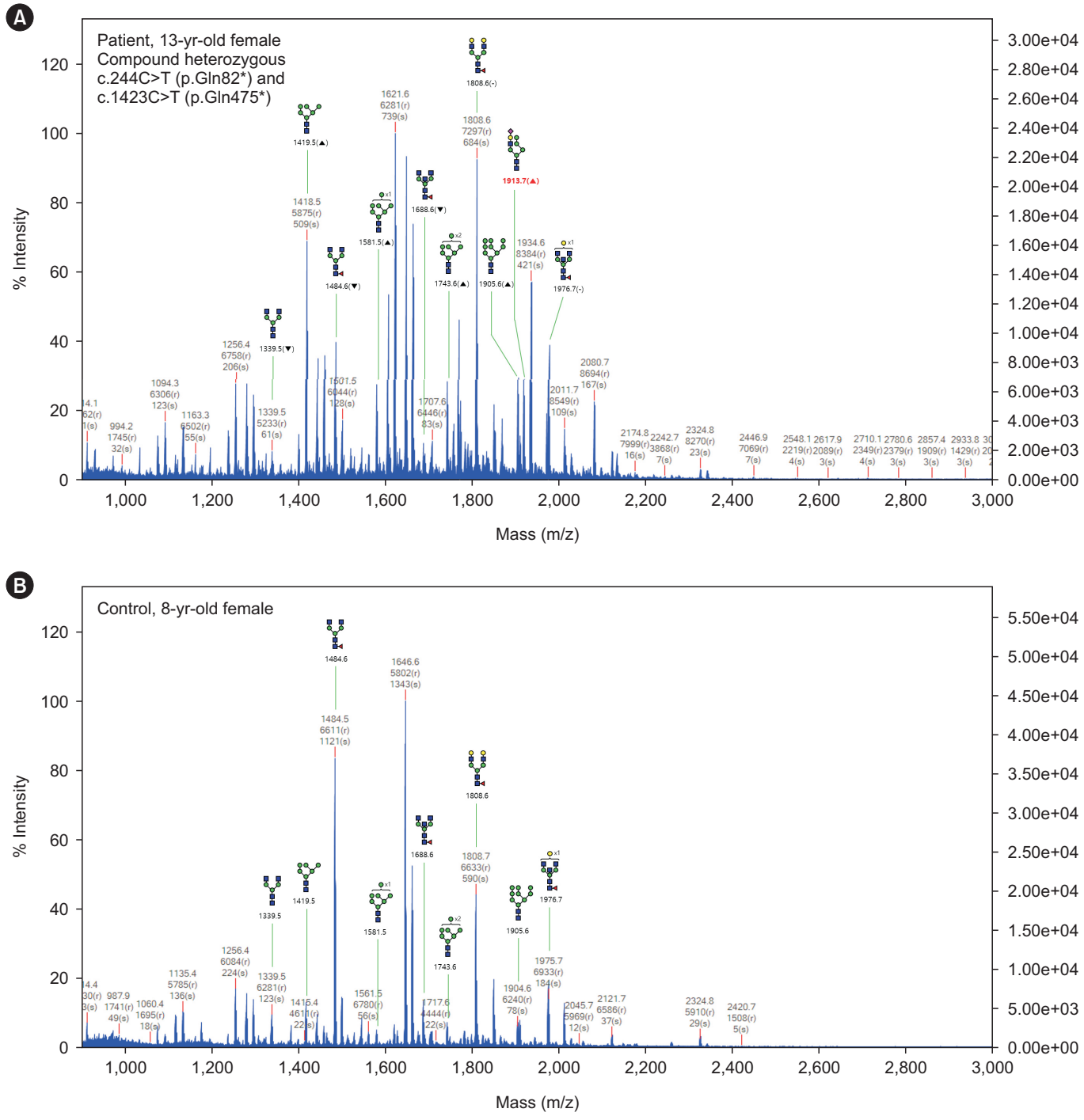

Fig. 2. Mass spectra of *N*-glycan profiling using Matrix-assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry following peptide-N-glycosidase F treatment. The mass spectra of samples from the patient (A) revealed an increase in mannose-rich glycans (upward black triangle) and a decrease in *N*-acetyl glucosamine-binding glycans (downward black triangle) compared with that in the healthy control (B). A hybrid-type *N*-glycan was observed at *m/z* 1913.7 (red), which is specific to patients with *MAN1B1* deficiency. Green circles, mannose; yellow circles, galactose; blue squares, *N*-acetyl glucosamine; red triangles, fucose; and purple diamonds, sialic acid.

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The phenotypes associated with *MAN1B1* variants are relatively uniform [8-10]. They include mild to moderate ID, truncal obesity, muscular hypotonia, and modest dysmorphic features. However, various reports of *MAN1B1*-CDG, including ours, suggest that inconsistent phenotypes are not uncommon [10]. In our case, ID and facial dysmorphism were typical clinical presentations, but severe foot deformities are not commonly reported. Therefore, we suggest that WES is an efficient diagnostic tool in cases of unexplained ID with non-specific, heterogeneous clinical presentations. In addition, *N*-glycan profiling using MALDI-ToF MS can aid in the assessment and confirmation of CDGs with novel variants.

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AUTHOR CONTRIBUTIONS

KB Kim and JS Ha collected and summarized the literature, interpreted the test results, and wrote the manuscript draft. D Kim, D Seo, and H Kweon reviewed the literature, designed and conducted the MALDI-ToF MS experiment, and analyzed the MS data. GS Lee, S Shin, H Kang, S Park, and D Kim contributed to interpreting and reporting the test results. S Lee, N Ryoo, and JS Ha collected and summarized the literature studies, compared them with the present case, and reviewed the manuscript draft.

CONFLICTS OF INTEREST

None declared.

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