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 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. 

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.

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. 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
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.ibg.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. 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 (dSMAN); conditions originating from GARS mutations are called CMT2D or dSMAN depending on whether sensory nerves are affected. The majority of previously reported CMT2D/dSMAN cases involved adolescent onset with upper limb-dominant weakness, and the progression of symptoms was slow. Other organs including brain and
A novel mutation in GARS caused CMT2D with facial and respiratory muscle involvement.

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 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 al20. 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). (Note: numbering of residues starts from the alternative start codon after MTS in human protein). 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.

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 CMT1 and overexpression of wild-type GlyRS could not rescue the neuropathy phenotype in mouse models. 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. Recent study analyzing the tertiary structure of GlyRS using hydrogen-deuterium exchange revealed that all five mutations tested promote the same localized conformational opening. 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.

Table 1 In silico analysis of previously reported mutations.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Domains</th>
<th>Mutations</th>
<th>MUPro Method 1</th>
<th>MUPro Method 2</th>
<th>Polyphen-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rohkamm, et al. (2007)</td>
<td>WHEF-TRS</td>
<td>A57V</td>
<td>-0.13</td>
<td>-0.76</td>
<td>0.439</td>
</tr>
<tr>
<td>Antonellis, et al. (2003)</td>
<td></td>
<td>E71G</td>
<td>-0.76</td>
<td>-0.98</td>
<td>0.788</td>
</tr>
<tr>
<td>Antonellis, et al. (2003)</td>
<td></td>
<td>L129P</td>
<td>-1.00</td>
<td>-1.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Lee, et al. (2012)</td>
<td>Catalytic-1</td>
<td>D146N</td>
<td>-0.79</td>
<td>-0.86</td>
<td>1.000</td>
</tr>
<tr>
<td>Lee, et al. (2012)</td>
<td></td>
<td>S211F</td>
<td>0.19</td>
<td>0.55</td>
<td>1.000</td>
</tr>
<tr>
<td>Presented case</td>
<td></td>
<td>L218Q*</td>
<td>-1.00</td>
<td>-0.96</td>
<td>1.000</td>
</tr>
<tr>
<td>Antonellis, et al. (2003)</td>
<td></td>
<td>G240R</td>
<td>0.30</td>
<td>0.68</td>
<td>1.000</td>
</tr>
<tr>
<td>Abe, et al. (2009)</td>
<td>Catalytic-2</td>
<td>P244L</td>
<td>0.25</td>
<td>0.67</td>
<td>1.000</td>
</tr>
<tr>
<td>James, et al. (2006)</td>
<td></td>
<td>L280F</td>
<td>-1.00</td>
<td>-1.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Sivakumar, et al. (2005)</td>
<td></td>
<td>H418R</td>
<td>0.55</td>
<td>0.73</td>
<td>0.998</td>
</tr>
<tr>
<td>Del Bo, et al. (2006)</td>
<td></td>
<td>D500N</td>
<td>-0.53</td>
<td>-0.79</td>
<td>0.048</td>
</tr>
<tr>
<td>Antonellis, et al. (2003)</td>
<td></td>
<td>G526R</td>
<td>0.01</td>
<td>-0.51</td>
<td>1.000</td>
</tr>
<tr>
<td>James, et al. (2006)</td>
<td></td>
<td>S581L*</td>
<td>0.15</td>
<td>0.80</td>
<td>0.420</td>
</tr>
<tr>
<td>James, et al. (2006); Eskuri, et al. (2012)</td>
<td>Anticodon-binding</td>
<td>G598A*</td>
<td>0.86</td>
<td>0.82</td>
<td>0.013</td>
</tr>
</tbody>
</table>

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.
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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**


