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【 CASE REPORT 】

An MFN2-related Charcot-Marie-Tooth Disease Patient with Optic Nerve Atrophy, Neurogenic Bladder Dysfunction, and Diaphragmatic Weakness

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Abstract:

Charcot-Marie-Tooth disease (CMT) is a common hereditary peripheral polyneuropathy encompassing distinct monogenetic disorders. Pathogenic mutations in *mitofusin 2* (*MFN2*) are the most frequent cause of its axonal type, CMT type 2A, with diverse phenotypes. We herein report a Japanese patient with a novel heterozygous *MFN2* pathogenic variant (c.740 G>C, p.R247P) and severe CMT phenotypes, including progressive muscle weakness, optic atrophy, urinary inconsistency, and restrictive pulmonary dysfunction with eventration of the diaphragm that developed over her 60-year disease course. Our case expands the clinico-genetic features of *MFN2*-related CMT and highlights the need to evaluate infrequent manifestations during long-term care of CMT patients.

Key words: Charcot-Marie-Tooth disease, hereditary motor sensory neuropathy, mitofusin 2, diaphragmatic weakness, restrictive pulmonary dysfunction, neurogenic bladder

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Introduction

Charcot-Marie-Tooth disease (CMT), also known as hereditary motor sensory neuropathy, is one of the most common hereditary peripheral polyneuropathies, including distinct monogenetic disorders. The demyelinating form with mainly autosomal dominant (AD) inheritance is called CMT 1, the axonal type with mainly AD inheritance is called CMT2, and disease with autosomal recessive inheritance is called CMT4. To date, over 100 causative genes have been identified for CMT and related disorders (1).

Mitofusin 2 (MFN2) is an outer mitochondrial membrane GTPase critical for mitochondrial dynamics, distribution including axonal transport in the neurons, quality control, and function through its role in mitochondrial fusion and endoplasmic reticulum (ER)-mitochondria tethering (2). Mutations in *MFN2*, which alter GTP hydrolysis and the homo/hetero dimerization ability of MFN2 and impair mitochondrial fusion and ER-mitochondria tethering, resulting in axonal degeneration and CMT2 onset (3, 4). *MFN2* mutations have been identified in 10% to 30% of CMT2 probands and are known as the most frequent cause of CMT2 in Japan (5-8). Various heterozygous *MFN2* pathogenic or likely pathogenic variants have been identified in most probands, whereas biallelic *MFN2* mutations, including compound heterozygous mutations, have been detected in some cases. The cardinal symptom of *MFN2*-related CMT, or CMT2A, is progressive distal-dominant muscle weakness with more severe involvement of the lower limbs than the upper extremities. Sensory deficits are milder than motor deficits. Some patients have infrequent findings, including optic atrophy,

