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BRIEF COMMUNICATION

Charcot–Marie–Tooth disease type 2A with an autosomal-recessive inheritance: the first report of an adult-onset disease

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Abstract

Axonal Charcot–Marie–Tooth disease (CMT) is most frequently caused by mutations in the *MFN2* gene (CMT2A) that can lead to various clinical phenotypes. The age at disease onset varies, but most cases occur before adolescence. We report two Japanese sisters who presented with middle-age-onset peripheral neuropathy with distinct clinical features. In the affected sisters, a homozygous missense mutation, c.1894C>T, p.R632W, corresponding to the transmembrane domain of *MFN2* was identified; this mutation was heterozygous in another non-affected sibling, demonstrating co-segregation of the genotype and phenotype. The patients developed adult-onset slowly progressive muscle weakness that was predominant in the calf muscles and sensory disturbance. Magnetic resonance imaging revealed diffuse atrophy of the spinal cord, especially in the thoracic segment, and mild atrophy of the parietal lobe and the cerebellum in both patients. Electron microscopy of the sural nerve revealed clusters of round and swollen mitochondria. This is the first case report of adult-onset CMT2A with an autosomal-recessive inheritance pattern. The phenotype caused by the *MFN2* mutation in these cases is very mild, considering that the mutation causes middle-aged-onset Charcot–Marie–Tooth even in the homozygous state. The mechanism of *MFN2* mutation-induced toxicity is an interesting theme that awaits further investigations.

Introduction

Charcot–Marie–Tooth disease (CMT) is a group of hereditary motor sensory neuropathies. Among axonal CMT, designated as CMT2, the most prevalent phenotype is CMT2A, which is caused by mutations in the mitofusin 2 gene (*MFN2*; OMIM 608507) [1–5]. *MFN2* encodes a protein with a dynamin-like glutamyl transpeptidase located in the mitochondrial outer membrane, and this protein promotes the membrane fusion [6]. Although the mode of inheritance is mostly autosomal-dominant, autosomal-recessive cases have been reported only in early-onset CMT2A, and the symptoms tend to be severe [3, 7, 8]. We herein report two Japanese sisters carrying a novel homozygous *MFN2* mutation in the transmembrane domain who had late onset and a mild phenotype.

Case report

Three Japanese sisters, born from non-affected parents who were cousins, were examined. Two of the sisters developed

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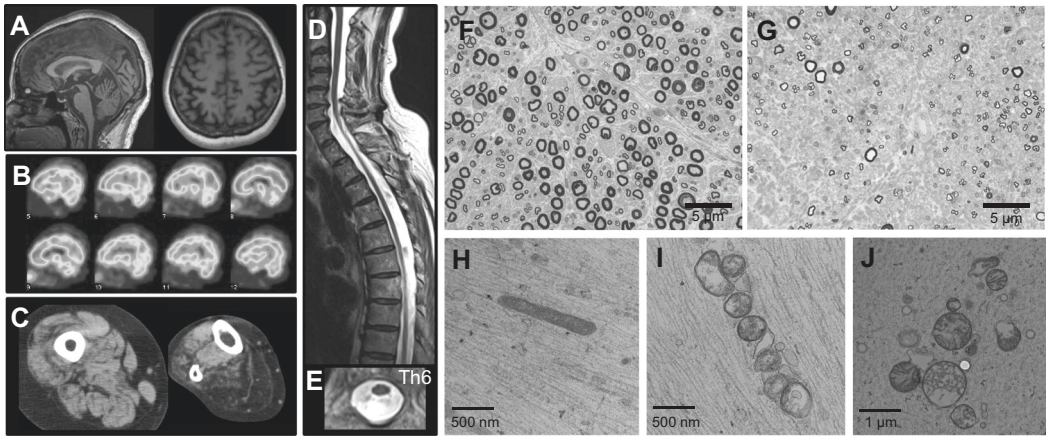

Fig. 1 Radiological and pathological findings of Patient 1. Brain MRI **a** T1-weighted images demonstrate atrophy of the parietal lobe and the anterior lobe of the cerebellum. SPECT (**b**) cerebellar blood flow is diminished. CT images of the lower extremity (**c**) disproportional muscle atrophy with fatty replacement is evident in the posterior compartment of the legs. Spinal cord MRI (**d**, **e**) midsagittal and axial T2-weighted images show diffuse atrophy of the spinal cord, which is more marked in the thoracic segment. Toluidine blue staining of the transverse sections of biopsied sural nerve from a control subject diagnosed with cerebral palsy (**f**) and Patient 1 (**g**); the density of myelinated fibers is markedly decreased. Scale bar: 5 μm. Electron micrographs of a control subject diagnosed with chronic inflammatory demyelinating polyneuropathy (**h**, longitudinal section) and Patient 1 ((**i**) longitudinal section, and (**j**) transverse section) show abnormally aggregated and round mitochondria. Scale bars: 500 nm (**h**, **i**) and 1 μm (**j**)

