Clinical Research

Two Novel De Novo GARS Mutations Cause Early-Onset Axonal Charcot-Marie-Tooth Disease

Yi-Chu Liao1,2, Yo-Tsen Liu1,2, Pei-Chien Tsai1,2, Chia-Ching Chang3,4, Yen-Hua Huang3,4, Bing-Wen Soong1,2,5, Yi-Chung Lee1,2,5

1 Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan, 2 Department of Neurology, National Yang-Ming University School of Medicine, Taipei, Taiwan, 3 Institute of BioMedical Informatics, National Yang-Ming University School of Medicine, Taipei, Taiwan, 4 Center for Systems and Synthetic Biology, National Yang-Ming University, Taipei, Taiwan, 5 Brain Research Center, National Yang-Ming University, Taipei, Taiwan

* ycl@vghtpe.gov.tw

Abstract

Background

Mutations in the GARS gene have been identified in a small number of patients with Charcot-Marie-Tooth disease (CMT) type 2D or distal spinal muscular atrophy type V, for whom disease onset typically occurs during adolescence or young adulthood, initially manifesting as weakness and atrophy of the hand muscles. The role of GARS mutations in patients with inherited neuropathies in Taiwan remains elusive.

Methodology and Principal Findings

Mutational analyses of the coding regions of GARS were performed using targeted sequencing of 54 patients with molecularly unassigned axonal CMT, who were selected from 340 unrelated CMT patients. Two heterozygous mutations in GARS, p.Asp146Tyr and p.Met238Arg, were identified; one in each patient. Both are novel de novo mutations. The p.Asp146Tyr mutation is associated with a severe infantile-onset neuropathy and the p.Met238Arg mutation results in childhood-onset disability.

Conclusion

GARS mutations are an uncommon cause of CMT in Taiwan. The p.Asp146Tyr and p.Met238Arg mutations are associated with early-onset axonal CMT. These findings broaden the mutational spectrum of GARS and also highlight the importance of considering GARS mutations as a disease cause in patients with early-onset neuropathies.
Introduction
Charcot-Marie-Tooth disease (CMT) comprises a clinically, pathologically and genetically heterogeneous group of hereditary neuropathies. Histologically, CMTs are categorized as either demyelinating or axonal according to their electrophysiological or pathological features. Following the advent of molecular genetics, particularly the development of next-generation sequencing, more than 80 disparate mutated genes have been associated with CMT, but only a few of them, including \textit{PMP22}, \textit{GJB1}, \textit{MPZ}, \textit{MFN2} and \textit{NEFL}, account for a significant percentage of CMT cases [1–2]. The rarity with which other mutated genes cause CMT hinders our understanding of their features. Intriguingly, six of the genes responsible for axonal CMT encode aminoacyl-tRNA synthetases (ARSs), which are responsible for charging tRNA molecules with cognate amino acids, suggesting that protein synthesis plays a vital role in axonal functioning. These six genes are glycyl-tRNA synthetase (\textit{GARS}; MIM *600287) [3], tyrosyl-tRNA synthetase (\textit{YARS}; MIM *603623) [4], alanyl-tRNA synthetase (\textit{AARS}; MIM *601065) [5], lysyl-tRNA synthetase (\textit{KARS}; MIM *601421) [6], histidyl-tRNA synthetase (\textit{HARS}; MIM *142810) [7], and methionyl-tRNA synthetase (\textit{MARS}; MIM *156560) [8]. Among them, \textit{GARS} was the first one identified to cause CMT when mutated.

