A missense, loss-of-function *YARS1* variant in a patient with proximal-predominant motor neuropathy

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Abstract Aminoacyl-tRNA synthetases (ARSs) are essential enzymes with a critical role in protein synthesis: charging tRNA molecules with cognate amino acids. Heterozygosity for variants in five genes (*AARS1*, *GARS1*, *HARS1*, *WARS1*, and *YARS1*) encoding cytoplasmic, dimeric ARSs have been associated with autosomal dominant neurological phenotypes, including axonal Charcot–Marie–Tooth disease (CMT). Missense variants in the catalytic domain of *YARS1* were previously linked to dominant intermediate CMT type C (DI-CMTC). Here, we report a patient with a missense variant of unknown significance predicted to modify residue 308 in the anticodon binding domain of *YARS1* (p.Asp308Tyr). Interestingly, p.Asp308Tyr is associated with proximal-predominant motor neuropathy, which has not been reported in patients with pathogenic *YARS1* variants. We demonstrate that this allele causes a loss-of-function effect in yeast complementation assays when modeled in *YARS1* and the yeast ortholog *TYS1*; structural modeling of this variant further supports a loss-of-function effect. Taken together, this study raises the possibility that certain *YARS1* variants cause proximal-prominent motor neuropathy and indicates that patients with this phenotype should be screened for genetic lesions in *YARS1*.

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[Supplemental material is available for this article.]

CASE PRESENTATION

Aminoacyl-tRNA synthetases (ARSs) are a group of ubiquitously expressed, essential enzymes that are responsible for charging transfer RNAs (tRNAs) with cognate amino acids in the cytoplasm and mitochondria, a process critical for establishing fidelity in protein synthesis. Variants in the 37 ARS genes (all of which are encoded in the nucleus) have previously been associated with a spectrum of genetic disorders (Meyer-Schuman and Antonellis 2017; Kuo and Antonellis 2020). Tyrosyl-tRNA synthetase (encoded by *YARS1*) is responsible for charging tyrosyl-tRNA with tyrosine in the cytoplasm via a two-step aminoacylation reaction. Importantly, two YARS1 subunits must form an asymmetric homodimeric complex with two tyrosyl tRNAs for the aminoacylation reaction to occur (Ward and Fersht 1988). Previously, homozygosity or compound heterozygosity for loss-of-function *YARS1* variants

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has been associated with severe, early-onset, multisystem disease, with phenotypes including liver dysfunction, developmental delay, brain anomalies, and sensorineural hearing loss (Nowaczyk et al. 2017; Williams et al. 2019; Averdunk et al. 2021; Estève et al. 2021). Furthermore, heterozygosity for missense variants or a small, in-frame deletion in *YARS1* have been associated with dominant intermediate Charcot–Marie–Tooth neuropathy type C (DI-CMTC; MIM #608323), which is characterized by progressive weakness and sensory loss in the distal upper and lower extremities, with intermediate motor nerve conduction velocities between 25 and 45 m/sec (Jordanova et al. 2003, 2006; Hyun et al. 2013).

Here, we present a 47-yr-old female proband of European ancestry. She first noted symptoms at 18 yr of age when she developed tremors, weakness, and cramping of her hands. At 22, neuromuscular examination revealed asymmetric weakness in the distal upper extremities and proximal lower extremities. Reflexes were absent at the triceps, but were otherwise normal. Serial examinations have demonstrated slow progression of weakness. At 47, her exam showed asymmetric upper extremity weakness affecting both proximal and distal muscles and proximal > distal lower extremity weakness (Fig. 1A–C). Hip flexion and extension measured the weakest at 2/5 bilaterally on manual muscle testing. Atrophy was prominent in her forearms and hands (Fig. 1A). Deep tendon reflexes were absent except at the ankles, where hyporeflexia was noted. Sensory exam was normal to touch, vibration, position, and pinprick. Additional findings included rotary nystagmus (present since 18 yr of age), kyphoscoliosis, and tongue fasciculations. Creatine kinase was mildly elevated (947–1692 units/L) at symptom onset, but was reduced to 300 units/L at last measurement (47 yr of age). Electrodiagnostic studies performed at 23 yr of age demonstrated signs of widespread denervation in the upper and lower extremities and increased amplitude of motor unit potentials, suggesting abnormalities in motor neurons of the spinal cord. The motor and sensory nerve conduction velocities were normal. A left deltoid biopsy obtained at 21 yr of age showed grouped angular fibers, consistent with a neurogenic process. Clinical findings are summarized in Table 1.