CMT, while the other did not. Patient 1, the third daughter, began to experience hypoesthesia in the bilateral soles and difficulty with toe-walking at the age of 41 years. She subsequently suffered from clumsiness in both hands. The symptoms gradually worsened, and she became cane-dependent within 5 years. Neurological examinations at the age of 52 years demonstrated muscle weakness and atrophy in the distal lower limbs, and areflexia. Her vibratory and superficial senses were decreased in all distal limbs and below the knees, respectively. No pathological reflex was present. Patient 2, the eldest daughter, began to experience slowly progressive gait disturbance at the age of 42 years. She subsequently experienced hypoesthesia in the bilateral soles, and became wheelchair-bound at the age of 65 years. Neurologically, distal muscle weakness with areflexia, sensory loss of all modalities in the bilateral lower limbs, and truncal instability were observed. No pathological reflex was present. In contrast to Patients 1 and 2, the second daughter presented no abnormal findings in neurological examinations at the age of 62 years, and all the children of the patients have not experienced any neurological symptom.

A magnetic resonance imaging study revealed mild atrophy of the parietal lobe and the cerebellum, and diffuse atrophy of the thoracic cord in both patients, although they were more prominent in Patient 1. In Patient 1, cerebellar hypoperfusion was evidenced by single photon emission computed tomography (CT), and muscle CT showed preferential atrophy of the bilateral gastrocnemius muscles (Figs. 1a–e, Supplementary Fig. 1). A nerve conduction study revealed sensory–motor axonopathy (Supplementary Table 1). For diagnostic purposes, a sural nerve biopsy was also performed in Patient 1. There was a marked decrease in the density of large myelinated fibers (2180/mm²; Fig. 1g), and electron microscopy revealed many round and aggregated mitochondria in the axon. The inner and outer mitochondrial membranes were irregular with disrupted cristae (Figs. 1i, j). These changes might have been a result of abnormal mitochondrial fusion and fission, suggesting the diagnosis of mitofusin 2-related neuropathy [6, 9].

After informed consent was obtained, genomic DNA was extracted from the peripheral blood leukocytes of the patients and the asymptomatic sibling. DNA analysis of 60 genes associated with hereditary motor and sensory neuropathies (Supplementary Appendix) revealed a novel missense mutation, c.1894C>T, p.R632W, in the *MFN2* gene (NM_014874.3; Figs 2a, b). This mutation was registered as single-nucleotide variation with minor allele frequency of 0.000008 in dbSNP150, but was not registered in the 1000 Genomes project, or the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/), which includes genetic variations determined by exome sequencing of 1208 Japanese individuals. The homozygosity of the mutation, but not the result of a large deletion of one allele, was confirmed by quantitative PCR analysis (Supplementary Fig. 2).

R632 is located in the transmembrane domain, which is highly conserved across various species (Fig. 2c). The homozygosity of the patients, the heterozygosity of the non-affected sibling, as well as the asymptomatic state of all of the children of the patients indicated the autosomal-recessive inheritance of this mutation.

Discussion

This is the first case report of adult-onset CMT2A with an autosomal-recessive inheritance, although >100 CMT2A disease-causing mutations have been described for the *MFN2* gene to date [2, 3, 7–10]. Many CMT-related genes display both dominant and recessive patterns, and the recessive forms show a trend to cause severe phenotypes with early onset [11]. Unlike the two cases described here, all previously reported cases carrying homozygous or compound heterozygous *MFN2* mutations presented with early-onset CMT (Fig. 3).

In addition, this is the third case report describing a mutation in the transmembrane domain. We described the precise clinical features, including segregation analysis, while the previously reported cases with a mutation in the

