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Novel Compound Heterozygous Nonsense *PRX* Mutations in a Korean Dejerine-Sottas Neuropathy Family

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Background Mutations in the gene encoding periaxin (*PRX*) are known to cause autosomal recessive Dejerine-Sottas neuropathy (DSN) or Charcot-Marie-Tooth disease type 4F. However, there have been no reports describing Korean patients with these mutations.

Case Report We examined a Korean DSN patient with an early-onset, slowly progressive, demyelinating neuropathy with prominent sensory involvement. Whole-exome sequencing and subsequent capillary sequencing revealed novel compound heterozygous nonsense mutations (p.R392X and p.R679X) in *PRX*. One mutation was transmitted from each of the patient's parents. No unaffected family member had both mutations, and the mutations were not found in healthy controls.

Conclusions We believe that these novel compound heterozygous nonsense mutations are the underlying cause of DSN. The clinical, electrophysiologic, and pathologic phenotypes in this family were similar to those described previously for patients with *PRX* mutations. We have identified the first *PRX* mutation in a Korean patient with DSN.

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Key Words Dejerine-Sottas neuropathy, Charcot-Marie-Tooth disease, periaxin, whole-exome sequencing, peripheral nerve.

Introduction

Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous disorder of the peripheral nervous system.¹ Demyelinating CMT neuropathies are classified as either autosomal dominant CMT type 1 or autosomal recessive CMT type 4 (CMT4).² CMT4 has been further divided into many subtypes from CMT4A to CMT4J according to clinical symptoms and genetic causes.³ Dejerine-Sottas neuropathy (DSN), which is also known as CMT type 3, is characterized by early

age at onset, very slow motor nerve conduction velocities (MNCVs), and severe demyelinating neuropathy.⁴ Previous reports have described several genes that are relevant to DSN, including those encoding early growth response 2, myelin protein zero, peripheral myelin protein 22, and periaxin (*PRX*).⁴⁻⁷

Mutations in *PRX* are known to cause DSN and CMT4F.^{6,7} Loss of periaxin function causes instability of Schwann cell myelination, which eventually leads to demyelination and peripheral neuropathy. It has been shown that *PRX*-knockout mice develop a spectrum of morphologic changes to the myelin sheath.⁸

Dozens of patients with *PRX* mutations have been described previously,⁹⁻¹⁴ but none of them were Korean. In this study we identified a Korean DSN patient with novel compound heterozygous nonsense mutations in *PRX* through

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whole-exome sequencing (WES).

Case Report

A 10-year-old girl (Fig. 1A, II-3) was the third child with two healthy older sisters born to nonconsanguineous parents (FC390). Neurologic and electrophysiologic examinations revealed that the parents (I-1 and I-2) and two sisters (II-1 and II-2) did not exhibit distal motor weakness, sensory deficit, or abnormal electrophysiologic findings. However, the patient had delayed motor milestones, with autonomous walking not present until 24 months of age. During childhood she developed gait difficulties with clumsiness and balance problems; however, the disease progression was very slow. A neurologic examination at 10 years of age revealed bilateral pes cavus, scoliosis, and a broad-based gait. She had severe weakness of foot eversion without proximal muscle involvement. Her sensitivities to pinprick, touch, position, and vibration were decreased; the vibration sensation was more severely disturbed than pain sensations in all limbs. Sensory ataxia and a positive Romberg sign were present. Deep tendon reflexes were absent and pathologic reflexes were not found. Her CMT neuropathy score was 20.¹⁵

Electrophysiologic studies were performed at 7 and 10 years of age. Sensory nerve action potentials were absent in the median, ulnar, and sural nerves. Compound muscle action potentials were severely reduced in the median nerve (range, 0.2–0.5 mV) and absent in the ulnar, peroneal, and tibial nerves. Median MNCVs were markedly reduced (range, 2.6–3.0 m/s), and distal latencies were dramatically prolonged (range, 39.6–44.8 ms). The needle electromyogram was consistent with chronic neuropathy.

MRI scans of the hip and thigh were normal (Fig. 2A, B, and C). Lower-calf MRI revealed hyperintense signal abnormalities (Fig. 2D and E). T1-weighted images demonstrated mild signal changes and atrophy in the peronei muscles; however, the tibialis anterior and soleus muscles were not involved.

