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A recurrent GARS mutation causes distal hereditary motor neuropathy
A recurrent *GARS* mutation causes distal hereditary motor neuropathy

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Abstract

We found a p.Gly327Arg mutation in *GARS* in two unrelated women, both of whom had a similar phenotype – motor weakness that began in late childhood, distal weakness in the arms and legs, a motor greater than sensory neuropathy with slowing of motor and not sensory conduction velocities. A *de novo* mutation was proven in one patient and suspected in the other. The p.Gly327Arg *GARS* variant did not support yeast growth in a complementation assay, showing that this variant severely impairs protein function. Thus, the p.Gly327Arg *GARS* mutation causes a distal motor neuropathy.

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Introduction

Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed proteins that encode the enzymes that ligate tRNAs to cognate amino acids (*Antonellis and Green, 2008*). ARSs are essential genes for protein synthesis in the cytosol and in mitochondria. Of the 37 ARS genes, 17 encode a cytoplasmic enzyme, 17 encode a mitochondrial enzyme, and 3 encode an enzyme that is both cytoplasmic and mitochondrial. Recessive mutations in ARS loci, both in humans and in model organisms, are associated with complex phenotypes affecting multiple organ systems (*Meyer-Schuman and Antonellis, 2017*), including recessive *GARS* mutations (*McMillan, et al., 2014; Oprescu, et al., 2017a; Taylor, et al., 2014*). Dominant mutations of five different ARS genes – *AARS*, *GARS*, *HARS, YARS*, and *WARS* – are associated with axonal neuropathies, labeled dominant intermediate Charcot-Marie-Tooth disease (CMT), CMT2, or distal hereditary motor neuropathy (dHMN), depending on the nerve conduction velocity and the presence or absence of sensory involvement (*Rossor, et al., 2012*). Approximately 14 different dominant *GARS* mutations have been described, typically causing a motor greater than sensory axonal neuropathy with onset in the teens (http://hihg.med.miami.edu/code/http/cmt/public_html/index.html#/). Here we report two young women with a similar clinical presentation, both of whom are likely to have a *de novo* p.Gly327Arg *GARS* mutation that causes loss of function in a biological assay.

Methods

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Patients 1 and 2 were examined by a neurologist at the University of Iowa (MES) and the University of Pennsylvania (SSS), respectively (Table 1), where nerve conduction studies were performed with standard techniques. A gene panel (patient 1) or whole exome sequencing (patient 2) revealed the same p.Gly327Arg variant in both patients. Using the cloud based variant analytic platform, GENESIS, the two groups were able to identify each other's patients.

Yeast complementation assays were performed as previously described using the human *GARS* open-reading frame (*Oprescu, et al., 2017b*). The p.Gly327Arg *GARS* variant was introduced into the ΔMTSΔWHEP *GARS* open-reading frame using site-directed mutagenesis (primers available upon request). The open-reading frame was fully sequenced to rule out cycle-induced errors and recombined into the pYY1 expression construct (adapted from (*Chien, et al., 2014*) using Gateway cloning technology (Invitrogen). The resulting *GARS* expression construct was transformed into a haploid yeast strain in which the endogenous yeast ortholog (*GRS1*) was deleted; cell viability was maintained with an exogenous copy of *GRS1* on pRS316, which is a *URA3*-bearing plasmid. Transformants were selected on media lacking uracil and leucine to confirm expression of both the *URA3* marker and the *LEU2* marker on the pYY1 plasmid. Individual colonies were then grown for two days at 37°C in liquid culture until saturation. One mL of the resulting culture was centrifuged at 15,000 rpm for 1 minute, re-suspended in 50 μl of Ultrapure water (Invitrogen), and a 10 μl aliquot of each culture was spotted (undiluted or diluted 1:10 in H_2O) on plates containing 0.1% 5-FOA (Teknova), which selects for cells that

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have spontaneously lost the *URA3*-bearing maintenance vector (*Boeke, et al., 1984*). Yeast growth was visually assessed after 5 days. Two independent transformations were performed, using four independently generated expression clones (A-D), and two independent colonies were selected from each transformation (Set 1 and Set 2).

Results

Patient 1 is an 18-year-old woman who was first seen at age 12 for difficulty walking and suspected CMT. At age 10, she had problems running, and by age 14, she was unable to run and her ankles "rolled out" when walking. Table 1 summarizes her clinical exam, and Figure 1 shows her pedigree. She had normal proximal strength, but was symmetrically weak (3/5 MRC score) in her median- and ulnar-innervated intrinsic hand muscles, 2-3/5 in ankle dorsi-flexion, and was 5/5 in ankle plantar flexion. Clinical electrophysiology showed a motor greater than sensory neuropathy, with slowing of motor and not sensory conduction velocities (Table 2). A gene panel revealed the c.979G>A/p.Gly327Arg variant in *GARS*, and segregation analysis showed that the variant was a *de novo* mutation.