Glycyl-tRNA synthetase protein (GlyRS) is ubiquitously expressed and highly evolutionarily conserved in eukaryotic cells [9]. It is essential for all living cells, including both eukaryotic and prokaryotic cells. The human cytoplasmic GlyRS consists of 685 amino acids, which encode an N-terminal WHEP-TRS domain (Met1-Asp62), a catalytic domain (Asp63-Val556) and a C-terminal anticodon-binding domain (Val557-Glu685) [10], and belongs to the class IIA aminoacyl-tRNA synthetases, which are characterized by three conserved motifs within the catalytic domain [10]. In addition, three insertion domains split the catalytic domain of human GlyRS, including insertion I (Ala145-Asn225), insertion II (His318-Asn349) and insertion III (Val440-Val504) [11]. Human GlyRSs functions as a homodimer, and insertions I and III participate in a series of conformational changes in the human GlyRS homodimer during the aminoacylation of tRNA substrates with glycine [11]. Mutations in \textit{GARS} were firstly reported in 2003, with four missense mutations (p.Glu71Gly, p.Leu129Pro, p.Gly240Arg and p.Gly526Arg) resulting in CMT type 2D (CMT2D; MIM #601472) or distal spinal muscular atrophy type V (dSMA-V; MIM #600794), which is also known as distal hereditary motor neuropathy type VA (dHMN5A) [3]. The clinical presentations of distal hereditary motor neuropathy are similar to CMT except for the absence of sensory involvement. Both CMT2D and dSMA-V (dHMN5A) are usually characterized by disease onset in adolescence or young adulthood and initial manifestations of weakness and atrophy of the hand muscles [3]. In the past 12 years, only 9 more pathogenic mutations in \textit{GARS} have been identified (Table 1) [12–21]. The small number of \textit{GARS} mutations limits our knowledge of the clinical and molecular spectrums of patients with \textit{GARS} mutations. The aim of this study was to ascertain the frequency and spectrum of \textit{GARS} mutations in our cohort of 340 unrelated CMT patients of Han Chinese origin in Taiwan. The clinical presentations of those patients with \textit{GARS} mutations were also characterized.

Methods
Patients
Fifty-four patients of Han Chinese descent with molecularly-unassigned CMT2 were recruited in this study. These patients were selected from a consecutive series of 340 unrelated CMT patients at the Neurology Clinics of Taipei Veterans General Hospital who were assessed using standard clinical and electrophysiological evaluations. Among the 340 CMT patients, 103 have
axonal CMT (CMT2), with 27 already demonstrated as having a mutation in GJB1, MFN2, NEFL, HSPB1, AARS, or TFG, and another 22 had previously been shown to have no mutation in GARS via Sanger sequencing [22,23]. The remaining 54 patients with an age range from 8 to 70 years received GARS analysis in this study. CMT2 was diagnosed according to the guidelines described in the report of the 2nd Workshop of the European CMT consortium [24]. Sensory involvement was demonstrated in all the 54 patients by nerve conduction studies (NCS), and the 17p12 duplication or deletion, mutations in the GJB1 gene or its promoter and mutations in the transthyretin gene had been excluded in advance. Peripheral blood samples were collected after written informed consent was obtained from the participants or their parents on behalf of them for those younger than 18 years. All protocols for this study were approved by the Institutional Review Board of Taipei Veterans General Hospital. The individuals have

Table 1. Clinical and molecular features of mutations in the GARS gene.

