JCN Open Access ORIGINAL ARTICLE

pISSN 1738-6586 / eISSN 2005-5013 / J Clin Neurol 2021;17(4):534-540 / https://doi.org/10.3988/jcn.2021.17.4.534

A Compound Heterozygous Pathogenic Variant in *B4GALNT1* Is Associated With Axonal Charcot-Marie-Tooth Disease

Ji-Man Hong[a*]
Hyeonjin Jeon[b,c*]
Young-Chul Choi[d]
Hanna Cho[d]
Young Bin Hong[b,c]
Hyung Jun Park[d]

[a]Department of Neurology, Yongin Severance Hospital, Yonsei University College of Medicine, Yongin, Korea
[b]Department of Biochemistry, College of Medicine, Dong-A University, Busan, Korea
[c]Department of Translational Biomedical Sciences, Graduate School of Dong-A University, Busan, Korea
[d]Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Background and Purpose Pathogenic variants in *B4GALNT1* have been reported to cause hereditary spastic paraplegia 26. This study has revealed that a novel compound heterozygous pathogenic variant in *B4GALNT1* is associated with axonal Charcot-Marie-Tooth disease (CMT).

Methods Whole-exome sequencing (WES) was used to identify the causative factors and characterize the clinical features of a Korean family with sensorimotor polyneuropathy. Functional assessment of the mutant genes was performed using a motor neuron cell line.

Results The WES revealed a compound heterozygous pathogenic variant (c.128dupC and c.451G>A) in *B4GALNT1* as the causative of the present patient, a 53-year-old male who presented with axonal sensorimotor polyneuropathy and cognitive impairment without spasticity. The electrodiagnostic study showed axonal sensorimotor polyneuropathy. *B4GALNT1* was critical to the proliferation of motor neuron cells. The compensation assay revealed that the pathogenic variants might affect the enzymatic activity of *B4GALNT1*.

Conclusions This study is the first to identify a case of autosomal recessive axonal CMT associated with a compound heterozygous pathogenic variant in *B4GALNT1*. This finding expands the clinical and genetic spectra of peripheral neuropathy.

Keywords Charcot-Marie-Tooth disease; whole-exome sequencing; *B4GALNT1*.

Received March 16, 2021
Revised June 8, 2021
Accepted June 8, 2021

Correspondence
Hyung Jun Park, MD, PhD
Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea
Tel +82-2-2019-3329
Fax +82-2-3462-5904
E-mail hjpark316@yuhs.ac

Young Bin Hong, PhD
Department of Biochemistry, College of Medicine, Dong-A University, 32 Daesingongwon-ro, Seo-gu, Busan 49201, Korea
Tel +82-51-240-2762
Fax +82-51-240-2971
E-mail ybhong@dau.ac.kr

*These authors contributed equally to this work.

INTRODUCTION

A hereditary motor and sensory neuropathy commonly known as Charcot-Marie-Tooth disease (CMT) is a heterogeneous disorder of the peripheral nervous system.[1,2] CMT is mainly divided into demyelinating and axonal types according to the location of the main pathogenesis.[3-5] The former is associated with demyelination in the Schwann cells and the latter is caused by aberrations in the integrity of peripheral axons. The main symptoms of CMT are motor deficit and sensory loss due to peripheral degeneration, gait disturbance, and walking disability. More than 100 genes have been reported to be associated with the CMT phenotype, and their number continues to increase with the application of efficient analysis tools such as whole-exome sequencing (WES).[6,7]

The beta-1,4-N-acetyl galactosaminyltransferase 1 gene (*B4GALNT1*) transfers GalNAc to LacCer, GM3, GD3, or GT3 to generate GA2, GM2, GD2, and GT2, respectively.[8-10] Gangliosides are glycosphingolipids that are highly expressed in the nervous system and are involved in various critical roles such as synaptic plasticity and signal transduction.[11-13] Alterations in ganglioside metabolism affect neuronal function and are associated with neurodegenerative diseases.[14] Changes in the concentrations of gangliosides are involved in the pathogen-

esis of Alzheimer's disease, Huntington's disease, and gangliosidosis.[15-17]

Pathogenic variants in *B4GALNT1* are involved in hereditary spastic paraplegia subtype 26 (SPG26) in an autosomal recessive manner. The clinical symptoms of SPG26 caused by pathogenic variants in *B4GALNT1* include lower extremity spasticity, muscle weakness, and gait abnormality, while extrapyramidal and cerebellar signs, intellectual disability, and dysarthria have also been reported.[18-20]

Here we report the clinical features of an autosomal recessive CMT patient with a novel compound heterozygous pathogenic variant in *B4GALNT1*.