Based on the observations above, the patient was thought to have symptoms consistent with spinal muscular atrophy type III; however, genetic testing of *SMN1* was normal (further genetic testing information provided below). Family history was negative for similar disease, and the patient's mother had no symptoms of neuropathy; however, the patient's father developed a sensory peripheral neuropathy in adulthood without symptoms of weakness. He did not undergo electrodiagnostic testing. The patient's two daughters (ages 20 and 22 yr) were examined by the proband's neuromuscular physician and had normal strength and reflexes.

TECHNICAL ANALYSIS

Based on clinical presentation, genetic testing was performed using the Invitae Comprehensive Neuropathies Gene Panel (Invitae), which evaluates 111 genes associated with hereditary neuropathies for (1) single-nucleotide variants and deletions/insertions <15 nt in length by targeted next-generation sequencing of coding exons plus 10–20 bp of flanking adjacent intronic sequence, and (2) duplications and deletions at single-exon resolution (Invitae 03200, https://www.invitae.com/en/providers/test-catalog/test-03200). This analysis identified a missense variant in *YARS1* (NM_003680.4:c.922G > T [p.Asp308Tyr]) (see Table 2). Importantly, familial testing demonstrated that this variant was absent in the patient's mother and two daughters, who are not affected with the neuropathy phenotype present in the patient (Fig. 1D). The patient's father was not available for genetic testing. Given these findings, it is possible that the variant observed in the proband may have arisen de novo, but further haplotype analysis would be required to rule out paternal inheritance.
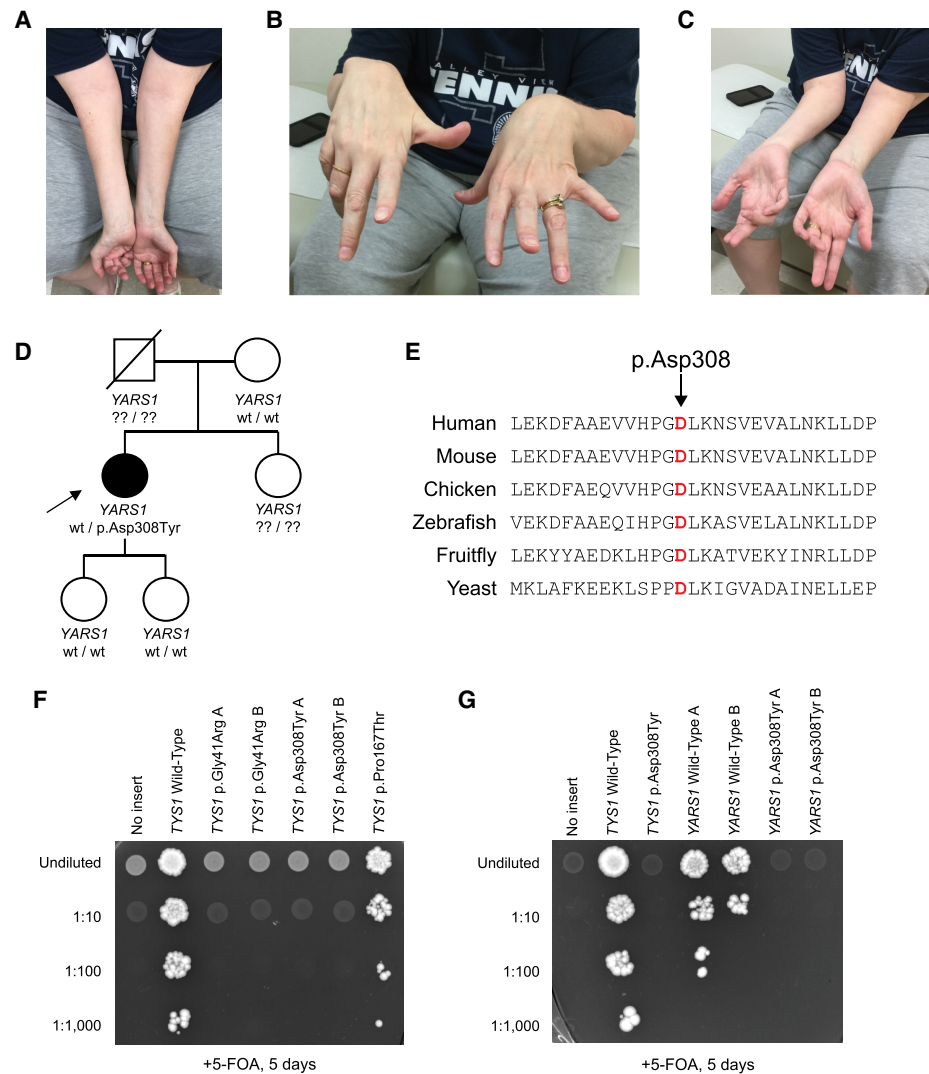