| Mutation | Protein Structure | Origins     | Age of onset | Phenotype | Inheritance | First symptom                                      | References |
|----------|------------------|-------------|--------------|-----------|-------------|----------------------------------------------------|------------|
| Ala57Val | W                | Ghana       | 12 y         | CMT2/D/  | Unknown     | atrophy and weakness of the hand muscles            | [12]       |
| Glu71Gly | C                | Monogolia   | 18 y*        | CMT2/D/  | AD          | atrophy and weakness of the hand muscles            | [3, 13]   |
| Leu129Pro| C, DI            | Bulgaria    | 16.9 y*      | dSMA-V   | AD          | atrophy and weakness of the hand muscles            | [3, 13]   |
| Asp146Asn| Ins I            | Korea       | 15 y         | dSMA-V   | AD          | atrophy and weakness of the hand muscles            | [14]       |
| Asp146Tyr| Ins I            | Taiwan      | <6 m         | CMT2     | De novo     | delayed milestones, severe generalized weakness     | this study |
| Ser211Phe| Ins I            | Korea       | 13 y         | dSMA-V   | AD          | atrophy and weakness of the hand muscles            | [14]       |
| Leu218Gln| Ins I            | Japan       | <2 y         | CMT2     | Unknown     | delayed onset of walking, slow running              | [15]       |
| Met238Arg| C, DI            | Taiwan      | 2 y          | CMT2     | De novo     | delayed onset of walking, unsteady gait             | this study |
| Gly240Arg| C, DI            | North America | 23 y*       | CMT2/D/  | AD          | atrophy and weakness of the hand muscles            | [3, 13]   |
| Pro244Leu| C                | Japan       | 10 y         | CMT2     | NA          | slow running                                       | [16]       |
| Ile280Phe| C, DI            | UK          | 11–18 y      | dSMA-V   | AD          | distal limb muscle atrophy and weakness             | [17]       |
| Ile280Phe| Ins II           | USA         | 34 y         | CMT2     | AD          | progressive unsteadiness walking                    | [18]       |
| His418Arg| C                | UK/Australia | 26 y*       | dSMA-V   | AD          | atrophy and weakness of the hand muscles            | [13]       |
| Asp500Asn| Ins III          | Italy       | 10–35 y      | CMT2/D/  | AD          | atrophy and weakness of the hand muscles            | [19]       |
| Gly526Arg| C                | Sephardic Jewish | 13–26 y     | dSMA-V   | AD          | atrophy and weakness of the hand muscles            | [3]        |
| Gly526Arg| C                | France      | 23.3 y*      | dSMA-V   | AD          | atrophy and weakness of the hand muscles or distal four limbs | [20] |
| Gly598Ala| ACBD            | UK          | <6 m         | Infantile SMA | De novo | delayed milestones, severe generalized weakness | [17]   |
| Gly598Ala| ACBD            | USA         | <6 m         | Infantile SMA | De novo | delayed milestones, severe generalized weakness | [21]   |

a C: Catalytic domain; DI: dimer interface; W: WHEP domain; Ins I: Insertion I domain; Ins II: Insertion II domain; Ins III: Insertion III domain; ACBD: anticodon binding domain
b * average age of disease onset
c CMT: Charcot-Marie-Tooth disease; SMA: spinal muscular atrophy; dSMA: distal SMA;
d AD: autosomal dominant; NA: not available

doi:10.1371/journal.pone.0133423.t001
provided written consent for the use of their information and images as per the consent form for publication in a PLOS Journal.

**Mutation analyses**

Genomic DNA was extracted from peripheral blood using standard protocols. A high-throughput targeted sequencing panel covering all coding exons of GARS was developed to screen the 54 patients with CMT2. The NimbleGen Design (2.1) online software (http://www.nimblegen.com/products/nimbledesign/) was used to design a library of oligonucleotide probes to capture targeted regions. The NimbleGen SeqCap EZ Choice Library system (Roche NimbleGen, Madison, WI) was used to enrich for custom regions of interest. Following the manufacturer’s instructions, 1 μg of genomic DNA was used for sample library preparation and then hybridized to the capture probes. The final enriched samples were sequenced using the HiSeq2000 platform (Illumina, San Diego, CA). High-throughput sequencing was performed, and the raw image files were processed by Illumina base calling software 1.7 for base calling with default parameters. The sequences of each library were generated as 100-bp paired-end reads. The reference genome for sequence read alignment and variant calling was the Human Genome version 19 (hg19/GRCh37). The BaseSpace pipeline (https://basespace.illumina.com/) was used for variant calling. The Illumina VariantStudio data analysis software (http://variantstudio.software.illumina.com/) was used to annotate variants. All identified GARS mutations were validated by Sanger sequencing with intronic primers flanking the coding regions of GARS using the Big Dye 3.1 dideoxy terminator method (Applied Biosystems, Foster City, CA) on an ABI Prism 3700 Genetic Analyzer (Applied Biosystems). Amplicon sequences were compared with the reference GARS coding sequence (NM_002047.2) and the standard sequence of the short isoform of the human cytoplasmic GlyRS (AAA57001.1) was used to annotate amino-acid changes. Both reference sequences were retrieved from the NCBI Database (http://www.ncbi.nlm.nih.gov). The pathogenic properties of the validated novel variants were further assessed by segregation analysis in patients’ families. Then, the putative pathogenic variants were further discriminated by their absence in 1,000 control chromosomes derived from 500 neurologically healthy individuals of Han Chinese origin recruited at our hospital. The Exome Aggregation Consortium Sequencing Project (ExAC; http://exac.broadinstitute.org) and dbSNP (Build 144; https://www.ncbi.nlm.nih.gov/snp) databases were also queried for these variants. *In silico* prediction of the functional effects of the missense mutations were conducted using Combined Annotation Dependent Depletion (CADD) (http://cadd.gs.washington.edu) [25] and Mutation Taster (http://www.mutationtaster.org) [26]. Evolutionary conservation of the mutation sites was analyzed by aligning amino-acid sequences using the UniProt website (http://www.uniprot.org) [27].