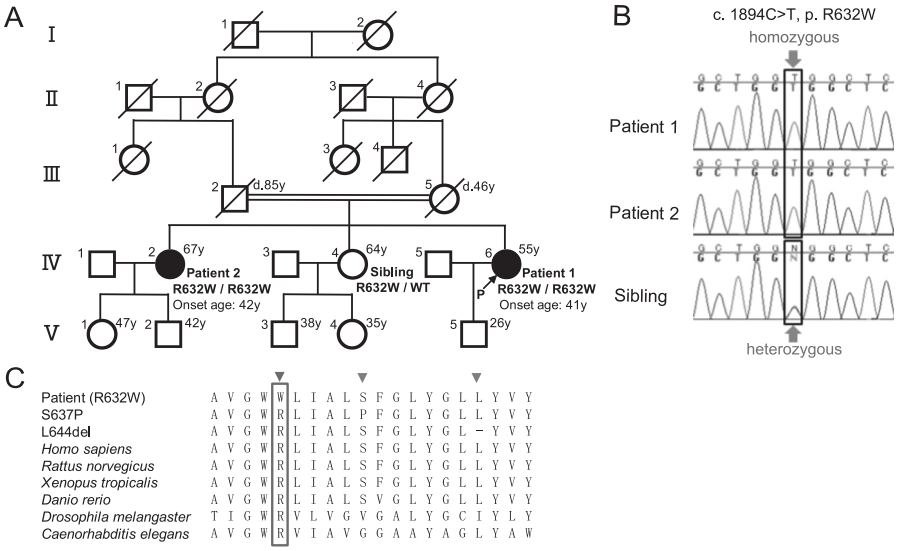

Fig. 2 Identification of a novel *MFN2* mutation. **a** Pedigree of the family. Solid symbols represent clinically affected individuals. **b** DNA sequence analysis of *MFN2* showing the c.1894C>T (p.R632W) mutation. **c** Alignment of the amino-acid sequences of MFN2 within the transmembrane domain. Phylogenetic conservation of the amino-acid residue R632 (red arrowhead) is observed across diverse species in contrast to the previously reported residues S637 and L644 (blue arrowhead) [4, 12]

Mutation(s)	Domains	Age at onset	Reference
Homozygous			
R632W	TM	41y, 42y	This report
F216S	GTPase	early-childhood	Vallat JM, et al. 2008
R707W	Cc2	2y	Nicholson GA, et al. 2008
Compound Heterozygous			
K38del, T362M	Neck(1) Neck(2)	3y, 3y	J.M. Polke, et al. 2011
Del ex 7–8, F216S	NMD, GTPase	12m, 18m	
Q308X, R519P	Neck(2) Trunk	14m	
A164V, T362M	GTPase Neck(2)	3y	Nicholson GA, et al. 2008
D214N, C390R	GTPase Neck(2)	3y	
R250W, R400X	GTPase Neck(2)	4y	Verhoeven, et al. 2006
G108R, R707W	GTPase Cc2	< 10y	Calvo J, et al. 2009
G80V, R104Q	Neck(1) GTPase	Early onset	Bombelli, et al. 2014
Del ex 7–8, T362M	NMD, Neck(2)	18m, 18m	Carr, et al. 2015
Del ex 7–8, R707W	NMD, Cc2	24m	

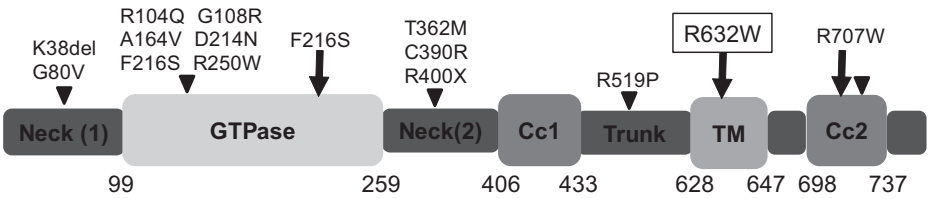

Fig. 3 Previously reported homozygous and compound heterozygous mutations of the *MFN2* gene. "GTPase" indicates the glutamyl transpeptidase domain. Numbers under the protein indicate the amino-acid residue positions delimiting the different domains. Arrows indicate homozygous mutations, while arrowheads indicate compound heterozygous mutations. *Cc1* and *Cc2* coil-coiled domains, *TM* transmembrane domain, *NMD* nonsense-mediated decay, *y* years, *m* months

transmembrane domain did not necessarily demonstrate co-segregation [4, 12].

Our cases indicated that the mutation in the transmembrane domain at R632 has mild pathogenicity, since only homozygous carriers showed adult-onset and slowly progressive paralysis, while the heterozygous individual remained unaffected clinically. We pointed out no distinct abnormalities, except for bilateral mild carpal tunnel syndrome by a nerve conduction study of the sibling at the age of 64 years, although we could not rule out a possibility of a subclinical neuropathy (Supplementary Table 1). The patients also presented diffuse atrophy of the spinal cord and mild atrophy of the parietal lobes and cerebellum. These findings can be caused by the *MFN2* mutation because various types of involvement of the central nervous system, especially spinal cord abnormalities, have been reported in CMT2A [12], although there may be a homozygous mutation in other genes responsible for them as the patients have consanguineous parents.

The mechanism underlying how *MFN2* mutations cause disease has been extensively investigated. It has been shown that only one *MFN2* null allele does not cause disease, so MFN2 is not sensitive to haploinsufficiency, and the disease is considered to be caused by a dominant-negative or a toxic gain-of-function effect [8]. In addition, the extent of disease severity might be associated with dysfunction of the GTPase domain because most cases with mutations within or near the GTPase domain experienced severe disease courses. In our histopathological study, morphologically abnormal mitochondria were aggregated, as has been previously reported [9], implying that the missense mutation in the transmembrane domain also caused an imbalance of mitochondrial fusion and fission [13]. Further studies on whether the mutation in the transmembrane domain of our cases affects the function of the GTPase domain and how the mutation causes toxicity may help to elucidate the disease mechanism of CMT.

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Compliance with ethical standards

Conficlt of interests The authors declare that they have no competing financial interests.

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