A novel mutation in glycyl-tRNA synthetase caused Charcot-Marie-Tooth disease type 2D with facial and respiratory muscle involvement

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Abstract: BACKGROUND: Charcot-Marie-Tooth disease (CMT) is a hereditary peripheral neuropathy; symptoms include distal wasting and weakness, usually with some sensory impairment. The clinical course is typically benign and the disease is not life threatening; however, in some cases, severe phenotypes include serious respiratory distress. CASE REPORT: Here we describe a 45-year-old woman with a long course of motor-dominant neuropathy. Distal weakness appeared in childhood and became worse with age. After a diagnosis of CMT type 2, the symptoms progressed, and in her fourth decade, facial and respiratory muscle weakness appeared, ultimately requiring non-invasive mechanical ventilation. There was no family history of CMT. Comprehensive analysis of known CMT-related genes revealed a novel heterozygous c.815T>A, p.L218Q mutation in glycyl-tRNA synthetase (GARS), a causative gene for both CMT type 2D (CMT2D) and distal spinal muscular atrophy type V (dSMA-V). This mutation was considered pathogenic based on molecular evidence; notably, it was unique in that all other reported GARS mutations associated with severe phenotypes are located in an anticodon-binding domain, while in this case in an apparently non-functional region of the GARS gene. Not a simple loss-of-function mechanism, but rather gain-of-function mechanisms have also been reported in GARS mutations. This case provided useful information for understanding the mechanism of CMT2D/dSMA-V.

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Case report

A 45-year-old woman initially presented with distal dominant muscle atrophy, which progressed, and facial muscle atrophy and respiratory muscle impairment, and a novel mutation was found in the glycyl-tRNA synthetase gene (the gene is abbreviated as GARS and the protein as GlyRS), which is a causative gene for both CMT type 2D (CMT2D) and distal spinal muscular atrophy type V (dSMA-V).
respiratory failure developed subsequently. The patient was born after 32 weeks of gestation without any abnormality. Walking was slightly delayed and running speed was slow through her preschool years. Bilateral foot drop developed around age 7, and distal muscle atrophy developed in all limbs by age 10. She was wheelchair-bound in her third decade. At age 29, she was admitted to our hospital for three months; muscle and nerve biopsies were performed, and she subsequently received a diagnosis of CMT type 2 (CMT2) with evidence of axonal sensorimotor neuropathy. At age 36, respiratory muscle dysfunction developed and non-invasive mechanical ventilation was started. At age 45, she was admitted for re-evaluation. Her parents were unrelated to unknown causes. Her father and brother were alive and healthy at the writing of this report. Physical examination showed severe atrophy in all skeletal muscles, including limb, truncal, facial and tongue muscles (Fig. 1B). Dysphagia and nasal voice were evident. Her muscle strength scores, which were based on the Medical Research Council scale, were 2 out of 5 for proximal muscles and 1 out of 5 for distal muscles. Deep tendon reflexes were absent. Sensory disturbance was mild and only evident with distal lower limbs.

In nerve conduction studies, compound muscle action potentials (CMAPs) were not evoked from routinely examined muscles, including the abductor pollicis brevis, abductor digiti minimi and flexor hallucis brevis. CMAPs from the flexor carpi radialis had extremely low amplitudes, but the distal latency was normal, and conduction velocities were only slightly decreased (42 m/s) relative to normal values. Sensory nerve action potentials (SNAPs) and sensory conduction velocities (SCVs) from the median nerve were normal. SNAPs from the sural nerve had been recorded when the patient was 29 years old; these SNAPs had very low amplitudes (2.1 μV), but the SCVs were normal (55 m/s). Needle electromyography showed chronic neurogenic patterns.

A muscle biopsy from triceps brachii was performed at age 29. The majority of the muscle fibers ranged from 70 to 100 μm in diameter. Pyknotic clamp was present in the rim of a fascicle. Necrotic or regenerating fibers were not observed. Islands of groups of extremely atrophic fibers and spindles were present in epimysium. Internal nuclei were moderately increased. Muscle fibers occasionally showed fiber splitting. Fatty connective tissue was increased in perimysium and more markedly in epimysium. Trichrome staining added no information. Intermyofibrillar network was preserved in NADH dehydrogenase-stained sections. Every fascicle of fibers showed fiber-type grouping as assessed by ATPase staining. These findings were consistent with chronic denervation.

A sural nerve biopsy also taken at age 29 revealed moderate loss of myelinated fibers; however, axonal degeneration and active demyelination were not evident. Perivascular mononuclear cells were observed in epineurium, but these cells had not infiltrated the endoneurium. Substantial deposition of fat droplets was observed at the tunica media-externa of small arteries (Fig. 1C). Electron microscopy revealed no obvious mitochondrial abnormalities.

Lung CT scan revealed no abnormalities. Electrocardiogram showed normal sinus rhythm with a tall P wave and right axis deviation. Echocardiogram appeared normal.