METHODS

Clinical and electrophysiological assessments

The clinical information used in the phenotype assessment included age at symptom onset, age at examination, family history, muscle impairments, joint contracture, sensory deficit, and deep tendon reflexes. Physical disability was quantified by scoring the patient on two scales. Disease severity was assessed according to the 9-point Functional Disability Scale (FDS) from 0 to 8 as follows: 0=normal; 1=normal except for cramps and fatigability; 2=inability to run; 3=difficulty walking unaided, but still possible; 4=can walk with a cane; 5=can walk with crutches; 6=can walk with a walker; 7=wheelchair-bound; and 8=bedridden.[21] The CMT neuropathy score was determined based on the symptoms as well as the results of a neurological examination and nerve conduction study (NCS).[22] NCS and needle electromyography were performed at both 41 and 53 years of age. The Mini-Mental State Examination (MMSE) and neuropsychological tests were performed at 53 years of age. This research protocol was approved by the Institutional Review Board of Gangnam Severance Hospital, Korea (IRB No: 3-2021-0014). Written informed consent was exempted by the board because this was a retrospective study.

Isolation of genetic cause

The genetic cause of peripheral neuropathy was determined by applying WES to the patient (III-2). The total sequencing yield was 18.87 Gbp/sample, and the coverage rate of the targeted exon regions (≥10×) was 99.0%. The average read depth of the target regions was 253.0 reads. The total number of single-nucleotide variants (SNVs) and indels was 111,802 per sample, of which 43,662 SNVs were coding variants. We identified seven functionally significant variants of neuromyopathy-relevant genes (Table 1).

Generation of *B4GALNT1* mutant

The plasmid containing human *B4GALNT1*, pCMV6-myc-B4GALNT1, was obtained from OriGene (Rockville, MD, USA). Site-directed mutagenesis was performed to generate c.128dupC and c.451G>A using the following primers: B4GALNT1 forward, 5'-GGA GAT CTG CCG CCG CGA TCG CCA TGT GGC TGG GCC GCC GGG CCC-3'; B4GALNT1 reverse, 5'-CTG CTC GAG CGG CCG CGT ACG CGT CTG GGA GGT CAT GCA CTG-3'; B4GALNT1-128dupC forward, 5'-CTT GCG CCG TGG GCG CCC CCC GCA AAG CCC CCG CAG-3'; B4GALNT1-128dupC reverse, 5'-CTG CGG GGG CTT TGC GGG GGG CGC CCA CGG CGC AAG-3'; B4GALNT1-451G>A forward, 5'-CTC CAG TAC CCC CTA CAG AGT GTG GAA GTT CAG CCC C-3'; and B4GALNT1-451G>A reverse, 5'-GGG GCT GAA CTT CCA CAC TCT GTA GGG GGT ACT GGA G-3'. All pathogenic variants were confirmed using capillary sequencing.

Cell viability assay

The NSC34 mouse motor neuron cell line was used to monitor the effect of *B4galnt1* knockdown as described previously.[23,24] To determine cell proliferation, cells (4×10^4) were transfected using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol

Table 1. Functionally significant variants of neuromyopathy-relevant genes

Gene	Accession	Variant		Zygosity	dbSNP138	gnomAD exomes (version 2.11)	SIFT	PolyPhen2	ACMG classification
		Nucleotide	Amino acid						
*B4GALNT1**	NM_001478.5*	c.128dupC*	p.Gln44AlafsTer14*	Hetero*	-	-	-	-	Pathogenic*
*B4GALNT1**	NM_001478.5*	c.451G>A*	p.Gly151Ser*	Hetero*	rs750664123*	0.00000399*	0.000*	1.000*	Likely pathogenic*
AFG3L2	NM_006796.3	c.242A>C	p.Lys81Thr	Hetero	-	-	0.016	0.594	VOUS
WASHC5	NM_014846.4	c.1708G>A	p.Glu570Lys	Hetero	-	-	0.242	0.099	VOUS
SACS	NM_014363.6	c.6751C>A	p.Gln2251Lys	Hetero	rs747293426	-	0.163	0.965	VOUS
NDUFV1	NM_007103.4	c.218C>T	p.Pro73Leu	Hetero	-	0.00000398	0.181	0.005	VOUS
ABHD12	NM_015600.5	c.718G>A	p.Val240Met	Hetero	rs572997548	0.000211	0.003	0.992	VOUS

*Pathogenic or likely pathogenic variants. SIFT: <0.05 indicates prediction of deleterious. PolyPhen2: ~1 indicates prediction of pathogenicity. ACMG classification refers to the classification of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[46] VOUS, variant of uncertain significance.

with the following *B4galnt1*-specific siRNAs (Bioneer, Daejeon, Korea): *B4galnt1*-siRNA#1, 5'-CAG UUC UGG AUA AAC UCA A-3'; *B4galnt1*-siRNA#2, 5'-CUU CUG UCC AGG AGA UAU A-3'; and *B4galnt1*-siRNA#3, 5'-CUG AUA GCU CCC GCC AAC U-3'. After 3 days of knockdown, cell proliferation was quantified by direct counting under a microscope.