Patient 2 is a 22-year-old woman who first presented with weakness in her distal legs and arms at age 13. Table 1 summarizes her clinical exam, and Figure 1 shows her pedigree. She had normal proximal strength, but was weak (2-3/5 MRC score) in her median and ulnar-innervated intrinsic hand muscles, 3/5 in ankle dorsi-flexion, and 4-/5 in ankle plantar flexion. Clinical

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electrophysiology showed a purely motor neuropathy with slowing of motor and not sensory conduction velocities (Table 2), and EMG showed severe, chronic denervation in distal arm muscles. Whole exome sequencing revealed a c.979G>A/p.Gly327Arg variant in *GARS*, and segregation analysis showed that her unaffected mother and sister did not have the variant. Her father was not available for analysis, but was not known to have neuropathy.

Thus, the p.Gly327Arg variant arose independently in these two patients who have a phenotype that is similar to other patients who have dominant *GARS* mutations. Further, like most dominant *GARS* mutations that are associated with neuropathy (p.Glu125Gly, p.Pro152Leu, p.Leu183Pro, p.Asp200Asn, p.Asp215His, p.Ser265Phe, p.Leu272Gln, p.Pro298Leu, p.Glu333Gly, p.Ile334Phe, p.His472Arg, p.Gly580Arg, p.Gly652Ala), the p.Gly327Arg variant is not present in gnomAD (p.Gly294Arg has an allele count on 1). Because the p.Gly327Arg mutation affects a Gly residue that is conserved from yeast to mammals (Supplemental Figure 1), we assessed the functional consequences of p.Gly327Arg mutation in a yeast complementation assay that has been previously used to test the functional consequences of disease-associated ARS variants, including those identified in *GARS* (*Abbott, et al., 2018; Oprescu, et al., 2017a*). The p.Gly327Arg *GARS* variant did not support any yeast growth, indicating that this variant severely impairs protein function (Figure 2). These data support the pathogenicity of the p.Gly327Arg *GARS* mutation.

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Discussion

We found a previously unreported *GARS* p.Gly327Arg variant in two individuals with a predominately motor axonal neuropathy. The identification of this novel variant was facilitated by the sharing of data provided by the Inherited Neuropathy Consortium. The similar clinical presentation of the two patients to other patients with dominant *GARS* mutations, the proximity of the p.Gly327Arg mutation to known pathogenic mutations (Supplemental Figure 2), the conservation of the affected amino acid amongst species, the segregation analysis (documenting a *de novo* mutation in one patient, and consistent with a *de novo* mutation in the other), and the results of the yeast complement assay, taken together, make a strong case that this variant caused the neuropathy observed in these two patients. Yeast complementation assays provide a strong correlation between loss-of-function effects in yeast and pathogenicity of ARS alleles in dominant axonal neuropathies (*Oprescu, et al., 2017b*). However, this assay has limitations and the results from such functional studies build an argument for or against, but not to prove, pathogenicity.

The clinical phenotype we describe is similar to that described in other patients with dominant *GARS* mutations (*Sivakumar, et al., 2005*), including the age of onset, pronounced upper limb involvement, and a paucity of sensory findings. Similarly, reduced CMAP amplitudes and normal sensory responses are typical for dominant *GARS* mutations (*Antonellis, et al., 2003; Dubourg, et al., 2006; Eskuri, et al., 2012; James, et al., 2006; Lee and al., 2012; Nan, et al.,*

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2018b; Sivakumar, et al., 2005; Sun, et al., 2017). The slowed conductions that we documented in the median and ulnar motor responses, however, are unusual: the velocities in most patients are normal or slightly slowed (>40 m/sec; (*Antonellis, et al., 2003; Dubourg and al., 2006; Eskuri, et al., 2012; James, et al., 2006; Lee and al., 2012; Sivakumar, et al., 2005*), but velocities as slow as 33 and 29-36 m/sec were found in patients with a p.Ser265Phe (*Sun, et al., 2017*) or a p.Asp215His (*Nan, et al., 2018a*) mutation, respectively. Because the slowed motor conductions in *Gars* mutant mice results from smaller axonal diameters and not de/remyelination (*Achilli, et al., 2009; Seburn, et al., 2006*), we suspect that smaller motor axons are the likely explanation in these patients, too.

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Table 1. Summary of clinical characteristics.
Vibration was measured with a Rydell-Seiffer tuning fork; strength was scored using the MRC scale.

patient	age	CMTES (Rasch)	CMTNS (Rasch)	FDI (L/R)	ankle dorsiflexion	pin prick	vibration (L/R toes)	reflexes	foot deformities
1	14	11 (17)	15 (22)	3/3	3/2	reduced on toes	6/5	absent in arms and legs	pes cavus
2	22	7 (11)	10 (15)	3/3	3/3	normal	7/7	absent at ankles	pes cavus hammertoes

AFOs: ankle-foot orthoses; CMTES: CMT exam score; CMTNS: CMT Neuropathy Score version 2; (*Murphy, et al., 2011*); FDI: first dorsal interosseous

Table 2. Summary of nerve conductions.