Figure 1. A loss-of-function *YARS1* variant in a patient with proximal-prominent neuropathy. (*A–C*) Clinical findings noted on the patient's physical exam, including atrophy of the distal upper extremities and hand muscles (*A*) and finger extensor weakness demonstrated by finger drop (*B,C*). (*D*) Pedigree of the proband's (arrow) family. Genotypes at p.Asp308 *YARS1* are indicated below each individual (??/?? indicates an unknown genotype). Filled objects indicate that the individual is affected, whereas unfilled objects indicate that the individual is unaffected. A slash through an individual indicates that they are deceased. (*E*) YARS1 protein sequence alignment, including six evolutionarily diverse species centered around the affected p.Asp308 residue (indicated in red). (*F*) Haploid yeast lacking endogenous *TYS1* were transformed with a *LEU2*-bearing pRS315 vector to express wild type, p.Gly41Arg, p.Asp308Tyr, or p.Pro167Thr *TYS1*, or with a vector containing no *TYS1* insert ("No Insert"). Resultant yeast cultures were plated undiluted or diluted (1:10, 1:100, or 1:1000) on solid media without leucine or uracil (−leu −ura) (see Supplemental Fig. 1A) or on solid media containing 5-FOA and grown at 30°C. Replicates A and B indicate two separate colonies from a single transformation. Image shown is representative of two independent transformations using two different plasmid DNA preparations (p.Asp308Tyr *TYS1* total *n* = 8). (*G*) Haploid yeast lacking endogenous *TYS1* were transformed with a *LEU2*-bearing pAG425-GPD vector to express wild-type *TYS1*, p.Asp308Tyr *TYS1*, wild-type *YARS1*, or p.Asp308Tyr *YARS1*, or with a vector containing no *TYS1* insert ("No Insert"). Resultant yeast cultures were plated undiluted or diluted (1:10, 1:100, or 1:1000) on solid media without leucine or uracil (−leu −ura) (see Supplemental Fig. 1B) or on solid media containing 5-FOA and grown at 30°C. Replicates A and B indicate colonies from transformations using two different plasmid DNA preparations. Image shown is representative of two independent transformations using two different plasmid DNA preparations (p.Asp308Tyr *YARS1* total *n* = 6).