The knockdown of *B4galnt1* in NSC34 cells was confirmed using the reverse-transcription polymerase chain reaction (RT-PCR). Total mRNA was purified using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The cDNA obtained by applying reverse transcription using SuperScript™ II reverse transcriptase (Invitrogen) was used as a template for PCR amplification. Transfections of human wild-type and c.128dupC and c.451G>A mutant *B4GALNT1* plasmids were performed in combination with *B4galnt1*-siRNA#3. After overexpression and knockdown for 3 days, the total number of NSC34 cells was counted.

RESULTS

Clinical manifestations

A 53-year-old male (Fig. 1A, III-2) presented to our neurological clinic with gait disturbance. He did not have diabetes mellitus or alcohol abuse, and was only a carrier of hepatitis B. He first noticed a steppage gait at an age of 20 years, after which his muscle weakness progressed very slowly. When he was first examined at the age of 41 years, he displayed motor weakness and hypesthesia of the distal leg muscles. At the last examination at 53 years of age, he was able to ambulate independently. A neurological examination revealed motor weakness and atrophy of the bilateral distal leg muscles. Ankle contractures were also observed. Pain sensation was preserved, but the vibration and position senses were reduced. Knee and ankle jerks were absent, as were Babinski's sign and ankle clonus. The patient did not exhibit facial weakness or a high-arched palate. He had an FDS score of 4 and a CMT neuropathy score of 6, and was categorized as having mild disability. He had received 12 years of education and scored 25 of 30 on the Korean version of the MMSE. Neuropsychological tests revealed cognitive impairments in multiple domains (language, ideomotor praxis, calculation, visuospatial, and memory) without any limitation in performing the activities of daily living.

Electrodiagnostic studies were performed at ages of 41 and 53 years (Table 2). NCSs showed reduced sensory nerve action potentials in the median, ulnar, superficial peroneal, and sural nerves. Needle electromyography showed mild denervation potentials in the bilateral tibialis anterior and gastrocnemius muscles at 53 years of age. These findings were con-

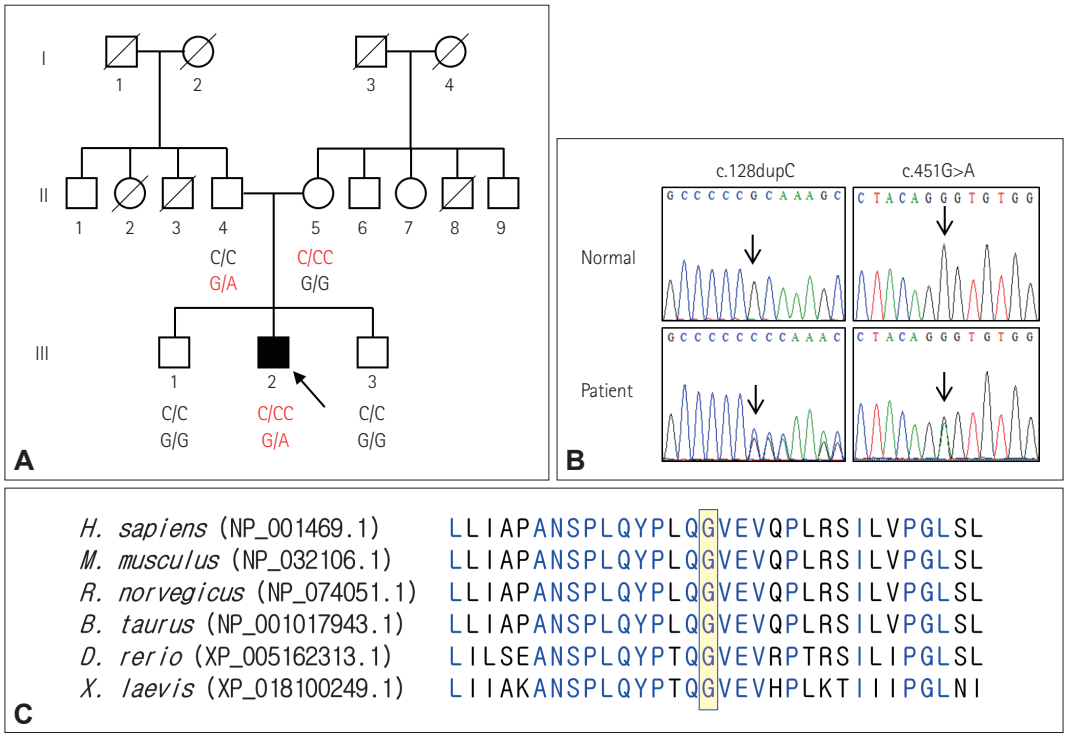

Fig. 1. Pedigree and *B4GALNT1* variants in the family with axonal Charcot-Marie-Tooth disease. A: Alleles of two pathogenic variants of *B4GALNT1*. Open symbols, unaffected; filled symbol, affected; arrow, proband. B: Sequencing chromatograms of c.128dupC and c.451G>A variants. Arrows indicate the pathogenic variant sites. C: Conservation analysis for amino acid sequences of *B4GALNT1* among species. Yellow highlighting indicates the variant-site p.Gly151Ser (c.451G>A); blue text indicates completely conserved amino acids.

