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ORIGINAL ARTICLE

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Periaxin mutation causes early-onset but slow-progressive Charcot-Marie-Tooth disease

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Abstract Periaxin (PRX) plays a significant role in the myelination of the peripheral nerve. To date, seven nonsense or frameshift *PRX* mutations have been reported in six pedigrees with Dejerine-Sottas neuropathy or severe Charcot-Marie-Tooth neuropathy (CMT). We detected a *PRX* mutation in three patients in the screening of 66 Japanese demyelinating CMT patients who were negative for the gene mutation causing dominant or X-linked demyelinating CMT. Three unrelated patients were homozygous for a novel R1070X mutation and presented early-onset but slowly progressive distal motor and sensory neuropathies. Mutations lacking the carboxyl-terminal acidic domain may show loss-of-function effects and cause severe demyelinating CMT.

Keywords Periaxin · Charcot-Marie-Tooth neuropathy · Dejerine-Sottas neuropathy · Congenital hypomyelination · Peripheral nerve

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Introduction

Charcot-Marie-Tooth disease (CMT) is one of the most common but heterogeneous inherited neuropathy. CMT has been classified into two types, the demyelinating form and the axonal form. At present, at least 11 different genes have been identified as causing demyelinating CMT, including autosomal dominant, autosomal recessive, and X-linked types (Young and Suter 2003). To identify the responsible gene mutation, we previously examined 143 Japanese demyelinating CMT patients for autosomal dominant CMT type 1 (CMT1) or X-linked CMT genes including a duplication of 17p11.2–p12 and mutations of peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), connexin 32 (Cx32), early growth response 2 (EGR2), and lipopolysaccharide-induced tumor necrosis factor-α factor (LITAF) genes (Young and Suter 2003; Street et al. 2003). However, we were unable to identify the responsible mutation in 66 (46%) of 143 Japanese demyelinating CMT patients. Recently, the candidates for autosomal recessive demyelinating CMT (CMT4) have been identified as follows: ganglioside-induced differentiation-associated protein-1 (GDAP1), myotubularin-related protein-2 (MTMR2), SET binding factor 2 (SBF2), N-myc downstream-regulated gene 1 (NDRG1), and periaxin (PRX) genes (Boerkoel et al. 2001; Guilbot et al. 2001; Senderek et al. 2003).

In the present report, we studied *PRX* in Japanese patients with demyelinating CMT carrying no identified responsible mutations and detected homozygous novel R1070X mutation in three unrelated patients.

Materials and methods

We screened the mutation of *PRX* by denaturing high-performance liquid chromatography analysis (DHPLC) in 66 Japanese patients who were clinically diagnosed with demyelinating CMT including Dejerine-Sottas syndrome and congenital hypomyelination and who

were negative for 17p11.2–p12 duplication and mutation of *PMP22*, *MPZ*, *LITAF*, *Cx32*, and *EGR2* (Numakura et al. 2002; Numakura et al. 2003).

Patients

Case 1

The patient was the first child of healthy, consanguineous parents. She had one brother who had similar symptoms as described in our previous report (Sawaishi et al. 1995). The patient showed gross motor delay since early infancy and walked without support at 3 years. On neurological examination at age 3.5, she had distal dominant muscle weakness and absence of all deep-tendon reflexes. Responses to light touch and painful stimuli appeared normal. Cerebrospinal fluid analysis showed a mild increase in protein concentration (42 mg/dl). No motor or sensory action potentials were detected on nerve conduction velocity (NCV) studies. The electron micrographs of the biopsied sural nerve demonstrated an atypical onion-bulb formation consisting of double-layered, empty basement membranes, which surrounded thinly myelinated or naked axons. She had nonprogressive or slow-progressive disease course, and she could walk unaided at 18 years old.

Case 2

The patient was the first child of healthy, consanguineous parents. He had two sisters, and one of them had similar symptoms. The patient had gait problems from childhood. At age 40, he had muscle weakness of his hands and further difficulties in gait. On examination at age 51, he had absence of all deep-tendon reflexes, weakness of the hand and distal leg muscles, pes cavus, and decreased sensitivity to touch and vibration in the lower extremities. Electrophysiological studies of the median nerve showed slow motor NCV of 20 m/s and undetectable sensory nerve action potentials. Sural nerve biopsy revealed moderate loss of fibers with large diameters, thin myelinated fibers, and mild onion-bulb formation.