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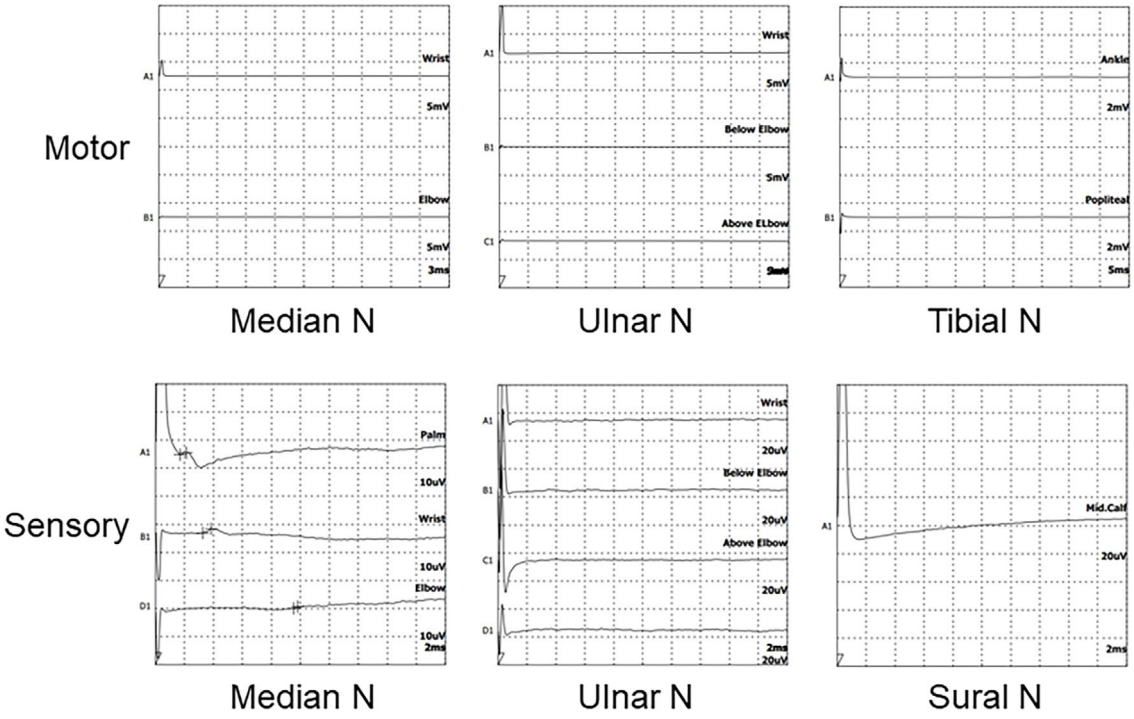

Figure 1. Nerve conduction study taken at 60 years after the onset. CMAPs and SNAPs were not evoked except for in a median sensory nerve. The amplitude of the median sensory nerve evoked by stimulation at the wrist was 1.5 μV. Nerve conduction velocities were 47.3 m/s from finger to palm, 50.5 m/s from palm to wrist, and 47.0 m/s from wrist to elbow.

hearing loss, vocal cord paralysis, vasomotor troubles, scoliosis, and pyramidal signs (5-8). Respiratory failure or diaphragmatic weakness has also been reported in very few cases (7-9).

Further expanding the phenotype and highlighting the respiratory dysfunction and lower urinary dysfunction in *MFN 2*-related CMT, we herein report a patient with a novel heterozygous *MFN2* mutation who developed optic atrophy, neurogenic bladder dysfunction, and eventration of diaphragm during her long disease course.

Case Report

A 64-year-old Japanese woman presented with slowly progressive muscle weakness, scoliosis, optic nerve atrophy, urinary incontinence by neurogenic bladder, constipation, and restrictive pulmonary dysfunction with eventration of the diaphragm. Distal dominant muscle weakness of the lower extremities had emerged at three years old and gradually worsened. She visited several hospitals and was clinically diagnosed with pediatric paralysis or possible CMT. She has been using an ankle-foot orthosis since elementary school and had undergone surgery for both feet in junior high school. She had started using a walker at 15 years old and a wheelchair at 20 years old. She had experienced constipation since her 20s.

At 38 years old, urinary incontinence emerged. She was diagnosed with and treated for neurogenic bladder by a urologist. At 47 years old, she was admitted to our hospital for a neurologic evaluation. Her manual muscle test (MMT) scores of the proximal muscles of the upper limbs were 3 to 4, and those of the distal muscles of the upper limbs were 0. The MMT scores of the lower extremities were 0. She had no facial palsy. Deep tendon reflexes were absent, and there was no apparent sensory deficit. The patient showed mild scoliosis. An ophthalmological evaluation revealed a reduced visual acuity with optic nerve atrophy. An audiogram showed normal auditory acuity. Compound muscle action potentials were not evoked in the median, ulnar, or tibial nerves according to a nerve conduction study. Sensory nerve action potentials (SNAPs) were reduced in the median and ulnar nerves, and no sural SNAP was evoked (Fig. 1). Electromyography revealed chronic neurogenic motor unit potentials with a high amplitude and long duration. No white matter lesions in the brain were identified on brain magnetic resonance imaging (Fig. 2). Based on these findings, the patient was clinically diagnosed with CMT.