Table 1. Summary of clinical findings in the proband described in this study compared with other patients with *YARS1*-associated dominant intermediate Charcot–Marie–Tooth neuropathy type C (DI-CMTC)

	c.922G>T (p.Asp308Tyr) current study	c. 121G>A (p.Gly41Arg) (Jordanova et al. 2003; Thomas et al. 2016)	c. 586G>A (p.Glu196Lys) (Jordanova et al. 2003; Thomas et al. 2016)	c.586G>C p.Glu196Gln (Gonzaga-Jauregui et al. 2015)	c. 458-469del (p.153-156del) (Jordanova et al. 2006)	c.241_242delGAinsAT (p.Asp81Ile) (Nam et al. 2022; Hyun et al. 2013)	c.497A>G (p.Tyr166Cys) (Nam et al. 2022)
Age of onset	18 yr	5–20 yr	7–59 yr	16 yr	NR	23 yr	17–27 yr
Country	United States	United States	Bulgaria	NR	Belgium	Korea	Korea
Inheritance	Unknown (possible de novo)	Autosomal dominant	Autosomal dominant	Autosomal dominant	De novo	Unknown	Autosomal dominant (2/3); unknown (presumed de novo) (1/3)
Presenting symptom	Distal upper extremity weakness	Ankle dorsiflexion weakness	Ankle dorsiflexion weakness	NR	NR	Distal muscle weakness	Distal muscle weakness and atrophy
Pes cavus (HP:0001761)/ hammertoe (HP:0001765)[a]	No/no	Yes (13/15)/ Yes (3/15)	Yes (22/27)/ Yes (3/27)	Yes/yes	NR	Yes	Yes (3/3)/NR
Upper limb distal weakness (HP:0008959)[a]	Yes	Yes (2/21)	Yes (14/27)	Yes	NR	Yes	Yes (3/3)
Upper limb proximal weakness (HP:0008997)[a]	Yes	Yes (a few, mild)	No	NR	NR	NR	NR
Lower limb distal weakness (HP:0009053)[a]	Yes	Yes (9/15)	Yes (16/27)	Yes	NR	Yes	Yes (3/3)
Lower limb proximal weakness (HP:0008994)[a]	Yes	Yes (2/15)	No	NR	NR	Yes	NR
Muscle atrophy (HP:0008944; HP:0007149)[a]	Yes (distal UE)	Yes (15/15 LE, 11/15 UE)	Yes (27/27 LE, 14/27 UE)	Yes	NR	Yes	Yes
Distal sensory impairment (HP:0002936)[a]	No	Yes (21/21)	Yes (20/27)	No	NR	Yes	Yes
		Yes (15/15)	Yes (26/26)	Yes	NR	No	Yes (1/3)

(Continued on next page.)

Table 1. (Continued)

	c.922G > T (p.Asp308Tyr) current study	c. 121G>A (p.Gly41Arg) (Jordanova et al. 2003; Thomas et al. 2016)	c. 586G>A (p.Glu196Lys) (Jordanova et al. 2003; Thomas et al. 2016)	c.586G > C p.Glu196Gln (Gonzaga-Jauregui et al. 2015)	c. 458-469del (p.153-156del) (Jordanova et al. 2006)	c.241_242delGAinsAT (p.Asp81Ile) (Nam et al. 2022; Hyun et al. 2013)	c.497A > G (p.Tyr166Cys) (Nam et al. 2022)
Areflexia/hyporeflexia (HP:0001284)[a]	Yes (at most recent exam)						
Hyperreflexia (HP:0001347)[a]	Yes (knees/ankles, at disease onset)	NR	NR	NR	NR	No	Yes (3/3; knees)
Hand tremor (HP:0002378)[a]	Yes	Yes (13/15)	No	NR	NR	NR	No
Other symptoms	Rotary nystagmus (16 y; HP:0001583), tongue fasciculations (HP:0001308), kyphoscoliosis (HP:0002751)[a]	—	Kyphoscoliosis (HP:0002751)[a] (2/27)	NR	—	—	—
Median motor NCVs (m/sec)	65.2	29.5–45.6 (25/25 abnormal)	24.7–57.8 (15/18 abnormal)	NR	NR	29.1 (left), 45.1 (right) (abnormal)	37.4–41.5 (3/3 abnormal)

(NR) Not reported, (UE) upper extremities, (LE) lower extremities, (NCV) nerve conduction velocity.
[a]Human Phenotype Ontology (HP) terms for peripheral neuropathy phenotypes.