**Results**

The average coverage and read depth of the target GARS sequence are 98% and 516.4X per targeted base, respectively. Mutational analyses of GARS in the 54 unrelated patients with CMT2 revealed three heterozygous missense variants, including p.Asp146Tyr (c.598G>T), p.Met238Arg (c.875T>G) (Fig 1A) and p.Asn208Asp (c.784A>G). The last variant was likely a benign polymorphism, as it was absent in two affected siblings of the proband. However, the first two variants, p.Asp146Tyr and p.Met238Arg, are *de novo* mutations (Fig 1B) and are absent in the 61,486 ethnically diverse individuals (122,972 chromosomes) in ExAC, which includes approximately 4,300 individuals from East Asian populations. These mutations are also absent from dbSNP and the 1,000 ethnically matched control chromosomes. Both variants are predicted to be disease-causing mutations by CADD and Mutation Taster programs. The
CADD v1.2 PHRED scores for both chr7:30642678G>T and chr7:30649340T>G are 33.0, which place GARS p.Asp146Tyr and p.Met238Arg in the top 0.05% most deleterious variants in the genome [25]. Mutation Taster predicted both variants to be disease-causing with strong probability values larger than 0.99; a probability value close to 1 indicates a high “security” of the prediction [26]. The 146th and 238th amino acid residues of the human cytoplasmic GlyRS protein, which is encoded by GARS, are also highly evolutionarily conserved (Fig 1C).

Each of the two de novo GARS mutations were identified in one single patient, and the paternities between the two index patients and their parents were confirmed by analyzing 14 microsatellite markers. Patient 1 is heterozygous for the GARS p.Asp146Tyr mutation. She was reported to have pneumonia with respiratory failure at 3 months of age. Axonal polyneuropathy was diagnosed at that time, and pneumonia with respiratory failure was cured with treatment. The patient had delayed motor milestones and was not able to sit by herself until 15

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Fig 1. **Two novel de novo GARS mutations.** (A) The novel GARS mutations identified in this study, p.Asp146Tyr and p.Met238Arg, with the sense strand electropherogram shown on the top and a limited reading frame depicting the corresponding amino acid substitutions shown below. (B) Pedigrees of the 2 patients harboring the novel GARS mutations. The arrows indicate the probands. “M” represents the GARS mutations and “W” means wild type. (C) The GARS p.Asp146Tyr and p.Met238Arg mutations reside in an evolutionarily conserved region, as shown by aligning the amino acid sequences of glycyl-tRNA synthetase protein (GlyRS) orthologs from various species.

doi:10.1371/journal.pone.0133423.g001
months of age. She could never stand, pinch, or move her toes. She learned to control an electrical wheelchair by using a joystick at age 7. Neurological examination at age 9 revealed pes planus, generalized areflexia and severe and symmetrical weakness and atrophy of the muscles in four limbs (Fig 2). The Medical Research Council (MRC) scale scores were grade 4 for muscles of the shoulder and pelvic girdles, grade 3 for flexors and extensors of the elbows and knees, grade 1–2 for the wrist muscles and finger flexors, and grade 0 for the intrinsic hand and feet muscles and muscles in the legs. All modalities of sensation were diminished in the regions distal to the knees and wrists. The NCS demonstrated a severe polyneuropathy with a failure to elicit any motor or sensory response in the limbs. Patient 2 is heterozygous for the GARS p. Met238Arg mutation. She had no delayed onset of ambulation but began to experience gait difficulty with frequent tripping at age 2. She developed feet drop and difficulty pinching at age 8.