Table 2. Electrophysiological features of patients with compound heterozygous *B4GALNT1* variants

	First examination	Second examination		Normal value
Age at examination (yr)	41	53		
Side	Right	Right	Left	
Median nerve				
TL (ms)	3.0	3.0		<3.9
CMAP (mV)	20.3	17.5		>6.0
MNCV (m/s)	57.1	58.0		>50.5
F-wave (ms)	27.2	26.9		<28.0
Ulnar nerve				
TL (ms)	2.3	2.3		<3.0
CMAP (mV)	16.2	14.8		>8.0
MNCV (m/s)	57.1	60.0		>51.1
F-wave (ms)	26.5	27.1		<29.0
Peroneal nerve				
TL (ms)	4.2	4.1	3.9	<5.3
CMAP (mV)	7.1	6.3	4.8	>1.6
MNCV (m/s)	48.2	48.0	46.0	>41.2
F-wave (ms)	43.4	49.5	49.5	<49.0
Tibial nerve				
TL (ms)	3.8	3.6	3.5	<5.4
CMAP (m V)	12.9	15.0	22.5	>6.0
MNCV (m/s)	47.2	47.0	46.0	>41.1
F-wave (ms)	40.1	45.2	44.7	<52.1
Median sensory nerve				
SNAP (μV)	6.8*	6.7*		>8.8
SNCV (m/s)	43.5	43.0		>39.3
Ulnar sensory nerve				
SNAP (μV)	5.4*	5.3*		>7.9
SNCV (m/s)	38.8	40.0		>37.5
Superficial peroneal nerve				
SNAP (μV)	8.4	5.2*	6.2	>6.0
SNCV (m/s)	37.7	38.0	36.0	>32.1
Sural nerve				
SNAP (μV)	6.3	5.0*	5.0*	>6.0
SNCV (m/s)	30.0*	33.0	31.0*	>32.1
H-reflex (ms)	A*	A*	A*	<30.2

*Abnormal values.
A, absent potentials; CMAP, compound muscle action potential; MNCV, motor nerve conduction velocity; SNAP, sensory nerve action potential; SNCV, sensory nerve conduction velocity; TL, terminal latency.

sistent with axonal sensorimotor polyneuropathy. MRI of the brain and spinal cord did not reveal any parenchymal abnormalities.

Identification of a compound heterozygous pathogenic variant in *B4GALNT1*

From the unreported functionally significant SNVs in dbSNP138 and the Genome Aggregation database (gnomeAD, https://gnomad.broadinstitute.org), we identified a pair of compound heterozygous pathogenic variants transmitted from each of the parents: c.128dupC and c.451G>A in *B4GALNT1* (NM_001478.5) (Fig. 1A and B). Although the parents carried a copy of each mutant allele, they did not exhibit an axonal CMT phenotype. The siblings carried wild-type alleles. The c.128dupC variant was classified as a pathogenic variant based on the following evidence: 1) it is a null variant of a gene where loss of function is a known disease mechanism, 2) the variant is not found in gnomAD exomes and genomes, 3) there are multiple lines of computational evidence for a deleterious effect on the gene or protein, and 4) an in vitro functional study supports a damaging effect on the gene. The c.451G>A change causes the p.Gly151Ser variant. Gly151 is located in a highly conserved region among different species (Fig. 1C), and in silico analyses (using SIFT and PolyPhen2) predict that it affects functional integrity. This missense variant was classified as a likely pathogenic variant based on the following evidence: 1) the variant is not found in gnomAD exomes and genomes, 2) the variant is detected in trans with a pathogenic variant, 3) there are multiple lines of computational evidence for a deleterious effect on the gene or protein, and 4) an in vitro functional study supports a damaging effect on the gene.

Mutant protein inhibits cell proliferation and viability

To investigate the role of *B4GALNT1* in motor neurons, we measured its effect on cell proliferation after abrogation. Transfection of mouse *B4galnt1*-specific siRNAs for 72 h affected the number of NSC34 cells (Fig. 2A). RT-PCR showed that all of the *B4galnt1*-specific siRNAs were effective in reducing the mRNA levels in NSC34 cells (Fig. 2B). Direct cell counting showed that abrogation of *B4GALNT1* significantly reduced the proliferation of NSC34 cells (Fig. 2C). The cell numbers were reduced to 50.6% in *B4galnt1*-siRNAs#3 treated cells compared with negative-control siRNA (NC-siRNA) treatment. This implies that *B4GALNT1* is crucial for the proliferation of motor neurons.