At 62 years old, she experienced dyspnea. A chest radiograph and computed tomography (CT) revealed eventration of the diaphragm (Fig. 2). She had restrictive pulmonary dysfunction with a vital capacity of 38% and a 1-s forced expiratory volume (FEV1.0%) of 82%. An arterial blood gas analysis revealed the following: pH, 7.427; pCO_2, 46.3 Torr; pO2, 64.4 Torr; and HCO_3-, 30.0 mEq/L. Thus, she was diagnosed with developed restrictive lung dysfunction, probably due to the phrenic nerve involvement of CMT, and was advised to use noninvasive positive pressure ventilation.

At 63 years old, she agreed to undergo a genetic test for a

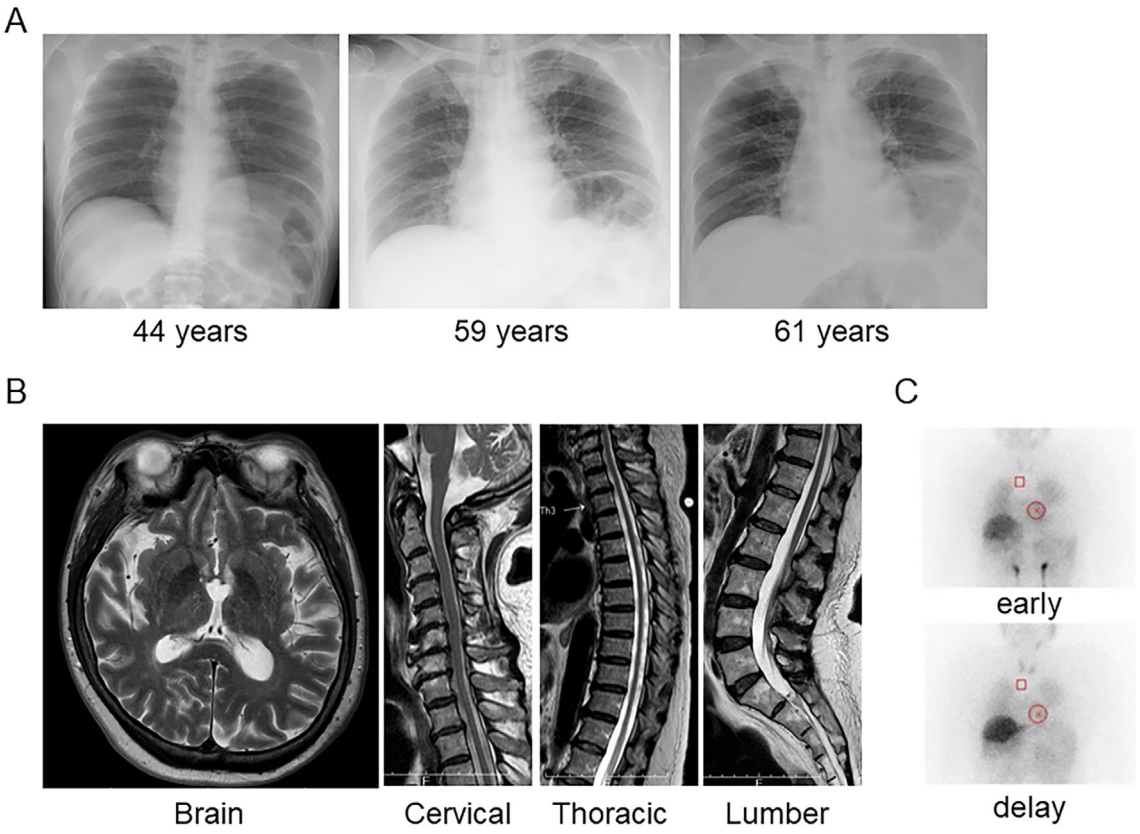

Figure 2. **(A) Chest radiograph taken at 44, 59, and 61 years after the onset. (B) Brain and spine T2-weighted MRI taken at 60 and 61 years after the onset. (C) I-123 MIBG scans taken at 61 years after the onset. The early and late H/M ratios were 2.78 and 2.35, respectively. The washout ratio was 41.8%.**