Fig 2. Clinical features of the patient harboring the GARS p. Asp146Tyr mutation. (A) Severe weakness and atrophy of the muscles in the feet and legs, pes planus and (B, C) severe intrinsic hand muscle atrophy with claw hand deformity.

doi:10.1371/journal.pone.0133423.g002
and became almost wheelchair-bound at age 11. Neurological examination at age 11 revealed pes cavus, generalized areflexia, and severe weakness and atrophy of the intrinsic feet and hand muscles and the muscles in the legs. The MRC scores were grade 4 for flexors and extensors of the knees, grade 3 for the wrist flexor and extensor and finger flexor muscles, and grade 0 for the intrinsic hand and feet muscles and muscles in the legs. The strength of muscles of the pelvic and shoulder girdles and the elbow flexors or extensors was normal. All modalities of sensation were diminished in the regions distal to the ankles. The NCS demonstrated a severe axonal polyneuropathy with right ulnar nerve motor conduction velocity of 43.5 m/s and a compound motor action potential of 0.1 mV. Other motor or sensory responses in the NCS were absent.

Discussion

In this study, we screened a Taiwanese cohort of 54 unrelated patients with molecularly unassigned CMT2 for mutations in the GARS gene and identified two novel de novo missense mutations, p.Asp146Tyr and p.Met238Arg, in two patients with early onset CMT2. In addition to the nature of de novo mutation, the pathogenicity of both mutations is supported by the following facts. First, the mutations are absent in the 1,000 ethnically matched control chromosomes and two large genetic polymorphisms databases (i.e., ExAC and dbSNP). Second, the mutations are predicted to be pathogenic by two bioinformatics tools, CADD and Mutation Taster. Third, the p.Asp146Tyr and p.Met238Arg mutations lead to non-conservative substitutions of a negatively charged aspartic acid with a nonpolar tyrosine and a nonpolar methionine with a positively charged arginine at evolutionarily conserved sites in the human GlyRS protein.

The pathogenic effects of these two mutations appear to be related to their roles in the protein conformational dynamics and homodimeric protein–protein interactions of human GlyRSs. On the basis of the three-dimensional structure of the human GlyRS dimer, Asp146 is located within the insertion I domain and Met238 is situated in the catalytic domain and also at the dimer interface (Fig 3) [10]. Insertion I domain is involved in multiple conformational changes in GlyRS during catalysis, and an altered GlyRS dimer interface has been recognized as a common feature of previously identified CMT-associated GlyRS mutants [10, 11, 28].

Fig 3. The crystal structure of human glycyl-tRNA synthetase (GlyRS) (PDB ID: 2PME). (A) Mapping of the two GARS mutation sites (p.Asp146Tyr and p.Met238Arg) on one subunit of the human GlyRS dimer. (B) A view of the human GlyRS dimers demonstrating that Met238 is located on the interface between the two subunits. Catalytic, Insertion I and Insertion II domains are colored as indicated.

doi:10.1371/journal.pone.0133423.g003
Therefore, GARS p.Asp146Tyr and p.Met238Arg mutations might cause CMT by compromising the enzymatic function of human GlyRS and influencing its functional conformations or disrupting the dimer interface.