A comprehensive sequence analysis of CMT-related genes revealed a novel heterozygous c.815T>A, p.L218Q mutation in the GARS gene (Fig. 1D). The patient’s unaffected father and brother did not carry this mutation. HomoloGene (http://www.ncbi.nlm.nih.gov/homologene) was used to conduct a sequence homology search; we found that leucine 218 in GlyRS was highly conserved among species (Fig. 1E). The computational protein function-predicting algorithm MUPro score was –1; this value indicated that the mutant protein was less stable than the wild-type protein (http://www.igb.uci.edu/~baldig/mutation.html). Moreover, the PolyPhen-2 score was 1.0; this score indicated that the mutant GlyRS protein was pathogenic (http://genetics.bwh.harvard.edu/pph2/).

Discussion

We present a unique case of CMT that involved a new mutation in GARS; the patient initially developed moderate CMT2 symptoms and subsequently developed facial and respiratory muscle impairment.

GARS is one of 37 aminoacyl-tRNA synthetases (ARSs). ARSs are divided into two groups, based upon their cytoplasmic or mitochondrial localization. Among them, GARS and tyrosyl-tRNA synthetase (YARS) are localized to both the cytoplasm and mitochondria. GlyRS, the product protein of GARS gene, is ubiquitously expressed, including the brain and spinal cord. It has two isoforms, with and without an N-terminal mitochondrial targeting sequence (MTS), localizing in the mitochondria and cytoplasm, respectively. GlyRS catalyzes attachment of glycine to its cognate tRNA for protein synthesis and non-translational functions of GlyRS include tumor suppression when secreted. Remarkably, all known disease-associated mutations in cytoplasmic ARSs are associated with CMT and related neuropathies, and the causative genes include GARS, KARS, tyrosyl-tRNA synthetase (YARS), and alanyl-tRNA synthetase (AARS). GARS is also one of the genes that, when mutant, can cause CMT2 or distal spinal muscular atrophy (dSMA); conditions originating from GARS mutations are called CMT2D or dSMA-V, depending on whether sensory nerves are affected. The majority of previously reported CMT2D/dSMA-V cases involved adolescent onset with upper limb-dominant weakness, and the progression of symptoms was slow. Other organs including brain and
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Muscle were not involved. Even though mitochondrial isoform of GlyRS localizes in mitochondria, mitochondrial disorders like myopathy and MELAS are not reported in GlyRS mutations, unlike mutations of other mitochondrial ARSs. Neither muscle or nerve biopsy in the presented case showed mitochondrial abnormalities.

Fig. 1 Clinical, pathological and molecular features of the patient.

(A) Pedigree. (B) Facial involvement with weakness of the orbicularis oris and atrophy of the temporalis and masseter muscles. The patient was instructed to close her mouth. Limbs showed severe muscle atrophy. (C) The sural nerve biopsy at age 29 showed moderate loss of myelinated fibers. Axonal degeneration and active demyelination were not evident. Bar = 20 μm. (D) Chromatogram of the heterozygous c.815T>A (p.L218Q) mutation in exon 7 of \textit{GARS}; the patient and two unaffected relatives. (E) Comparison of GlyRS from different species. Arrowhead on top of the alignment indicates amino acid position 218 (Note: numbering differences from related species are because the human annotation does not consider the N-terminal mitochondrial targeting sequence appended through alternative start codon usage). (F) The GlyRS protein contains four functional domains and three dimer interface regions. Mutations identified in GlyRS are distributed across the entire protein; modified from Motley, et al\textsuperscript{13}. L218Q, the mutation found in our patient is shown in purple. It is located in an apparently non-functional region. In contrast, both of two other known mutations that cause early onset and severe clinical phenotypes, shown in red, are located in an anticodon-binding domain.
The GlyRS protein comprises four functional domains and three dimer interface regions (Fig. 1F). Among 13 reported GARS mutations, two mutations caused early-onset clinical phenotypes in four patients. One patient developed facial and respiratory muscle involvement, and another developed vocal cord dysfunction. Both mutations are located in an anticodon-binding domain. In contrast, the mutation described in the current study was located in neither of the functional domains. Even so, we still consider this L218Q mutation a pathogenic mutation based on the following reasons: 1) its close location to the dimer interface region; 2) the high conservation of the affected amino acid; and 3) the fact that neither the unaffected parent nor the unaffected brother carried this mutation. In silico prediction using MUPro and Polyphen-2 suggests pathogenicity of the mutation, but the results from other reported mutations using these algorithms do not necessarily correlate with clinical severity (Table 1) and this approach may not be suitable as far as this gene is concerned. Although both loss-of-function and gain-of-function mechanisms were likely to synergistically give rise to severe phenotypes in the previous cases with mutations in an anticodon-binding domain, gain-of-function predominantly appears to have led to severe phenotypes in our case. Data from this unique case provided new information for understanding the mechanism of CMT2D/dSMA-V and for drug discovery as well.
A novel mutation in GARS caused CMT2D with facial and respiratory muscle involvement

The patient and family members included in this study gave written informed consent, and the study was approved by the Kyoto University and the Institutional Review Board of Kagoshima University.

Abstract of this work was presented at the 98th Kinki Regional Meeting of the Japanese Society of Neurology and recommended by the conference chairperson for the publication to Rinsho Shinkeigaku.

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※ The authors declare there is no conflict of interest relevant to this article.

References