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A novel p.Leu699Pro mutation in *MFN2* gene causes Charcot-Marie-Tooth disease type 2A

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Axonal Charcot-Marie-Tooth disease (CMT2) has most frequently been associated with mutations in the *MFN2* gene. *MFN2* encodes mitofusin 2, which is a mitochondrial fusion protein that plays an essential role in mitochondrial function. We report CMT2 in a Korean father and his son that manifested with gait difficulties and progressive atrophy of the lower legs. Molecular analysis revealed a novel heterozygous c.2096T>C (p.Leu699Pro) mutation in the exon 18 of *MFN2* in both subjects. We suggest that this novel mutation in *MFN2* is probably a pathogenic mutation for CMT2.

Key words: Charcot-Marie-Tooth disease; *MFN2*; Mutation; Phenotype

INTRODUCTION

Charcot-Marie-Tooth disease (CMT) is a hereditary neuropathy that is both clinically and genetically heterogeneous. The main clinical features of CMT are progressive distal limb weakness and sensory loss. CMT is classified according to the mode of inheritance and the electrophysiological criteria. For autosomal dominant forms, demyelinating CMT (CMT1) is more frequent than axonal CMT (CMT2). CMT2 is genetically heterogeneous with 11 identified genes. CMT2A is the most prevalent type of CMT2 and has most frequently been associated with mutations in the *MFN2* gene (NM_014874).[1] *MFN2* encodes mitofusin 2, which plays an essential role in the functions performed by mitochondria. *MFN2* mutation results in a defect in mitochondrial fusion, leading to a loss of mitochondrial DNA and impairment in oxidative phosphorylation.[2] To date, more than 100 *MFN2* mutations have been reported to be associated with CMT2.[3] Here, we report on a Korean family, in which the father and his son carried the novel heterozygous mutation c.2096T>C (p.Leu699Pro) in *MFN2*.

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CASE

A 16-year-old man presented with gait difficulty and slowly progressive weakness of the lower limb. The symptoms had first appeared a few years previously. He complained of gait unsteadiness and moderate distal lower limb weakness, but he had no sensory symptoms. The family history was unremarkable and he had no siblings. A motor examination showed reduced muscle strength with MRC score of 3 in the lower extremities. He exhibited steppage gait, severe lower limb atrophy, areflexia, and bilateral pes cavus. The findings of a sensory examination and cerebellar function test were unremarkable. Nerve conduction studies (NCS) revealed a normal motor nerve conduction velocity, but low sensory nerve action potentials and slightly slow sensory nerve conduction velocities in the upper limbs. In addition, neither compound motor action potentials or sensory nerve action potentials of lower limbs were elicited, these NCS results were consistent with sensorimotor axonal polyneuropathy (Table 1). The findings of laboratory tests and an ophthalmologic evaluation were also unremarkable. MRI of the lower extremities in the proband revealed a fatty atrophy almost throughout the muscles of the lower leg, but with this being less severe in the posterior tibial muscle (Fig. 1A). His parents were healthy, except that his father demonstrated reduced deep tendon reflexes. NCS in his father revealed axonal polyneuropathy similar to that in the proband.

Since the clinical features of the two patients were compatible with CMT2, molecular studies were performed with the aim of identifying a possible genetic etiology. Genomic DNA was purified from peripheral blood using a QIAamp blood DNA purification kit (Qiagen, Hilden, Germany). Exomes were captured with the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA), and whole exome sequencing was performed with a genome analyzer (HiSeq 2500, Illumina, San Diego, CA, USA). A previously unreported heterozygous c.2096T>C (p.Leu699Pro) mutation was detected in the exon 18 of *MFN2* (Fig. 1B) in both the proband and his father. Sequencing of the *PMP22* and *MPZ* genes did not reveal any mutations.