Table 2. Summary of the identified *YARS1* variant

Gene	Genomic location	HGVS[a] cDNA	HGVS[a] protein	Zygosity	Parent of origin	Variant interpretation
YARS1	Chr 1:32,782,524 (GRCh38); Chr 1:33,248,125 (GRCh37)	NM_003680.4: c.922G > T	NP_003671.1: p.Asp308Tyr	Heterozygous	Paternal or de novo	Uncertain significance, with an effect on gene function

[a]Human Genome Variation Society accepted nomenclature.

VARIANT INTERPRETATION

The p.Asp308 YARS1 Residue Is Located in a Highly Conserved Structure Involved in tRNA-Tyr Anticodon Recognition

The p.Asp308 YARS1 residue is located within a stretch of conserved amino acid residues in the anticodon binding domain that show high sequence similarity among a range of species (Fig. 1E); importantly, this amino acid residue is conserved between yeast and humans. Consistent with this high degree of conservation, PolyPhen-2 analysis of sequence, phylogenetic, and structural data (Adzhubei et al. 2010) predicts that the p.Asp308Tyr allele is "probably damaging." Previously determined crystal structures for tyrosyl-tRNA synthetases from multiple species indicate that the p.Asp308 residue is located at the beginning of a highly conserved α-helical structure that is present in archaea and bacteria tyrosyl-tRNA ligases as well as tyrosyl-tRNA synthetase homologs in yeast and higher eukaryotes (Qiu et al. 2001; Yaremchuk et al. 2002; Tsunoda et al. 2007). Although the structure of human YARS1 complexed with tRNA-Tyr has not been solved, the yeast p.Asp321 residue (equivalent to p.Asp308) (see Supplemental Table 1) forms direct hydrogen bonds with the guanine-34 residue of the tRNA-Tyr-GTA anticodon triplet for substrate discrimination (Tsunoda et al. 2007). Given the high degree of structural similarity between different tyrosyl-tRNA synthetase homologs (Tsunoda et al. 2007), it is possible that this residue is also important for tRNA-Tyr substrate discrimination in higher eukaryotes, including humans.

The p.Asp308Tyr *YARS1* Allele Is Present as a Singleton in Population Sequence Databases

To assess the frequency of the p.Asp308Tyr *YARS1* allele in the general population, we queried the 1000 Genomes and gnomAD databases at the appropriate chromosomal position (see Table 2). At the time of initial variant evaluation, the p.Asp308Tyr allele was not present in the 1000 Genomes database (Auton et al. 2015) or in the gnomAD database (Karczewski et al. 2020). However, during the preparation of this manuscript, a single observation of the p.Asp308Tyr allele in the heterozygous state was reported in the gnomAD database (1-33248125-C-A, GRCh37; allele frequency 1/251,452; 0.000003977). Importantly, this allele frequency is consistent with other pathogenic ARS variants associated with dominant neuropathy (e.g., p.Gly294Arg *GARS1*, 1-33248125-C-A, GRCh37; allele frequency 1/249,268; 0.000004012) (Antonellis et al. 2003). Furthermore, the individual reported in gnomAD is in the <30-yr age category; thus, it is possible that this individual may develop neuropathy symptoms at a later age.

