SHORT COMMUNICATION

The GARS gene is rarely mutated in Japanese patients with Charcot–Marie–Tooth neuropathy

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Charcot–Marie–Tooth neuropathy (CMT) is an extremely common but heterogeneous inherited neuropathy. It has been classified into two forms: demyelinating and axonal. The dominant axonal form, CMT2, has been further subdivided through linkage study and 15 loci and 10 genes have been reported. For the glycyl-tRNA synthetase (GARS) gene, a CMT2-causing gene, 10 mutations have been reported to date. We studied the *GARS* in 89 Japanese patients with axonal CMT and detected a novel heterozygous Pro244Leu (c.893C>T) mutation in a patient showing adolescent onset and early upper limb involvement. Results of our study indicate that *GARS* mutation is a rare cause of CMT2 among Japanese patients.

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INTRODUCTION

Charcot–Marie–Tooth neuropathy (CMT) is an extremely common, but heterogeneous inherited neuropathy. It has been classified into two forms: demyelinating and axonal. The dominant axonal form, CMT2, has been further subdivided through linkage study and 15 loci and 10 genes have been reported to date (<http://www.molgen.ua.ac.be/CMTMutations/Mutations>). Among the CMT2-causing genes, *MFN2* mutation is the most common, detected in 10–30% of the CMT2 patients.^{1–3} Moreover, each of the *MPZ* and *GJB1* mutations is found in less than 5% of axonal CMT including CMT2 patients. Patients with *MFN2* mutations show symptoms in early childhood, whereas axonal CMT patients with *MPZ* or *GJB1* mutations generally present the symptoms during adolescence to adulthood. Distal spinal muscular atrophy (dSMA), an exclusively motor neuropathy, is also a clinically and genetically heterogeneous neuropathy. In fact, CMT2 cannot be distinguished clearly from dSMA because sensory disturbances are often lacking in patients with CMT2. Recently, it has been revealed that *HSPB1*, *HSPB8* and glycyl-tRNA synthetase (*GARS*) gene mutations can cause both of these neuropathies.^{4–6}

We initially studied 110 Japanese patients with axonal CMT, and found an *MFN2* mutation in 12 patients, *MPZ* mutation in 4 patients and a *GJB1* mutation in 5 patients. In this report, we describe our study of *GARS* in 89 patients who had no mutations of *MFN2*, *MPZ*, *GJB1*, *HSPB1* and *HSPB8* and describe the detection of a novel mutation in a male patient showing early upper-limb involvement.

MATERIALS AND METHODS

We screened 89 Japanese patients with axonal CMT who had no mutations of *MFN2*, *MPZ*, *GJB1*, *HSPB1* and *HSPB8*, for *GARS* mutation. The *GARS*

mutation was identified in a male patient with early upper-limb involvement in adolescence. His clinical report would be described elsewhere.

Gene analyses and secondary structure analysis

The Ethics Committee of the Yamagata University School of Medicine approved this study. Peripheral blood specimens were taken for genomic DNA extraction after obtaining written informed consent from the patients. DNA was also extracted from the Japanese medical students and co-workers who agreed to the study protocol. All coding exons except for a mitochondrial targeting-like sequence in exon 1 and the exon–intron boundaries of *GARS* were amplified using polymerase chain reaction with primers designed according to the data of the *Homo sapiens* chromosome 7 genomic contig (NC_000007.12). We screened for the mutation using denaturing high-performance liquid chromatography analysis (DHPLC, WAVE System; Transgenomic Inc., Omaha, NE, USA). Fragments showing a heteroduplex were sequenced using the Dye Deoxy Terminator Cycle method on a Genetic Analyzer (ABI Prism 310; PE Applied Biosystems, Foster City, CA, USA).

The secondary structure of the peptides was predicted using the Chou–Fasman algorithm with a software (Genetix-Mac 14.0; Genetix Corp., Tokyo, Japan).

RESULTS

By screening all the coding exons using DHPLC, except the mitochondrial targeting-like sequence of *GARS*, we detected DNA fragments showing a heteroduplex and determined these sequences of these fragments. Figure 1 shows that we detected a novel Pro244Leu (c.893C>T) (named based on NP_002038) in one patient. This mutation was not detected in the 100 healthy controls, and Pro244 was conserved in the *GARS* of other species: human (NP_002038), rat (AC091711), chicken (AC092081), frog (AAI42564.1), zebra fish (AC099322), fruit fly (AAF49668.1) and fission yeast (NP_593935.1). The Pro244Leu mutation was predicted to alter the secondary structure of the peptide considerably (data not shown).

We also detected the following polymorphic nucleotide substitutions: c.93G>C (rs2529438), c.124C>G (rs1049402), c.222+5C>T (rs2072236), c.222+93_95delCCT, c.1032-23A>T (rs2527878), c.1359+97A>G (rs2709772), c.1716G>A (Pro518Pro), c.2094+26T>G, c.2095-6C>T (rs2240401) and c.2212G>A (Glu684Lys).

DISCUSSION

We studied the GARS in 89 patients with axonal CMT who had no mutations of *MFN2*, *MPZ*, *GJB1*, *HSPB1* and *HSPB8* and detected a disease-causing mutation in one patient, 0.9% of 110 patients with axonal CMT. The patient carried a novel Pro244Leu (c.893C>T) mutation in the catalytic domain. To date, 10 mutations of the

GARS have been reported in patients with CMT2 or dSMA (Figure 2).^{4,7-10} The patient showed early upper-limb involvement in adolescence, similar to the patients carrying eight different GARS mutations, except for two mutations in the anticodon-binding domain.^{4,7-10} The patients carrying the mutations in the anticodon-binding domain presented a predominant lower-limb involvement in infancy or during early childhood.⁹ Our study confirmed that GARS mutation is a rare cause of axonal CMT among Japanese patients.

Although GARS is ubiquitously expressed, patients carrying GARS mutations have no other symptoms except for peripheral neuropathy. In fact, GARS might have specific roles, such as cell-cycle regulation and signal transduction in peripheral nervous systems.¹¹ In yeast, GARS plays a role in mRNA 3'-end formation.¹² Peripheral neurons can be vulnerable because of their characteristically long structures, which require the transport of organelles and proteins over long distances to maintain their function. In fact, GARS is a homodimer, and, on the basis of results of *in vitro* expression experiments, the GARS mutations causing CMT or dSMA are predicted to alter the dimer interface and engender a neurite distribution defect.^{13,14} Further study is necessary to clarify the precise mechanism of peripheral neuropathy attributable to GARS mutation.

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Figure 1 Sequence chromatography of the patient. Sequence chromatography of the sense chain of the patient revealed a heterozygous mutation of GARS.

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Figure 2 Localization of GARS mutations. The stippled and hatched regions indicate each functional domain: WHEP-TRS enzyme conjugation domain, core catalytic domains and Gly-tRNA anticodon binding domain. The numbers under the box indicate the amino-acid sequence numbers of GARS except for a mitochondrial targeting-like sequence in exon 1.

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