DISCUSSION

We have identified a novel heterozygous c.2096T>C (p.Leu-

Table 1. The results of nerve conduction studies in proband and his father

Motor NCS	Proband		Father	
	Amplitude (mV)	NCV (m/sec)	Amplitude (mV)	NCV (m/sec)
Median (R/L)				
TL (ms)		3.2/3.2 (3.6)		3.7/3.4
F-W	15.3/13.9 (5)		17.9/16.8	
W-E	14.0/13.5	56/58 (49.9)	17.2/15.9	57/59
E-A	14.0/13.1	67/67 (55.9)	14.3/14.6	69/67
Ulnar (R/L)				
TL (ms)		2.3/2.4 (2.5)		2.3/2.3
F-W	13.7/16.3 (5)		20.1/19.8	
W-BE	12.9/15.0	60/54 (50.6)	19.9/19.1	56/57
BE-AE	12.7/14.7	53/46 (42.8)	19.5/18.4	60/59
E-A	12.1/13.6	64/67 (52.6)	17.2/17.6	69/65
Peroneal (R/L)				
TL (ms)		-/- (4.7)		5.9/5.7
Ankle	NP/NP		0.9/1.1	
Knee	NP/NP	-/- (41.8)	0.9/0.8	39/40
Tibial (R/L)				
TL (ms)		-/- (5.1)		-/-
Ankle	NP/NP		NP/NP	
PF	NP/NP	-/- (40)	NP/NP	-/-
Sensory NCS	**Amplitude (μV)**	**NCV (m/sec)**	**Amplitude (μV)**	**NCV (m/sec)**
Median (R/L)				
F-W	5.0/5.8 (10)	41/38 (41)	4.3/6.1	44/46
W-E	6.0/6.8	55/56 (49)	5.3/5.9	52/48
E-A	64.7/17.6	63/63 (53)	29.2/31.6	58/57
Ulnar (R/L)				
F-W	4.7/NP (10)	38/- (39)	3.4/4.8	41/43
W-E	9.0/8.1	56/51 (47)	15.3/13.6	53/51
E-A	25.1/27.6	67/59 (48)	23.2/26.5	56/59
Sural (R/L)	NP/NP (6)	-/- (34)	NP/NP	-/-

NCS, nerve conduction study; NCV, nerve conduction velocity; R/L, right/left; TL, terminal latency; F-W, finger-wrist; W-E, wrist-elbow; E-A, elbow-axilla; W-BE, wrist-below elbow; BE-AE, below elbow-above elbow; NP, no potentials; PF, popliteal fossa.

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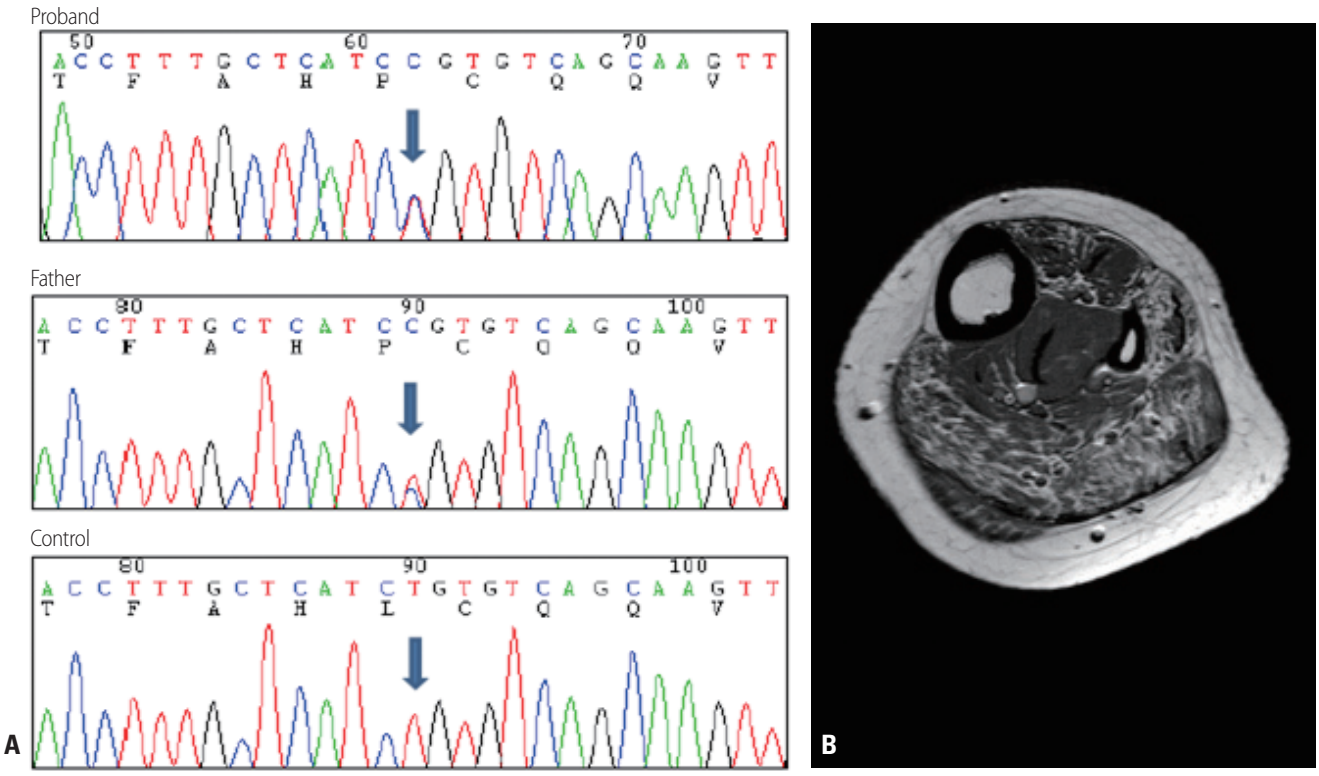