The p.Asp308Tyr *YARS1* Allele Does Not Support Viability in Yeast Complementation Assays

Previously reported neuropathy-associated ARS alleles demonstrated loss-of-function effects in biochemical, yeast, and mouse models; however, not all loss-of-function ARS alleles cause

dominant neuropathy. Yeast is particularly useful as an in vivo model to assess ARS variant effects on gene function, and previous studies demonstrated successful modeling of human *YARS1* variants using the yeast ortholog *TYS1* via yeast complementation assays (Jordanova et al. 2006; Gonzaga-Jauregui et al. 2015; Williams et al. 2019). We therefore used this model to determine the ability of wild-type and p.Asp308Tyr *TYS1* alleles to complement *TYS1* deletion in a previously validated haploid yeast strain; viability of this strain is maintained via a pRS316 vector containing wild-type *TYS1* and *URA3* (Antonellis et al. 2006; Oprescu et al. 2017; Williams et al. 2019). To allow comparison with previously identified pathogenic alleles, we included p.Gly41Arg *TYS1* (a loss-of-function allele identified in a patient with dominant neuropathy [Jordanova et al. 2006]) and p.Pro167Thr *TYS1* (a hypomorphic allele identified in a patient with a recessive syndromic phenotype [Williams et al. 2019]) in these analyses. Yeast cells were transformed with wild-type, p.Asp308Tyr, p.Gly41Arg, or p.Pro167Thr *TYS1* expression constructs or with a pRS315 construct with no *TYS1* insert. Resultant yeast strains were grown on media without leucine or uracil as a positive growth control (Supplemental Fig. 1A) and on media containing 5-FOA, which selects for cells that have spontaneously lost the *URA3*-bearing maintenance vector (Fig. 1F; Boeke et al. 1987). Importantly, the pRS315 vector with no insert was not sufficient to support yeast growth on 5-FOA media, confirming that *TYS1* is essential for yeast survival. Yeast cells transformed with wild-type *TYS1* showed appreciable growth at 30°C. The previously characterized p.Gly41Arg and p.Pro167Thr *TYS1* alleles show the expected loss-of-function effects: (1) The hypomorphic p.Pro167Thr allele results in reduced, but not ablated, yeast growth, and (2) the loss-of-function p.Gly41Arg allele is unable to support any yeast growth. Finally, the p.Asp308Tyr *TYS1* variant reported in this study did not support any yeast growth, indicating that p.Asp308Tyr *YARS1* is a loss-of-function allele in this assay, similar to previously reported pathogenic, neuropathy-associated ARS alleles. To further characterize the effects of the p.Asp308Tyr variant in the context of human *YARS1*, we repeated this assay using the same haploid yeast strain with *TYS1* deleted and transformed it with wild-type *YARS1* or p.Asp308Tyr *YARS1* cDNA expression constructs (Fig. 1G; Supplemental Fig. 1B). Yeast cells transformed with wild-type human *YARS1* demonstrated growth on 5-FOA, supporting conservation of function between yeast *TYS1* and human *YARS1*. Furthermore, the human p.Asp308Tyr *YARS1* allele was not able to support yeast growth in this assay, consistent with a loss-of-function effect. Importantly, yeast transformed with wild-type *YARS1* or p.Asp308Tyr *YARS1* showed stable expression of YARS1 (Supplemental Fig. 1C), suggesting that the loss-of-function effect is not due to protein instability.

Taken together with our findings regarding YARS1 structure and conservation as well as *YARS1* population data, we conclude that the p.Asp308Tyr *YARS1* allele is a variant of unknown significance according to current variant interpretation guidelines using evidence criteria PS3 and PP3 (Richards et al. 2015) However, the data presented in this study provide significant supporting evidence for pathogenicity, particularly with respect to the importance of the affected amino acid residue in tRNA binding and the observed loss-of-function effect in in vivo functional studies, which is similar to other neuropathy-associated ARS alleles. Although it is possible that this variant arose de novo in the proband, lack of availability of a paternal sample for genetic testing made it impossible to clarify the inheritance pattern. Further genetic evidence (e.g., reports of additional patients with variants at this residue) may allow for reclassification in the future.