Case 3

The patient was the fourth child of healthy, consanguineous parents. No family members had similar symptoms. He had walking difficulty from childhood. On neurological examination at age 60, he showed absence of all deep-tendon reflexes, muscle weakness and atrophy of distal extremities, pes caves, and loss of sensation in both extremities. Electrophysiological studies of the median and ulnar nerves showed decreased motor NCVs of 8 and 10 m/s, respectively. Sural nerve biopsy revealed prominent loss of fibers with large diameters, and mild onion bulb and tomacula formation.

Gene analyses

The Ethics Committee of the Yamagata University School of Medicine approved this study. With written informed consent from the patients and their families, peripheral blood specimens were used for genomic DNA extraction. DNA of healthy controls was also prepared from Japanese medical students and coworkers who agreed to the study protocol. All exons, including exon–intron boundaries of *PRX*, were amplified by polymerase chain reaction with the primers designed based on the published sequences (Boerkoel et al. 2001). The mutations were screened by DHPLC analysis (Transgenomic WAVE system). The fragments showing heteroduplex were sequenced by the Dye Deoxy Terminator Cycle method on an ABI PRISM Genetic Analyzer 310 (PE Applied Biosystems, Foster City, CA, USA).

Results

We screened the mutation of *PRX* by DHPLC in 66 Japanese patients with demyelinating CMT who were negative for the mutation of CMT1 and X-linked CMT genes. We detected heteroduplex in several fragments and identified a novel mutation in three unrelated patients. Cases 1, 2, and 3 had a homozygous C-to-T mutation at nucleotide 3208 leading to an R1070X mutation (Fig. 1). This mutation was not detected in 100 healthy controls.

Discussion

By screening the *PRX* mutation, we detected three unrelated patients with homozygous R1070X mutation who showed early onset but slowly progressive symptoms.

PRX is alternatively spliced and encodes two PDZ domain proteins, L-periaxin and *S*-periaxin. L-periaxin is expressed on the abaxonal surface of myelinated Schwann cells and forms a complex with dystrophin-related protein 2 linking the basal lamina outside the cell to the cytoskeleton within the cell (Scherer et al. 1995; Sherman and Brophy 2000; Sherman et al. 2001). From experiments with knockout mice, L-periaxin is known to play a significant role in the maintenance and repair of peripheral nerve myelin (Gillespie et al. 2000; Williams and Brophy 2002). However, expression of L-periaxin in Schwann cells moves from the nucleus to the adaxonal or periaxonal cytoplasm, then to the abaxonal membrane of myelinating Schwann cells with the maturation of myelin. In addition, all reported patients with *PRX* mutations had an early onset of severe symptoms. These observations suggest that periaxin alters the role in the stage of myelination and plays significant roles in the formation and maintenance of myelin and in the signal transduction from the extracellular matrix to the cytoskeleton of myelinating Schwann cells.

