Brief Clinical Note

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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Key words：Charcot-Marie-Tooth disease, hereditary sensory and motor neuropathy, glycine-tRNA ligase, spinal muscular atrophy, respiratory distress

Introduction

Charcot-Marie-Tooth disease (CMT) is a hereditary peripheral neuropathy presenting distal wasting and weakness, usually with some distal sensory impairment. In most cases, the clinical course is benign and the disease is not life threatening; however, in some cases, severe phenotypes can include respiratory distress, which, in relation to adults, is not widely recognized in the literature[1)]. We describe a unique case characterized by progression of serious symptoms; ultimately, these included facial 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).

Case report

A 45-year-old woman initially presented with distal dominant muscle atrophy, which progressed, and facial muscle atrophy and

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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 one another (Fig. 1A). Her mother had died at age 46 due 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 μV 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[2)3)] 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 *lysyl-tRNA synthetase (KARS)* are localized to both the cytoplasm and mitochondria. GlyRS, the product protein of *GARS* gene, is ubiquitously expressed, including the brain and spinal cord[4)]. 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[5)6)]. 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)*[6)]. *GARS* is also one of the genes that, when mutant, can cause CMT2 or distal spinal muscular atrophy (dSMA)[3)]; 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[4)7)~12)]. Other organs including brain and