We next investigated the function of mutant *B4GALNT1* after the generation of two mutants of *B4GALNT1* (c.128dupC and c.451G>A) from the wild-type gene. After the knockdown of endogenous *B4galnt1* in NSC34, human *B4GALNT1* plasmids were introduced. Overexpression of wild-type and mutant (c.128dupC and c.451G>A) *B4GALNT1* did not affect cell proliferation in combination with NC-siRNA transfection, indicating that the mutant proteins did not show a dominant-negative effect. In the *B4galnt1* cells knocked down by *B4galnt1*-siRNAs#3, overexpression of wild-type *B4GALNT1* significantly increased cell proliferation to the control level,

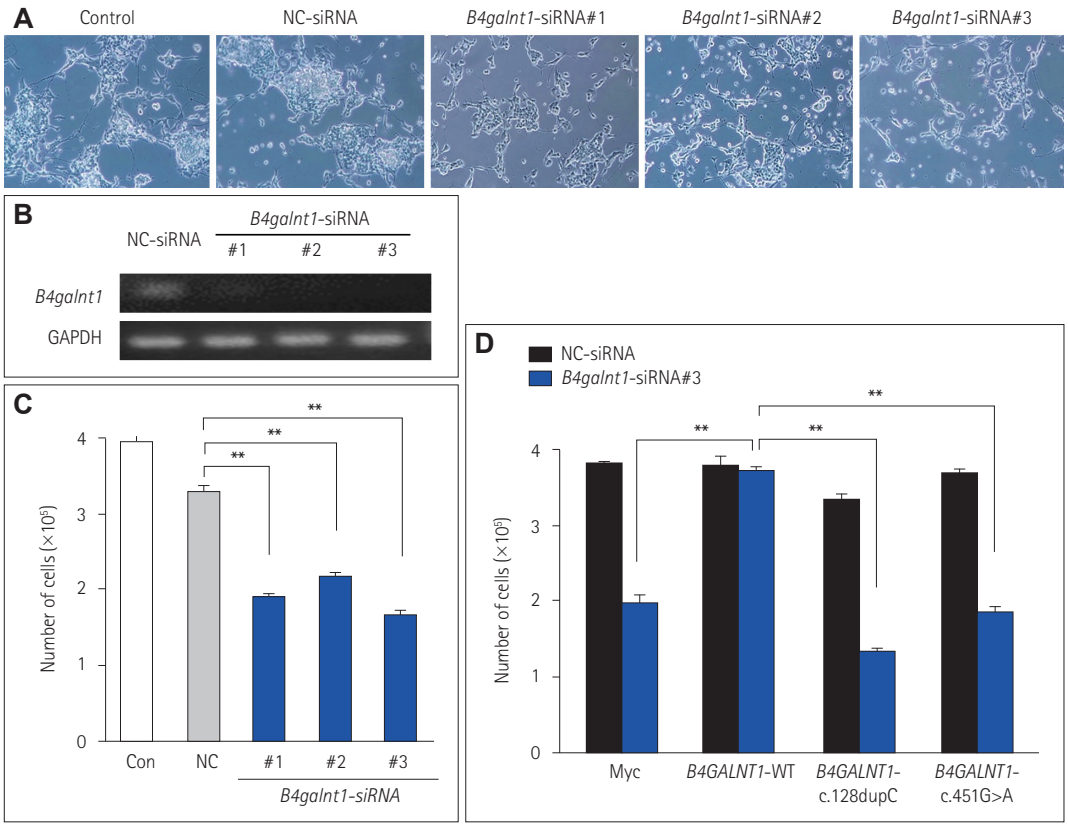

Fig. 2. Knockdown of *B4GALNT1* and cell proliferation. A: Representative images of cells from the NSC34 mouse motor neuron cell line, after mouse *B4galnt1*-specific siRNAs. B: Confirmation of *B4galnt1* knockdown in NSC34 by reverse-transcription polymerase chain reaction. C: Proliferation changes after *B4galnt1* knockdown. D: Compensation of *B4galnt1* knockdown with overexpression with human *B4GALNT1* (wild type and mutants). Data are mean and standard-error-of-the-mean values. Student's *t*-test: $^{**}p<0.01$. Con, control; NC, negative control; WT, wild type.

whereas overexpression of the mutant genes had no effect (Fig. 2D). These results are consistent with the reduced expression of *B4GALNT1* in affected patients.