The results of a histopathologic study performed at age 10 years are summarized in Table 1. Light-microscopy examination of longitudinal and cross-sections of nerve fibers revealed nerve fascicles that were markedly decreased in size, diffuse subendoneurial edema, and moderately to markedly decreased numbers of myelinated fibers (MFs) of all calibers, with suggestion of onion-bulb formation and endoneurial fibrosis (Fig. 1C). The mean diameter of MFs (3.14 μ m) and the percentage area of MFs (1.39%) were also lower than in age-matched controls (4.5 μ m and 25.2%, respectively) (Fig. 1D). Electron-microscopy examination revealed findings consistent with abnormal myelin, such as focally folded, uncompacted, or deteriorating myelin, irregular myelin thickness, or fragmented

myelin structures in Schwann cells or nearby macrophages (Fig. 1E and F).

Mutational analysis

Exomes were captured using a SeqCap EZ device (version 3.0, Roche-NimbleGen, Madison, WI, USA), and sequencing was performed using a genome analyzer (HiSeq 2000, Illumina, San Diego, CA, USA). The University of California, Santa Cruz assembly hg19 (National Center for Biotechnology Information build 37.1) was used as the reference sequence. The candidate variants considered to be causative were confirmed by Sanger sequencing. The total sequencing yield of WES was approximately 10.47 Gbp, with a 91.72% coverage rate for the targeted exon regions ($\geq 10\times$). The total number of observed variants was 69,592 single-nucleotide polymorphisms, of which 349 variants were observed in CMT-relevant genes that have been reported previously.¹⁶ Subsequent filtering was achieved for functionally significant variants, where the functionally significant variants indicate all variants that change the amino acid, such as nonsynonymous, splicing site, stop-gain/-loss, and coding indels. Filtering isolated 25 functionally significant variants in the CMT-relevant genes. Most variants have been reported in the dbSNP137 or 1000 Genome databases, except for three variants [one in the gene encoding dynactin subunit 1 (*DCTN1*) and one in *PRX*]. Mutation in *DCTN1* c.2054T>G (p.V685G) was excluded because the variant was detected in unaffected family members (father and an elder sister) as well as a healthy control by capillary sequencing. However, a pair of compound heterozygote nonsense mutations, c.1174C>T (p.R392X) and c.2035C>T (p.R679X), in exon 7 of *PRX* were well fitted with an autosomal-recessive inheritance model within the family, and neither was found in normal controls ($n=300$). The c.1174C>T and c.2035C>T mutations were inherited from the patient's mother and father, respectively (Fig. 1B). Thus, it was postulated that both mutations lead to enzymatic defects, and that these compound heterozygous mutations were the underlying cause of the DSN phenotype in this patient.

Discussion

We have identified novel compound heterozygous mutations in *PRX* from a Korean DSN family. The phenotype of the affected patient was similar to that of previously described *PRX* cases,^{10–14} with early age at onset, sensory ataxia, very slow MNCVs, and severe demyelinating neuropathy consistent with DSN. One of the R392X and R679X mutations was detected in each of the patient's asymptomatic parents, and neither of the mutations was observed in the 300 healthy controls.