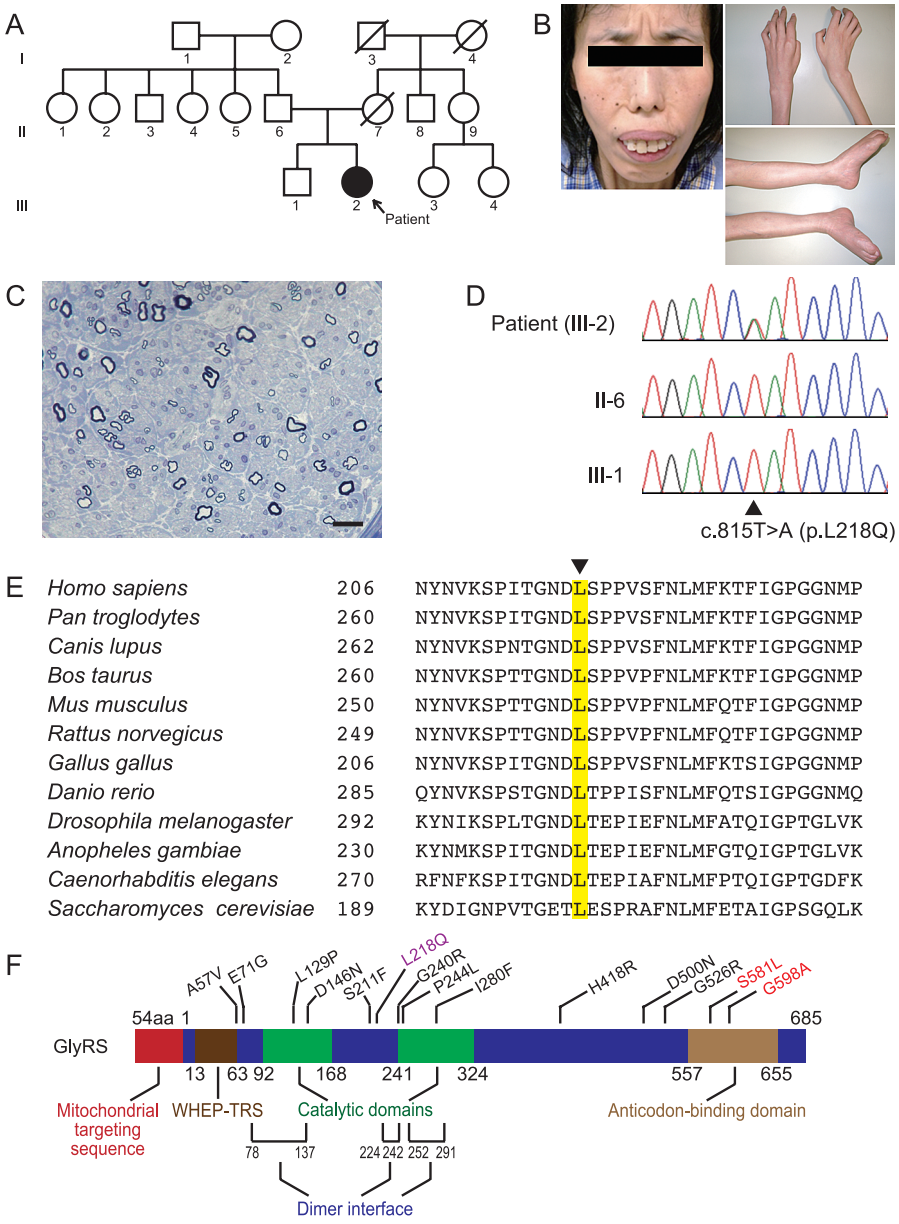

Fig. 1 Clinical, pathological and molecular features of the patient.

(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 *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[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.

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[6]. Neither muscle or nerve biopsy in the presented case showed mitochondrial abnormalities.

Table 1　*In silico* analysis of previously reported mutations.

Authors	Domains	Mutations	MUPro		Polyphen-2
			Method 1	Method 2	
Rohkamm, *et al.* (2007)[8]	WHEP-TRS	A57V	–0.13	–0.76	0.439
Antonellis, *et al.* (2003)[4]		E71G	–0.76	–0.98	0.788
Antonellis, *et al.* (2003)[4]	Catalytic-1	L129P	–1.00	–1.00	1.000
Lee, *et al.* (2012)[9]		D146N	–0.79	–0.86	1.000
Lee, *et al.* (2012)[9]		S211F	0.19	0.55	1.000
Presented case		L218Q*	–1.00	–0.96	1.000
Antonellis, *et al.* (2003)[4]		G240R	0.30	0.68	1.000
Abe, *et al.* (2009)[10]	Catalytic-2	P244L	0.25	0.67	1.000
James, *et al.* (2006)[7]		I280F	–1.00	–1.00	1.000
Sivakumar, *et al.* (2005)[11]		H418R	0.55	0.73	0.998
Del Bo, *et al.* (2006)[12]		D500N	–0.53	–0.79	0.048
Antonellis, *et al.* (2003)[4]		G526R	0.01	–0.51	1.000
James, *et al.* (2006)[7]	Anticodon-binding	S581L*	0.15	0.80	0.420
James, *et al.* (2006)[7]; Eskuri, *et al.* (2012)[14]		G598A*	0.86	0.82	0.013

Asterisks indicate mutations associated with severe phenotypes. MUPro scores range between –1 and 1. A score less than 0 means that the mutation decreases the protein stability, and *vice versa*. A larger absolute value indicates more confident prediction. Polyphen-2 scores range between 0 and 1. A larger score indicates that the mutation is more pathogenic. Underlines indicate high scores, predicting unstability and pathogenicity of the mutated proteins.

The GlyRS protein comprises four functional domains and three dimer interface regions[13] (Fig. 1F). (Note: numbering of residues starts from the alternative start codon after MTS in human protein). Among 13 reported *GARS* mutations[7~10,14], two mutations caused early-onset clinical phenotypes in four patients. One patient developed facial and respiratory muscle involvement[7], and another developed vocal cord dysfunction[14]. 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.

Mechanisms underlying CMT2D/dSMA-V caused by *GARS* mutations have been examined from various aspects, including enzyme activity, protein stability and dimerization, but those properties considerably depend on individual mutations and none of these approaches reached consistent results. Moreover, heterozygous mice with a single loss-of-function *GARS* allele exhibited reduced synthetase activity but none of the symptoms of CMT[15] and overexpression of wild-type GlyRS could not rescue the neuropathy phenotype in mouse models[16]. These experimental results, together with the observations of scattered locations of the mutations throughout the gene and the dominant inheritance pattern lead to a consequence that not a simple loss-of-function, but rather a gain-of-function mechanism significantly contributes to the pathogenesis of the disease[5,6,13]. Recent study analyzing the tertiary structure of GlyRS using hydrogen-deuterium exchange revealed that all five mutations tested promote the same localized conformational opening[17]. All other mutations untested are also within the opened-up areas, except for some mutations which are not covered in that analysis. They argued that those opened-up areas provide unique surfaces for potential novel interactions that lead to pathological consequences. The mutation of our case is also within the "opened-up areas" and that may account for the pathogenicity.

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.

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.

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