DISCUSSION

In this study, we identified a previously unreported *YARS1* variant that affects an amino acid residue in the anticodon binding domain. This variant was observed in a patient with a neuropathy phenotype that is clinically distinct from previously described *YARS1*-related

dominant intermediate CMT (Table 1). If this variant is confirmed as pathogenic, this report will have expanded the allelic and phenotypic spectrum of *YARS1*-related dominant disease. Notably, a similar range of dominant neuropathy phenotypes have been described in association with loss-of-function missense variants and small, in-frame deletions in four other cytoplasmic, homodimeric ARSs (*AARS1*, *GARS1*, *HARS1*, and *WARS1*) (Antonellis et al. 2003; Latour et al. 2010; McLaughlin et al. 2012; Vester et al. 2013; Safka Brozkova et al. 2015; Meyer-Schuman and Antonellis 2017; Tsai et al. 2017).

As noted, the patient reported here has several phenotypic features not previously seen in patients with *YARS1*-associated DI-CMTC (Table 1). This case is unique in that the patient presented with upper extremity weakness, whereas all prior patients presented with lower extremity involvement prior to developing upper extremity symptoms; however, such upper limb phenotypes have been reported in patients with pathogenic *GARS1* variants (Antonellis et al. 2003). The pattern of muscle weakness also differs from that seen in other patients with *YARS1* variants. The patient presented here has predominant proximal lower extremity weakness with minimal distal weakness of these extremities. Although some patients with DI-CMTC have been reported to have proximal lower extremity weakness, it was reported as less severe than the distal weakness (Thomas et al. 2016). The patient reported here also lacks foot deformities and sensory abnormalities seen in most patients (Thomas et al. 2016; Nam et al. 2022). In addition, median motor nerve conduction velocities (NCVs) were normal on three separate electrodiagnostic studies in the third decade of life, whereas most previously reported adult DI-CMTC patients presented with reduced NCVs. The patient reported here was also identified to have persistent elevation of creatine kinase (CK) early in her disease course, which has not been reported in association with DI-CMTC and is rarely associated with CMT disease (Rudnik-Schöneborn et al. 2016).

To our knowledge, the p.Asp308Tyr variant described in this study is the first potentially pathogenic *YARS1* variant identified in the anticodon binding domain; in contrast, all previously identified *YARS1* variants map to the catalytic domain (Supplemental Fig. 1D). Importantly, variants in the anticodon binding domain of other ARSs have been associated with a more severe, atypical dominant neuropathy, including an infantile-onset spinal muscular atrophy caused by *GARS1* variants (James et al. 2006; Eskuri et al. 2012; Markovitz et al. 2020). It will be interesting to test whether, and by what mechanism, mutations in the tRNA binding domain lead to differential clinical presentations.

In addition to aminoacylation activity, YARS1 has noncanonical functions that may be relevant for *YARS1*-related disease. First, a *Drosophila* model of *YARS1*-related CMT showed that YARS1 localizes to the nucleus and complexes with the scaffolding protein TRIM28 and histone deacetylase HDAC1 to enact a transcriptional regulation program, which is altered in the context of neurons expressing CMT-associated *YARS1* variants (Bervoets et al. 2019). Second, noncanonical cytokine activity by secreted YARS1 cleavage products plays a role in angiogenesis and platelet biogenesis (Wakasugi et al. 2002; Yang et al. 2002; Greenberg et al. 2008). Finally, noncanonical cytoplasmic mRNA binding has been reported for many cytoplasmic ARSs in yeast, often with downstream consequences on post-transcriptional gene regulation (Levi and Arava 2019; Levi et al. 2020; Garin et al. 2021). Therefore, it is possible that dysfunction in noncanonical activities—in concert with any effects on tRNA charging—could explain the clinical heterogeneity observed in *YARS1*-related neuropathy. Further studies will be needed to fully understand the molecular mechanisms underlying the range of *YARS1*-related phenotypes.

ADDITIONAL INFORMATION

Database Deposition and Access

The p.Asp308Tyr *YARS1* variant can be found in ClinVar (https://www.ncbii.nlm.nih.gov/clinvar/) under accession number VCV001681503.3.

Ethics Statement

Acknowledgments

Author Contributions

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