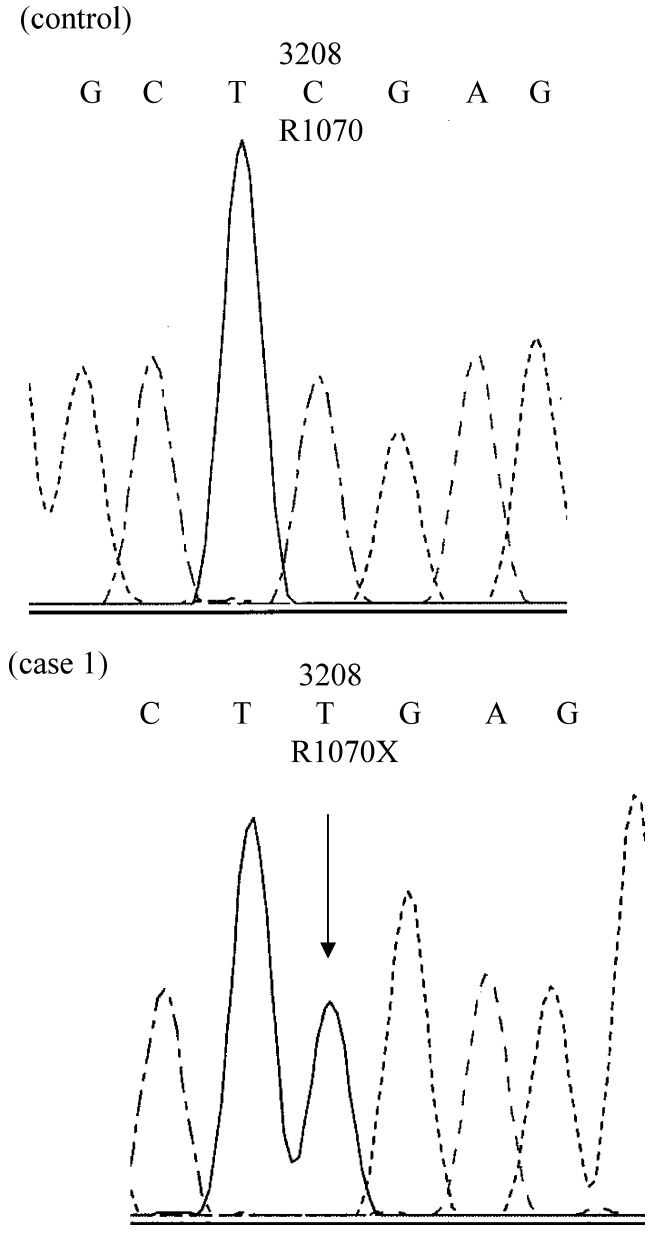

Fig. 1 Sequence chromatography of the sense chain of case 1 revealed a homozygous C-to-T mutation at nucleotide 3208 (*arrow*), resulting in an R1070X mutation

To date, six pedigrees carrying seven non-sense or frameshift mutations of *PRX* had been reported (Boerkoel et al. 2001; Guilbot et al. 2001; Takashima et al. 2002). The R1070X (C3208T) mutation was detected in three unrelated Japanese pedigrees. The C3208T mutation might arise as a result of a 5-methylcytosine to thymine transition at CpG dinucleotide. The R1070X mutation might be one of the hotspots for single base-pair substitutions or be transmitted to Japanese patients due to founder effects. Case 1 and her brother had similar severe symptoms as congenital hypomyelination (Sawaishi et al. 1995). However, they presented non-progressive or slow-progressive manifestations similar to cases 2 and 3. The R1070X mutation is probably associated with an early-onset but relatively slow-progressive distal motor and sensory neuropathy. Cases 1, 2, and 3 showed different histopathological findings of sural nerves: case 1 showed atypical onion-bulb formation; case 2, onion-bulb formation; and case 3, onion-bulb and tomacula formation. Onion-bulb and tomacula formation was previously reported in the patients with *PRX* mutations (Guilbot et al. 2001; Takashima et al. 2002). The specimens of case 1 demonstrated an atypical onion-bulb formation that consisted of double-layered empty basement membranes (Sawaishi et al. 1995). Similar atypical onion-bulb formation was reported in the patient with the R196X mutation (Guilbot et al. 2001). The difference in the histopathological findings of sural nerves from our three cases could not be definitely explained; however, they might be associated with the difference in their age at biopsy.

L-periaxin has four characteristic domains: PDZ, NLS, repeat, and acidic domains (Gillespie et al. 1994; Sherman and Brophy 2000; Sherman et al. 2001). All *PRX* mutations except the R82fsX96 mutation located in the 3′ terminal exon (exon 7) would probably escape from non-sense-mediated decay and are predicted to produce truncated proteins that lacke the carboxyl-terminal acidic domain. L-periaxin forms a complex with dystrophin-related-protein-2 and dystroglycan linking the cytoskeleton of the Schwann cell with the extracellular matrix (Sherman et al. 2001). The function of acidic domain of L-periaxin is not clear; however, acidic domains are known to mediate protein–protein interactions. L-periaxin lacking acidic domain may not be able to bind to the cytoskeleton of Schwann cells or to transmit the signals and cannot form stable compact myelins. All patients carrying the non-sense or frameshift mutations suggest that loss-of-function of L-periaxin is a major pathogenesis of the disease. Further analysis of *PRX* mutations would make clear the function of L-periaxin and the genotype–phenotype relationship.

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