The majority of previously identified GARS mutations are linked to CMT2D or dSMA-V and are characterized by autosomal dominant inheritance, disease onset in adolescence or young adulthood and initial clinical manifestation of hand muscle weakness and atrophy (Table 1). Only three of the mutations are associated with early-onset disease. Among them, the GARS p.Leu218Gln mutation was found in a patient with CMT2 who had a slightly delayed onset of walking and who developed feet drop at age 7 [15]; p.Gly598Ala mutations were identified in the patients with severe infantile-onset generalized limb muscle weakness and atrophy and a lifetime inability to walk [17,21]. The two novel GARS mutations reported here, p.Asp146Tyr and p.Met238Arg, also cause early-onset CMT2 with presentations of severe infantile-onset generalized weakness and early childhood-onset disability, respectively. Our findings stress the importance of considering mutations in GARS as a disease cause for patients with early-onset neuropathy.

Interestingly, the GARS p.Asp146Tyr mutation occurs at the same residue as the Asp164Asn mutation, which was reported in a Korean dHMN-V family. Korean patients with the Asp164Asn mutation display typical adolescent-onset dHMN-V, whereas our patient with the Asp164Tyr mutation had severe infantile-onset CMT2. The discrepancy between the clinical severities associated with the two mutations at the same residue may result from differences in the biochemical natures of asparagine and tyrosine. Given the rarity of GARS mutations, the Asp146 may represent a mutational hot-spot in the GARS gene.

The frequency of GARS mutations in Taiwanese patients with CMT is low (0.59%; 2/340). In previous studies, Saporta et al. identified three patients with GARS mutations from among 787 American patients with CMT (0.4%; 3/787) [29]. Fridman et al. analyzed clinical and genetic data from patients in the Inherited Neuropathies Consortium and found that two out of 1652 CMT patients had GARS mutations (0.12%; 2/1652) [30]. After pooling these data, the frequency of GARS mutations in CMT patients is approximately 0.25% (7/2779), indicating that GARS mutations are an uncommon cause of CMT. This study provides a rare chance to expand our knowledge of the genetic and clinical spectrum of GARS-associated neuropathy.

In conclusion, GARS p.Asp146Tyr and Met238Arg are novel causes of early-onset axonal CMT. Mutations in GARS are uncommon as the cause of CMT in Taiwan. This study illustrates the clinical and genetic features of GARS mutations in Taiwan, expands the spectrum of GARS mutations and highlights the role of GARS mutations in early-onset neuropathy.

Acknowledgments

We would like to thank the patients who participated in this study. We thank the High-throughput Genome Analysis Core Facility of National Core Facility Program for Biotechnology of Taiwan (NSC-101-2319-B-010-001) for the service of nucleotide sequencing. We also thank Bioinformatics Consortium of Taiwan of the National Core Facility Program for Biotechnology, Taiwan (MOST103-2319-B-010-002), National Research Program for Biopharmaceuticals (NRPB, NSC 1022325-B-492-001) and National Center for High-performance Computing (NCHC) of National Applied Research Laboratories (NARLabs) of Taiwan for bioinformatics support.

Author Contributions

Conceived and designed the experiments: Y-C Liao YTL BWS Y-C Lee. Performed the experiments: YTL PCT. Analyzed the data: Y-C Liao YTL CCC YHH. Contributed reagents/materials/analysis tools: Y-C Liao YHH Y-C Lee. Wrote the paper: Y-C Liao PCT BWS Y-C Lee.
References

1. Rosser AM, Polke JM, Houlden H, Reilly MM. Clinical implications of genetic advances in Charcot-Marie-Tooth disease. Nat Rev Neurol. 2013; 9: 562–571. doi:10.1038/nrneurol.2013.179 PMID: 24018473

2. Timmerman V, Strickland AV, Zuchner S. Genetics of Charcot-Marie-Tooth (CMT) Disease within the frame of the human genome project success. Genes (Basel). 2014; 5: 13–32.

3. Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin S-Q, et al. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. Am J Hum Genet. 2003; 72: 1293–1299. PMID: 12990580

4. Jordanova A, Irobi J, Thomas FP, Van Dijck P, Meerschaert K, Dewil M, et al. Disrupted function and axonal distribution of mutant tyrosyl-tRNA synthetase in dominant intermediate Charcot-Marie-Tooth neuropathy. Am J Hum Genet. 2010; 86: 77–82. doi:10.1016/j.ajhg.2009.12.005 PMID: 20045102

5. Latour P, Thauvin-Robinet C, Baudelet-Méry C, Soichot P, Cusin V, Faivre L, et al. A major determinant for binding and aminoacylation of tRNA(Ala) in cytoplasmic Alanyl-tRNA synthetase is mutated in dominant axonal Charcot-Marie-Tooth disease. Am J Hum Genet. 2010; 86: 77–82. doi:10.1016/j.ajhg.2009.12.005 PMID: 20045102

6. McLaughlin HM, Sakaguchi R, Igarashi T, Pehlivan D, Chu K, et al. Compound heterozygosity for loss-of-function lysyl-tRNA synthetase mutations in a patient with peripheral neuropathy. Am J Hum Genet. 2010; 87: 560–566. doi:10.1016/j.ajhg.2010.09.008 PMID: 20920668

7. Vester A, Velez-Ruiz G, McLaughlin HM, NISC Comparative Sequencing Program, Lupski JR, Talbot K, et al. A loss-of-function variant in the human histidyl-tRNA synthetase (HARS) gene is neurotoxic in vivo. Hum Mutat. 2012; 34: 191–199. doi:10.1002/humu.22210 PMID: 22930593

8. Gonzalez M, McLaughlin H, Houlden H, Guo M, Liu YT, Hadjivassiliou M, et al. Exome sequencing identifies a significant variant in methionyl-tRNA synthetase (MARS) in a family with late-onset CMT2. J Neurol Neurosurg Psychiatry. 2013; 84: 1247–1249. doi:10.1136/jnnp-2013-305049 PMID: 23729695

9. Griffin LB, Sakaguchi R, McGuigan D, Gonzalez MA, Searby C, Züchner S, et al. Impaired function is a common feature of neuropathy-associated glycyyl-tRNA synthetase mutations. Hum Mutat. 2014; 35: 1363–1371. doi:10.1002/humu.22681 PMID: 25168514

10. Xie W, Nangle LA, Zhang W, Schimmel P, Yang XL. Long-range structural effects of Charcot-Marie-Tooth disease-causing mutation in human glycyl-tRNA synthetase. Proc Natl Acad Sci U S A. 2007; 104: 9976–9981. PMID: 17545306

11. Qin X, Hao Z, Tian Q, Zhang Z, Zhou C, Xie W. Co-crystal structure of glycyl-tRNA synthetase in complex with tRNA suggest multiple conformational states in glycylation. J Biol Chem. 2014; 289: 20359–20379. doi:10.1074/jbc.M114.557249 PMID: 24898252

12. Rohkamm B, Reilly MM, Lochmüller H, Schlotter-Weigel B, Barisic N, Schöls L, et al. Further evidence for genetic heterogeneity of distal HMN type V, CMT2 with predominant hand involvement and Silver syndrome. J Neurol Sci. 2007; 263: 100–106. PMID: 17663003

13. Sivakumar K, Kyriakides T, Puls I, Nicholson GA, Funalot B, Antonellis A, et al. Phenotypic spectrum of disorders associated with glycyl-tRNA synthetase mutations. Hum Mutat. 2005; 128: 2304–2314. PMID: 16014653

14. Lee HJ, Park J, Nakhro K, Park JM, Hur YM, Choi BO, et al. Two novel mutations of GARS in Korean families with distal hereditary motor neuropathy type V. J Peripher Nerv Syst. 2012; 17: 418–421. doi:10.1111/j.1529-8027.2012.00442.x PMID: 23279345

15. Kawakami N, Komatsu K, Yamashita H, Uemura K, Oka N, Takashima H, et al. A novel mutation in glycyl-tRNA synthetase caused Charcot-Marie-Tooth disease type 2D with facial and respiratory muscle involvement. Rinsho Shinkeigaku. 2014; 54: 911–915. PMID: 25420567

16. Hamaguchi A, Ishida C, Iwasa K, Abe A, Yamada M, Charcot-Marie-Tooth disease type 2D with a novel glycyl-tRNA synthetase gene (GARS) mutation. J Neurol. 2010; 257: 1202–1204. doi:10.1007/s00415-010-5491-x PMID: 20169446