DISCUSSION

This study has identified a compound heterozygous pathogenic variant in *B4GALNT1* that is associated with axonal sensorimotor polyneuropathy and mild cognitive impairment without spasticity. Pathogenic variants in *B4GALNT1* has usually been reported to cause SPG26, which is associated with early-onset spastic paraplegia, intellectual disability, cerebellar ataxia, and peripheral neuropathy.[18-20,25] It has recently been reported that patients with a novel homozygous pathogenic variant (c.263dupG) in *B4GALNT1* exhibit glutaric acidemia type II, which results in a sudden metabolic crisis that includes acidosis and hypoglycemia.[26] The clinical findings of our patient have not been reported previously in other patients with pathogenic variants in *B4GALNT1*. However, many SPG-related genes, including *ATL1*, *KIF1A*, *KIF5A*, *SACS*, *SPG11*, and *TFG*, are also associated with spastic paraplegia, hereditary sensory neuropathy, or CMT diseases.[27-32]

Sphingolipids or gangliosides play a series of important functions in neurons, such as proliferation, differentiation, and synaptic transmission.[33-36] To date, the most well-described diseases with sphingolipid metabolism are lysosomal storage disorders such as Tay-Sachs disease and Niemann-Pick C disease.[37,38] Recent advances in causative gene isolation have revealed that sphingolipid metabolism is associated with various types of neurodegenerative diseases, including peripheral neuropathy. Pathogenic variants in *SPTLC1* (Serine palmitoyltransferase) and longchain base subunit and *SPTLC2* are associated with hereditary sensory and autonomic neuropathy.[39,40] Mutant *HSPB1*, a causative gene of CMT2F and distal hereditary motor neuropathy type IIB, was recently reported to decrease mitochondrial ceramide levels and modify the structural and functional changes in mitochondria via interactions with ceramide synthase 1.[41] Therefore, dysregulation of sphingolipid metabolism also affects neuronal activity in the peripheral nerves.

Previous studies have found that *B4galnt1* disruption in mice does not severely affect the nervous system, except for a slight reduction in neural conduction velocity from the tibial

nerve to the somatosensory cortex, which suggests that complex gangliosides are predominantly required in synaptic transmission.[42,43] These features are very similar to those observed in humans, such as reduced sensory nerve function, abnormal gait, tremor, and ataxia in age-related neurodegeneration. Eleven pathogenic variants in *B4GALNT1* have been reported, including two nonsense and three frameshift variants.[18-20,26] Predictions of the tertiary structure of *B4GALNT1* protein suggest that most missense variants will affect protein stability, which is also supported by immunostaining data.[44,45] Enzymatic assays have revealed that all of the pathogenic variants completely affect the activity, while two pathogenic variants (c.898C>T (p.Arg300Cys) and c.682C>T (p.Arg228)) show lower levels of enzymatic activity.[45]

To determine the effect of the newly identified pathogenic variants (c.128dupC and c.451G>A) on peripheral neurons, we evaluated the proliferation of motor neurons after abrogation of mouse *B4galnt1* and overexpression of human mutant *B4GALNT1*. Knockdown of *B4galnt1* significantly reduced the proliferation of mouse motor neurons. In the absence of mouse *B4galnt1*, overexpression of human wild-type *B4GALNT1* completely compensated for the cell proliferation. However, transfection of both mutant genes (c.128dupC and c.451G>A) did not affect cell proliferation, implying that these pathogenic variants might significantly affect the enzymatic activity of *B4GALNT1*. Collectively these data suggest that the loss-of-function variants in *B4GALNT1* can play a role in peripheral neuropathy by disturbing ganglioside metabolism in neurons.

In conclusion, here we report for the first time that a compound heterozygous pathogenic variant in *B4GALNT1* is associated with axonal CMT. The present findings suggest that alterations in sphingolipid metabolism are widely associated with peripheral neuropathy, and they expand the clinical spectrum of both *B4GALNT1*-associated diseases and hereditary motor and sensory neuropathy.

Availability of Data and Material

All data generated or analyzed during the study are included in this published article (and its supplementary information files).

ORCID iDs

Ji-Man Hong	https://orcid.org/0000-0002-0696-8448
Hyeonjin Jeon	https://orcid.org/0000-0002-8206-2349
Young-Chul Choi	https://orcid.org/0000-0001-5525-6861
Hanna Cho	https://orcid.org/0000-0001-5936-1546
Young Bin Hong	https://orcid.org/0000-0002-4333-3847
Hyung Jun Park	https://orcid.org/0000-0003-4165-8901

Author Contributions

Conceptualization: Young Bin Hong, Hyung Jun Park. Data curation: Hyeonjin Jeon, Ji-Man Hong. Formal analysis: Ji-Man Hong, Young Bin Hong, Hanna Cho, Hyung Jun Park. Funding acquisition: Ji-Man Hong. Supervision: Young-Chul Choi. Writing—original draft: Young Bin Hong, Hyung Jun Park. Writing—review & editing: Young Bin Hong, Hyung Jun Park.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Funding Statement

This study was supported by NRF grants funded by MSIP, Republic of Korea (2016R1A5A2007009 and NRF-2019R1F1A1060313) and faculty research grant of Department of Neurology of Yonsei University College of Medicine (2019).

Acknowledgements

The authors would like to thank the patient and his family for their help with this work.