Most reported causative *PRX* mutations have been non-

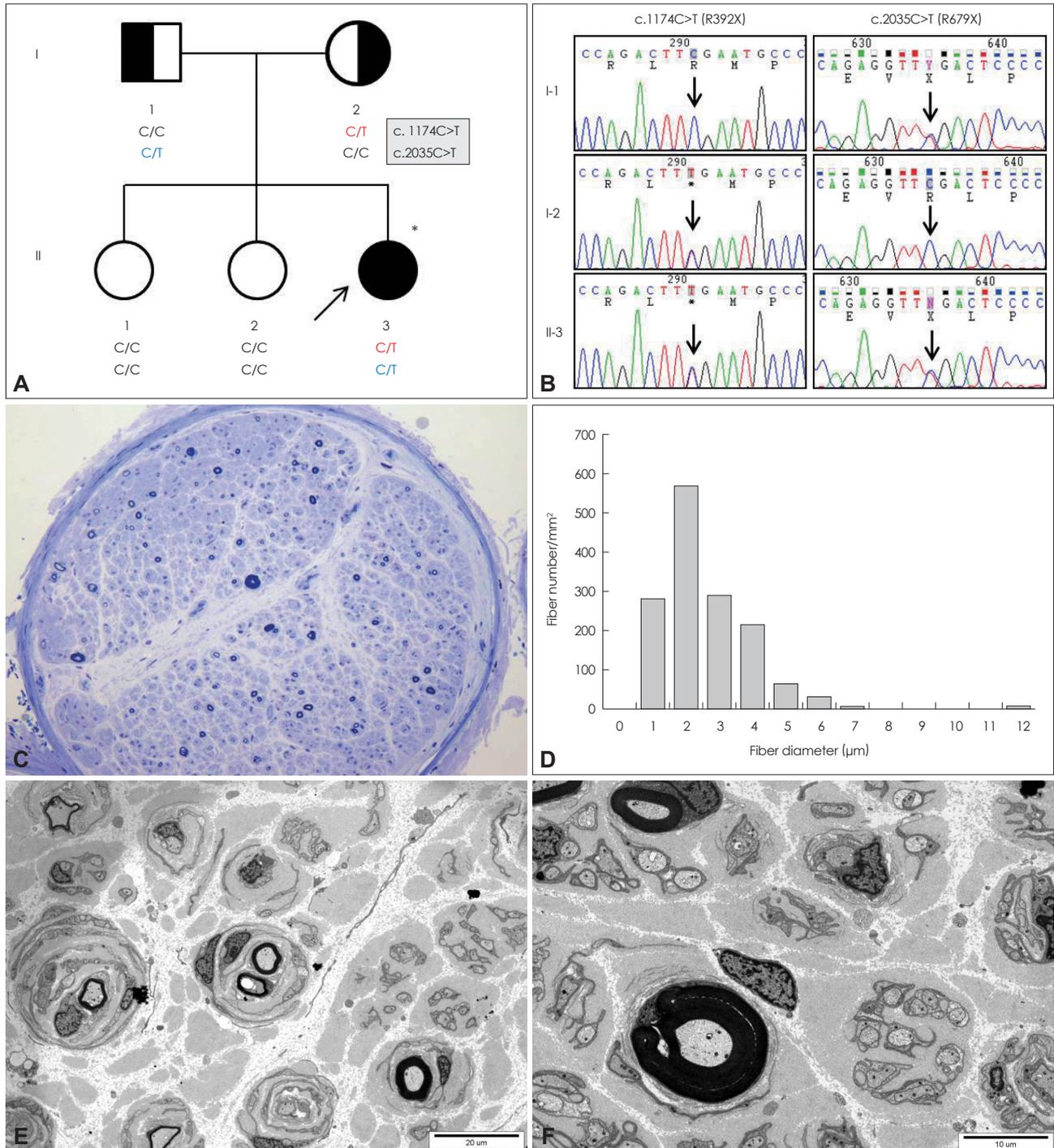


Fig. 1. Pedigree, sequencing analysis, and distal sural nerve biopsy of the proband with compound heterozygous mutations in *PRX*. **A:** Pedigree of patient's family. Genotypes of both *PRX* mutations were indicated at the bottom of each examined individual. Open symbols, unaffected; filled symbol, affected; half-filled symbols, individuals possessing only a heterozygous mutation; asterisk, whole-exome sequencing performed; and arrow, proband. **B:** Sequencing chromatograms from the proband and her parents. Vertical arrows indicate the mutation site. * (asterisk) and X indicate the mutation codon in I-2 and I-1, respectively. **C:** Transverse semi-thin sections with toluidine blue stain (×400). Light microscopy images revealed nerve fascicles that were markedly decreased in size, diffuse subendoneurial edema, and moderately to markedly decreased numbers of myelinated fibers (MFs) of all calibers with suggestive onion bulb formation and endoneurial fibrosis. **D:** Histogram of the sural nerve biopsy showing a unimodal distribution pattern. The mean diameter of MF (3.14 μm) and the percentage area of MFs (1.39%) were lower than in age-matched controls. **E** and **F:** Ultrastructural micrographs by electron microscopy (×1,000) revealed findings consistent with demyelinating neuropathy, such as focally folded, uncompacted, or deteriorating myelin, irregular myelin thickness, and fragmented myelin structures in Schwann cells or nearby macrophages.