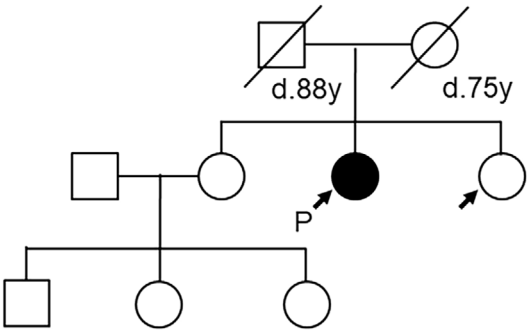

Figure 3. **Family tree. Genomes of the proband and healthy siblings (arrow) were analyzed.**

definitive diagnosis. Written informed consent for genetic analyses was obtained from the patient. The protocol was reviewed and approved by the institutional review board of Kagoshima University. DNA was extracted from blood leukocytes. Coding regions and splicing sites of 72 candidate genes listed in Table were sequenced by next-generation sequencing (NGS) with an Ion Proton (Life Technologies, Carlsbad, USA) as previously described (10, 11). The NGS analysis identified a novel heterozygous single nucleotide substitution of exon 8 of *MFN2* (c.740 G>C), resulting in a missense mutation (p. R247P) of the GTPase domain of MFN2. This mutation altered an amino acid residue conserved in many species, including *Macaca mulatta*, mouse (*Mus musculus*), rat (*Rattus norvegicus*), dog (*Canis familiaris*), and zebrafish (*Danio rerio*). An *in silico* analysis of p.R247P using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and PMut (http://mmb.irbbarcelona.org/PMut/) indicated that this mutation was pathologic. The patient's parents and her siblings had no history of neurological disorders, suggesting that this variant was *de novo* (Fig. 3). To further confirm the pathogenicity of the p.R247P mutation, a segregation study was performed. The patient's healthy sister, who had no neurological deficit, agreed to undergo a genetic test, and written informed consent was obtained. The p.R247P mutation was not detected in the sibling, indicating that this mutation was pathogenic, and the patient developed *MFN2*-related CMT with severe phenotypes and a long disease duration.

A follow-up examination was performed at 64 years old. Her MMSE score was 23/24, and her FAB score was 11/12, except for the items that required finger movement. Her visual acuity was classified into "hand motion", and VEP was impossible. She had no dysphagia. MMT showed no significant decline from that at 47 years old. Only SNAP of the median nerve was evoked by a nerve conduction study. Ultrasonography revealed that residual urine was approximately 120 mL. Cervical, thoracic, and lumbar MRI showed no apparent causative central nervous lesion for her neuro-

Table. **List of Target Genes for Analysis.**

AARS	*APTX*	*ARHGEF10*	*DHH*	*DNM2*	*EGR2*	*FGD4*	*FIG4*
GAN	*GARS*	*GDAP1*	*GJB1*	*HARS*	*HK1*	*HOXD10*	*HSPB1*
HSPB8	*KARS*	*LITAF*	*LMNA*	*MARS*	*MED25*	*MFN2*	*MPZ*
MTMR2	*NDRG1*	*NEFL*	*PMP22*	*PRPS1*	*PRX*	*RAB7A*	*SBF2*
SETX	*SH3TC2*	*SLC12A6*	*SOX10*	*TDP1*	*TRPV4*	*TTR*	*YARS*
BSCL2	*DCTN1*	*DHTKD1*	*DYNC1H1*	*FBLN5*	*FBXO38*	*GJB3*	*GNB4*
HSPB3	*IGHMBP2*	*INF2*	*KIF1A*	*LRSAM1*	*PDK3*	*REEP1*	*SBF1*
SLC5A7	*TFG*	*TRIM2*	*DCAF8*	*SURF1*	*SACS*	*GALC*	*PLEKHG5*

Sixty-four genes listed above and eight candidate genes were analyzed.

genic bladder (Fig. 2). No postural hypotension was observed. RR-CV was normal (4.86%), and an I-123 MIBG cardiac scan showed normal findings (Fig. 2). Her vital capacity decreased to 31% with an elevated pCO_2 of 50 Torr on room air. The apnomonitor indicated that her apnea hypopnea index was from 5 to 8 with morning hypoxemia (SpO_2: around 90%), and we advised her to use noninvasive positive pressure ventilation.