REFERENCES

1. Harding AE, Thomas PK. Genetic aspects of hereditary motor and sensory neuropathy (types I and II). *J Med Genet* 1980;17:329-336.
2. Klein CJ, Duan X, Shy ME. Inherited neuropathies: clinical overview and update. *Muscle Nerve* 2013;48:604-622.
3. Züchner S, Vance JM. Mechanisms of disease: a molecular genetic update on hereditary axonal neuropathies. *Nat Clin Pract Neurol* 2006;2:45-53.
4. Pareyson D, Marchesi C. Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. *Lancet Neurol* 2009;8:654-667.
5. Pareyson D. Axonal Charcot-Marie-Tooth disease: the fog is only slowly lifting. *Neurology* 2007;68:1649-1650.
6. Eggermann K, Gess B, Häusler M, Weis J, Hahn A, Kurth I. Hereditary neuropathies: clinical presentation and genetic panel diagnosis. *Dtsch Arztebl Int* 2018;115:91-97.
7. Pipis M, Rossor AM, Laura M, Reilly MM. Next-generation sequencing in Charcot-Marie-Tooth disease: opportunities and challenges. *Nat Rev Neurol* 2019;15:644-656.
8. Nagata Y, Yamashiro S, Yodoi J, Lloyd KO, Shiku H, Furukawa K. Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J Biol Chem* 1992;267:12082-12089.
9. Ruan S, Raj BK, Furukawa K, Lloyd KO. Analysis of melanoma cells stably transfected with beta 1,4GalNAc transferase (GM2/GD2 synthase) cDNA: relative glycosyltransferase levels play a dominant role in determining ganglioside expression. *Arch Biochem Biophys* 1995;323:11-18.
10. Yamashiro S, Haraguchi M, Furukawa K, Takamiya K, Yamamoto A, Nagata Y, et al. Substrate specificity of beta 1,4-N-acetylgalactosaminyltransferase in vitro and in cDNA-transfected cells. GM2/GD2 synthase efficiently generates asialo-GM2 in certain cells. *J Biol Chem* 1995;270:6149-6155.
11. Yu RK, Nakatani Y, Yanagisawa M. The role of glycosphingolipid metabolism in the developing brain. *J Lipid Res* 2009;50 Suppl:S440-S445.
12. Russo D, Parashuraman S, D'Angelo G. Glycosphingolipid-protein interaction in signal transduction. *Int J Mol Sci* 2016;17:1732.
13. She JQ, Wang M, Zhu DM, Sun LG, Ruan DY. Effect of ganglioside on synaptic plasticity of hippocampus in lead-exposed rats in vivo. *Brain Res* 2005;1060:162-169.
14. Ariga T. Pathogenic role of ganglioside metabolism in neurodegenerative diseases. *J Neurosci Res* 2014;92:1227-1242.
15. Ariga T, McDonald MP, Yu RK. Thematic review series: sphingolipids. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease—a review. *J Lipid Res* 2008;49:1157-1175.
16. Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Hunting-