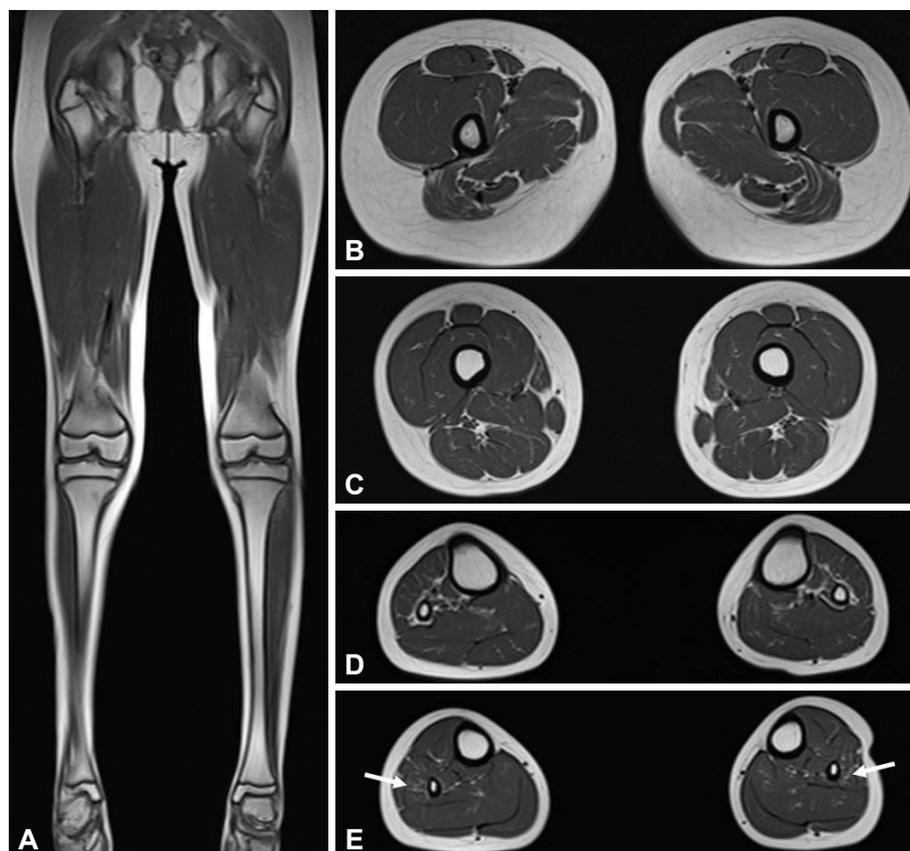


Fig. 2. Hip, thigh, and lower calf MRIs of the proband (II-3) with *PRX* mutation. A: Coronal scan of lower limbs. T1-weighted images demonstrated no definite fatty or atrophic changes in hip and thigh muscles. B-E: Axial scans of lower limbs. B and C: At the thigh levels, MR images were normal. D and E: On the lower calf levels, we found mild fatty changes in distal peronei muscles (arrows), but, the tibialis anterior and soleus muscles were not involved.

Table 1. Sural nerve biopsy findings of the patient (II-3) with *PRX* mutation

Findings	Patient	Normal distal sural nerve
Counting method	EM ($\times 1,000$, 12 fields)	
Onion bulbs (/mm ²)	410	
Several axonal clusters (/mm ²) (%)	494 (39.16)	
Thick MFs with axonal atrophy (/mm ²) (%)	46 (3.65)	
MFs with abnormal myelin (/mm ²) (%)	76 (6.03)	
MFs (/mm ²)	1,484	9,800
Diameter of MFs (μ m)		
Range (mean)	1.06-12.51 (3.14)	2-12.5 (4.5)
<3 μ m (%)	57.54	
<6 μ m (%)	96.65	
>8 μ m (%)	1.12	
Axonal diameter of MFs (μ m)		
Range (mean)	0.61-5.86 (1.94)	
Percentage area of MFs (%)	1.39	25.2
Axonal area (%)	0.45	
G-ratio (axonal diameter/MF diameter)		
Range (mean)	0.18-0.84 (0.63 \pm 0.12)	0.6-0.7
>0.7	31.28	
<0.4	3.91	
Myelin thickness		
Range (mean)	0.17-4.47 (0.60)	
>2 μ m (%)	1.12	At least 20% of MFs

EM: electron microscopic examination, MFs: myelinated fibers.

sense or frameshift mutations, and the novel mutations reported here in are also nonsense mutations.^{5-7,10-14} One exception was reported recently in a Japanese patient with a missense mutation in *PRX*,⁹ that patient exhibited an adult-onset phenotype that was milder than in patients with nonsense mutations.

It is well known that *PRX*-associated neuropathies are characterized by slow clinical progression.⁷ In spite of markedly severe electrophysiologic defects, it is surprising that the present patient was able to walk without orthopedic foot devices at 10 years of age and had exhibited very slow disease progression. Lower-limb MRIs revealed only mild fatty infiltrations of the bilateral peronei muscles, but the other muscles of the lower extremities were not involved. These MRI findings represented evidence of a mild phenotype, which was consistent with the clinical manifestations.

To the best of our knowledge this is the first patient of Korean origin with a *PRX* mutation. *PRX* mutation screening is recommended in Korean patients with early onset and slowly progressive demyelinating neuropathies.

Conflicts of Interest

The authors have no financial conflicts of interest.

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