Discussion

We herein report an *MFN2*-related CMT patient with a novel heterozygous *MFN2* pathogenic variant (c.740 G>C, p.R247P) and severe phenotypes, including diffuse muscle weakness, scoliosis, optic atrophy, urinary inconsistency with neurogenic bladder, constipation, and restrictive pulmonary dysfunction with eventration of the diaphragm, who sequentially developed in a 60-year disease course. Several disease-associated mutations in the MFN2 protein have been identified, with most localized inside or in the close vicinity of its GTPase or heptad repeat domains (6-9). The p.R247P mutation is located in this hot spot, and its pathogenicity was confirmed by a segregation study and *in silico* analysis. Genotype/phenotype correlation and diversities have been reported in *MFN2*-related CMT, with recent advances in the research of MFN2 proteins elucidating the mechanisms underlying the pathogenicity (2-4). Our case, with its validated pathogenic variant and long clinical history, will add important information to the clinico-genetic features of *MFN2*-related CMT.

Of note, the present patient developed diaphragmatic weakness and restrictive pulmonary dysfunction over a long disease duration. Although diaphragm ultrasound or cortical and posterior cervical magnetic stimulation were not evaluated in our case, chest radiography, spirometry, and an arterial blood gas analysis demonstrated progressive restrictive pulmonary dysfunction due at least in part to the phrenic nerve involvement. Pulmonary function disorders have long been recognized as infrequent complications of CMT (12). To our knowledge, three cases have been reported with respiratory impairment in *MFN2*-related CMT (7, 9). These patients had a severe CMT2 phenotype. For instance, one case of an autosomal recessive infant with hypotonia developed diaphragmatic weakness and respiratory compromise by eight months old. Diaphragm weakness is also reported in CMT1A and usually associated with severe disease manifestation. However, a recent study comprising 19 adult CMT1A patients demonstrated mild phrenic nerve involvement and respiratory muscle weakness in CMT1A patients with mild phenotypes (13). Considering the fundamental role of MFN2 in mitochondrial axonal transport and function, diaphragm weakness due to the phrenic nerve involvement might also occur in *MFN2*-related CMT patients with a mild phenotype and could become apparent during the long disease course. Thus, an evaluation of the pulmonary function should be considered, especially in *MFN2*-related CMT2 patients with severe phenotypes and/or a long disease duration.

Our patient developed urinary inconsistencies due to neurogenic bladder with an unknown etiology. This symptom usually reflects peripheral autonomic nerve impairment or spinal damage in S2-S4, and in our case, it might have been due to the autonomic nerve involvement, as no causative lesion was detected by brain or spine MRI. Lower urinary tract dysfunction might be underestimated in *MFN2*-related CMT, as previous case reports did not describe such symptoms, except for one abstract describing two *MFN2*-related CMT2 cases (14). Using questionnaires, Krhut et al. reported that the occurrence rate of lower urinary tract symptoms and bladder dysfunctions in patients with CMT was significantly higher than that in age-matched controls (15). Although a further study is needed to elucidate the relationship between autonomic dysfunction and *MFN2* mutations, urinary symptoms that impair patients' quality of life may be more prevalent in *MFN2*-related CMT than anticipated. Therefore, an evaluation should be included in the diagnostic approach and care of patients.

The findings in our patient with a novel *MFN2* mutation expand the clinical and genetic understanding of *MFN2*-related CMT. Considering the diagnostic difficulty due to the broad clinical variability and advances in research that may lead to the future development of disease-modifying therapy, genetic testing should be considered for the diagnosis of CMT and related disorders. This report highlighted the presumed infrequent complications that might be more common than those anticipated by clinicians. A comprehensive evaluation of the neurological symptoms, including restrictive lung dysfunction and lower urinary problems, should be considered in the diagnosis and care of patients

with CMT.

The authors state that they have no Conflict of Interest (COI).

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