17. James PA, Cader MZ, Muntoni F, Childs AM, Crow YJ, Talbot K, et al. Severe childhood SMA and axonal CMT due to anticodon binding domain mutations in the GARS gene. Neurology. 2006; 67: 1710–1712. PMID: 17101916

18. Klein CJ, Mittal S, Xuan D, Wu Y, Litchy WJ, Gu W, et al. Application of whole exome sequencing in undiagnosed inherited neuromuscular disorders. J Neurol Neurosurg Psychiatry. 2014; 85: 1265–1272. doi:10.1136/jnnp-2013-306740 PMID: 24604904

19. Del Bo R, Locatelli F, Corti S, Scarlato M, Ghezzi S, Prelle A, et al. Coexistence of CMT-2D and distal SMA-V phenotypes in an Italian family with a GARS gene mutation. Neurology. 2006; 66: 752–754. PMID: 16534118
20. Dubourg O, Azzedine H, Yaou RB, Pouget J, Barois A, Meininger V, et al. The G526R glycyl-tRNA synthetase gene mutation in distal hereditary motor neuropathy type V. Neurology. 2006; 66: 1721–1726. PMID: 16769947

21. Eskuri JM, Stanley CM, Moore SA, Mathews KD. Infantile onset CMT2/dSMA V in monozygotic twins due to a mutation in the anticodon-binding domain of GARS. J Peripher Nerv Syst. 2012; 17: 132–134. doi: 10.1111/j.1529-8027.2012.00370.x PMID: 22462675

22. Lin KP, Soong BW, Yang CC, Huang LW, Chang MH, Lee IH, et al. The mutational spectrum in a cohort of Charcot-Marie-Tooth disease type 2 among the Han Chinese in Taiwan. PLoS One. 2011; 6(12): e29393. doi: 10.1371/journal.pone.0029393 PMID: 22206013

23. Tsai PC, Huang YH, Guo YC, Wu HT, Lin KP, Tsai YS, et al. A novel TFG mutation causes Charcot-Marie-Tooth disease type 2 and impairs TFG function. Neurology. 2014; 83: 903–912. doi: 10.1212/WNL.0000000000000758 PMID: 25098539

24. De Jonghe P, Timmerman V, Van Broeckhoven C. 2nd Workshop of the European CMT Consortium: 53rd ENMC International Workshop on Classification and Diagnostic Guidelines for Charcot-Marie-Tooth Type 2 (CMT2-HMSN II) and Distal Hereditary Motor Neuropathy (distal HMN Spinal CMT) 26–28 September 1997, Naarden, The Netherlands. Neuromuscul Disord. 1998; 8: 426–431. PMID: 9713862

25. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014; 46: 310–315. doi: 10.1038/ng.2892 PMID: 24487276

26. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014; 11: 361–362. doi: 10.1038/nmeth.2890 PMID: 24681721

27. The Uniprot Consortium. Activities at the Universal Protein Resource (UniProt). Nucleic Acids Res. 2014; 42: D191–198. doi: 10.1093/nar/gkt1140 PMID: 24253303

28. Nangle LA, Zhang W, Xie W, Yang XL, Schimmel P. Charcot-Marie-Tooth disease-associated mutant tRNA synthetases linked to altered dimer interface and neurite distribution defect. Proc Natl Acad Sci U S A. 2007; 104: 11239–11244. PMID: 17595294

29. Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME. Charcot-Marie-Tooth disease subtypes and genetic testing strategies. Ann Neurol. 2011; 69: 22–33. doi: 10.1002/ana.22166 PMID: 21280073

30. Fridman V, Bundy B, Reilly MM, Pareyson D, Bacon C, Burns J, et al. CMT subtypes and disease burden in patients enrolled in the Inherited Neuropathies Consortium natural history study: a cross-sectional analysis. J Neurol Neurosurg Psychiatry. 2014 Nov 27. doi: 10.1136/jnnp-2014-308826. [Epub ahead of print].