ton's disease. *Neurobiol Dis* 2007;27:265-277.
17. Kolter T, Sandhoff K. Sphingolipid metabolism diseases. *Biochim Biophys Acta* 2006;1758:2057-2079.
18. Boukhris A, Schule R, Loureiro JL, Lourenço CM, Mundwiller E, Gonzalez MA, et al. Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. *Am J Hum Genet* 2013;93:118-123.
19. Harlalka GV, Lehman A, Chioza B, Baple EL, Maroofian R, Cross H, et al. Mutations in B4GALNT1 (GM2 synthase) underlie a new disorder of ganglioside biosynthesis. *Brain* 2013;136:3618-3624.
20. Wakil SM, Monies DM, Ramzan K, Hagos S, Bastaki L, Meyer BF, et al. Novel B4GALNT1 mutations in a complicated form of hereditary spastic paraplegia. *Clin Genet* 2014;86:500-501.
21. Birouk N, Gouider R, Le Guern E, Gugenheim M, Tardieu S, Maisonobe T, et al. Charcot-Marie-Tooth disease type 1A with 17p11.2 duplication. Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases. *Brain* 1997;120:813-823.
22. Shy ME, Blake J, Krajewski K, Fuerst DR, Laura M, Hahn AF, et al. Reliability and validity of the CMT neuropathy score as a measure of disability. *Neurology* 2005;64:1209-1214.
23. Hong YB, Kang J, Kim JH, Lee J, Kwak G, Hyun YS, et al. DGAT2 mutation in a family with autosomal-dominant early-onset axonal Charcot-Marie-Tooth disease. *Hum Mutat* 2016;37:473-480.
24. Choi YR, Hong YB, Jung SC, Lee JH, Kim YJ, Park HJ, et al. A novel homozygous MPV17 mutation in two families with axonal sensorimotor polyneuropathy. *BMC Neurol* 2015;15:179.
25. Wilkinson PA, Simpson MA, Bastaki L, Patel H, Reed JA, Kalidas K, et al. A new locus for autosomal recessive complicated hereditary spastic paraplegia (SPG26) maps to chromosome 12p11.1-12q14. *J Med Genet* 2005;42:80-82.
26. Rose L, Hall K, Tang S, Hasadsri L, Kimonis V. Homozygous B4GALNT1 mutation and biochemical glutaric acidemia type II: a case report. *Clin Neurol Neurosurg* 2020;189:105553.
27. Guelly C, Zhu PP, Leonardis L, Papić L, Zidar J, Schabhüttl M, et al. Targeted high-throughput sequencing identifies mutations in atlastin-1 as a cause of hereditary sensory neuropathy type I. *Am J Hum Genet* 2011;88:99-105.
28. Rivière JB, Ramalingam S, Lavastre V, Shekarabi M, Holbert S, Lafontaine J, et al. KIF1A, an axonal transporter of synaptic vesicles, is mutated in hereditary sensory and autonomic neuropathy type 2. *Am J Hum Genet* 2011;89:219-230.
29. Liu YT, Laurá M, Hersheson J, Horga A, Jaunmuktane Z, Brandner S, et al. Extended phenotypic spectrum of KIF5A mutations: from spastic paraplegia to axonal neuropathy. *Neurology* 2014;83:612-619.
30. Souza PVS, Bortholin T, Naylor FGM, Pinto WBVR, Oliveira ASB. Early-onset axonal Charcot-Marie-Tooth disease due to SACS mutation. *Neuromuscul Disord* 2018;28:169-172.
31. Montecchiani C, Pedace L, Lo Giudice T, Casella A, Mearini M, Gaudiello F, et al. ALS5/SPG11/KIAA1840 mutations cause autosomal recessive axonal Charcot-Marie-Tooth disease. *Brain* 2016;139:73-85.
32. Tsai PC, Huang YH, Guo YC, Wu HT, Lin KP, Tsai YS, et al. A novel TFG mutation causes Charcot-Marie-Tooth disease type 2 and impairs TFG function. *Neurology* 2014;83:903-912.
33. Sohn H, Kim YS, Kim HT, Kim CH, Cho EW, Kang HY, et al. Ganglioside GM3 is involved in neuronal cell death. *FASEB J* 2006;20:1248-1250.
34. Itokazu Y, Wang J, Yu RK. Gangliosides in nerve cell specification. *Prog Mol Biol Transl Sci* 2018;156:241-263.
35. Zitman FM, Todorov B, Verschuuren JJ, Jacobs BC, Furukawa K, Furukawa K, et al. Neuromuscular synaptic transmission in aged ganglioside-deficient mice. *Neurobiol Aging* 2011;32:157-167.
36. Ando S, Tanaka Y, Waki H, Kon K, Iwamoto M, Fukui F. Gangliosides and sialylcholesterol as modulators of synaptic functions. *Ann N Y Acad Sci* 1998;845:232-239.
37. Walkley SU, Siegel DA, Dobrenis K, Zervas M. GM2 ganglioside as a regulator of pyramidal neuron dendritogenesis. *Ann N Y Acad Sci* 1998;845:188-199.
38. Vance JE. Lipid imbalance in the neurological disorder, Niemann-Pick C disease. *FEBS Lett* 2006;580:5518-5524.
39. Dawkins JL, Hulme DJ, Brahmbhatt SB, Auer-Grumbach M, Nicholson GA. Mutations in SPTLC1, encoding serine palmitoyltransferase, long chain base subunit-1, cause hereditary sensory neuropathy type I. *Nat Genet* 2001;27:309-312.
40. Rotthier A, Auer-Grumbach M, Janssens K, Baets J, Penno A, Almeida-Souza L, et al. Mutations in the SPTLC2 subunit of serine palmitoyltransferase cause hereditary sensory and autonomic neuropathy type I. *Am J Hum Genet* 2010;87:513-522.
41. Schwartz NU, Linzer RW, Truman JP, Gurevich M, Hannun YA, Senkal CE, et al. Decreased ceramide underlies mitochondrial dysfunction in Charcot-Marie-Tooth 2F. *FASEB J* 2018;32:1716-1728.
42. Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proc Natl Acad Sci U S A* 1996;93:10662-10667.
43. Yao D, McGonigal R, Barrie JA, Cappell J, Cunningham ME, Meehan GR, et al. Neuronal expression of GalNAc transferase is sufficient to prevent the age-related neurodegenerative phenotype of complex ganglioside-deficient mice. *J Neurosci* 2014;34:880-891.
44. Dad R, Malik U, Javed A, Minassian BA, Hassan MJ. Structural annotation of beta-1,4-N-acetyl galactosaminyltransferase 1 (B4GALNT1) causing hereditary spastic paraplegia 26. *Gene* 2017;626:258-263.
45. Bhuiyan RH, Ohmi Y, Ohkawa Y, Zhang P, Takano M, Hashimoto N, et al. Loss of enzyme activity in mutated B4GALNT1 gene products in patients with hereditary spastic paraplegia results in relatively mild neurological disorders: similarity with phenotypes of B4galnt1 knockout mice. *Neuroscience* 2019;397:94-106.
46. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-424.