Fig. 1. (A) Molecular genetic analysis obtained from the sequencing of exon 18 of MFN2 show a heterozygous c.2096T>C (p.Leu699Pro) mutation in the proband and his father. (B) Magnetic resonance imaging of the lower extremities in the proband shows widespread fatty degeneration in most muscles of lower leg except posterior tibial muscle.

699Pro) mutation in *MFN2* causing CMT2 in a father and son. *MFN2* is associated with the most common form of autosomal dominant CMT2, with a reported prevalence of 8-30% among CMT2 patients.[3] Typical clinical symptoms of CMT2A caused by *MFN2* mutations are progressive distal limb muscle weakness, stepping gait, distal sensory loss, and impaired mobility. Intriguingly, the disease onset appears to be highly diverse, and a late onset seems to be associated with a less severe phenotype.[4] The phenotype seems to be classical CMT2 with axonal neuropathy in most patients, but there have been reports of more complex phenotypes involving optic atrophy,[5] cerebral white matter lesions,[6] cognitive impairment,[7] and upper motor neuron involvement.[8] Both of the patients in the present family showed the typical CMT2 phenotype without additional features. In addition, the proband's father had no atrophy of the lower legs or foot deformity despite his NCS findings being similar to those of his son. This suggest that the disease onset is closely related to its severity. The interesting hallmark of CMT2A is that it affects the longest axons. In our patient, MRI findings demonstrated widespread fatty degeneration in most muscles of the lower leg except the posterior tibial muscle.

MFN2 encodes mitofusin 2, which is a GTPase dynamin-like protein of the outer mitochondria membrane. Mitofusin 2 plays a major role in maintaining a functional mitochondrial network.[9] Mitofusin 2 not only has a mitochondria-shaping function, but also plays an essential role in almost all mitochondrial dynamics, including regulating the transport of mitochondria.[3] Accordingly, mutations of *MFN2* may cause mitochondrial dysfunction and a mitochondrial transport defect could be the cause of CMT2A. The overexpression of disease-associated mutant *MFN2* protein, both in vitro or in vivo, induces mitochondrial aggregation and disruption of axonal mitochondrial transport that might be due to a dominant-negative or toxic gain-of-function effect.[10] A mitochondrial transport defect should therefore be considered as a possible pathophysiological mechanism underlying the disease. The pathogenic *MFN2* mutations result in amino

acid substitution. In the present family, the c.2096T>C mutation changes an amino acid from leucine to proline and this mutation changed an evolutionarily conserved amino acid (with a Phylop score of 5.28). In addition, three computational tools (SIFT, PolyPhen-2 and MUpro) predicted p.Leu699Pro in *MFN2* to be a disease-causing mutation. The c.2096T>C substitution is not reported in the Exome Variant Server or 1000 genome databases, thus confirming that p.Leu699Pro is indeed a novel mutation.

We have reported on a family with one severely affected member with an early-onset CMT2 and another late-onset member with a mild phenotype. Both patients harbored a novel heterozygous *MFN2* mutation and this mutation results in a relatively typical CMT2 phenotype. We suggest that the c.2096T>C (p.Leu699Pro) mutation in *MFN2* is probably a pathogenic mutation for CMT2A.

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