	ulnar CMAP			median CMAP			tibial CMAP			peroneal CMAP	ulnar SNAP (O)		median SNAP (O)		radial SNAP (A)		sural SNAP (A)	
patient	DL <3.2 ms	amp ≥6 mV	CV ≥49 m/s	DL <4.4 ms	amp ≥4 mV	CV ≥49 m/s	DL <5.6 ms	amp ≥4 mV	CV ≥41 m/s	EDB amp≥2mV TA amp ≥5 mV	amp ≥7 µV	CV ≥50 m/s	amp ≥10 µV	CV ≥50 m/s	amp ≥15 µV	CV ≥50 m/s	amp ≥6 µV	CV ≥40 m/s
1 (18 y)	4.9	2.6	25	5.8	0.8	25	ND	ND	ND	EDB NR	7.3	41	13	43	ND	ND	NR	NR
2 (22 y)	4.0	1.5	34	8.8	0.2	26	13	0.3	26	EDB NR TA 0.2	28	50	25	50	60	56	26	43

A: antidromic; amp: amplitude; CMAP: compound muscle action potential; CV: conduction velocity; DL: distal latency; EDB: extensor digitorum brevis; ND: not done; NR: no response; O: orthodromic; SNAP: sensory nerve action potential; TA: tibialis anterior

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Figure legends

Figure 1: Pedigrees of patients 1 and 2.

Segregation analysis is noted on the pedigree by (+) indicating the variant is present and (-) variant is not present. The individuals in the brackets were adopted into the family.

Figure 2: The p.Gly327Arg *GARS* variant is a loss-of-function allele in yeast complementation assays. Haploid yeast lacking the endogenous *GRS1* were transformed with an empty expression construct, an expression construct with wild-type human ΔMTSΔWHEP *GARS*, or an expression construct with p.Gly327Arg ΔMTSΔWHEP *GARS*. After transformants were selected using plasmid markers, colonies were grown to saturation in 2 mL selective media. Subsequently, 1 mL of saturated culture was concentrated into 50 ul water, spotted (undiluted or diluted 1:10) on media containing 5-FOA to assess yeast growth in the absence of a *URA3*-bearing maintenance vector. Four independently generated p.Gly327Arg *GARS* expression clones were tested (A-D) and two independent colonies were evaluated per transformation (Set 1 and Set 2).

Supplemental Figure 1: Local alignment of the GARS protein across different species.

Human, yeast, mouse, C. elegans (CAEEL), rat, and orangutan (PONAB) are shown. The amino acid position that corresponds to 327 of human GARS is boxed in red and is completely conserved

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across these species. Shaded regions show other conserved regions. "*" indicates an exact match,
":" indicates a high match, and "." indicates a partial match.

Supplemental Figure 2: Crystal structure of the human GARS protein (*Zhang, et al., 2002*).
The orange highlighted portion represents the p.Gly327Arg variant site. Amino acids in red
represent known pathogenic variants and the catalytic core is represented by light gray.

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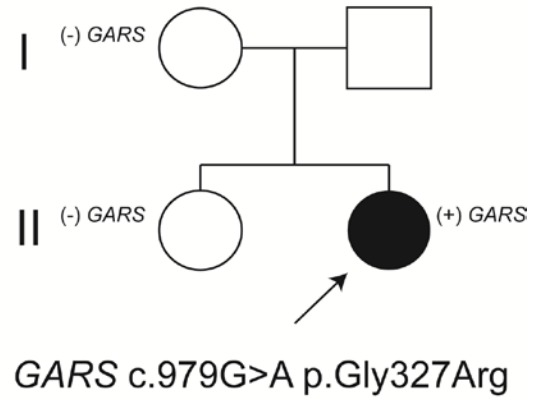A recurrent *GARS* mutation causes distal hereditary motor neuropathy

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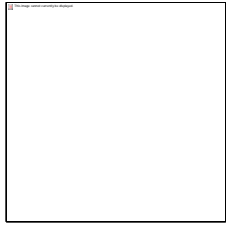A recurrent *GARS* mutation causes distal hereditary motor neuropathy
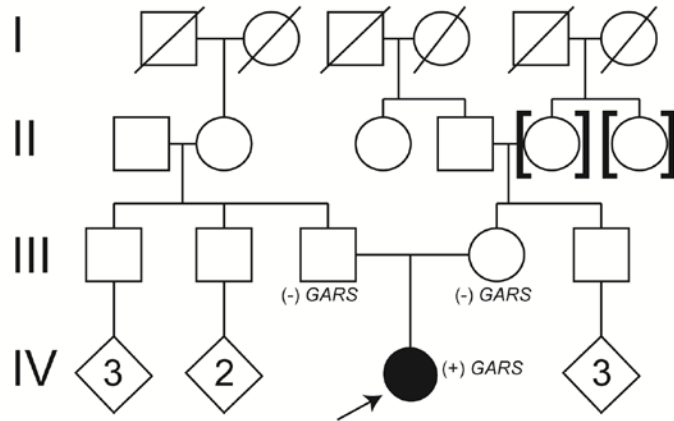
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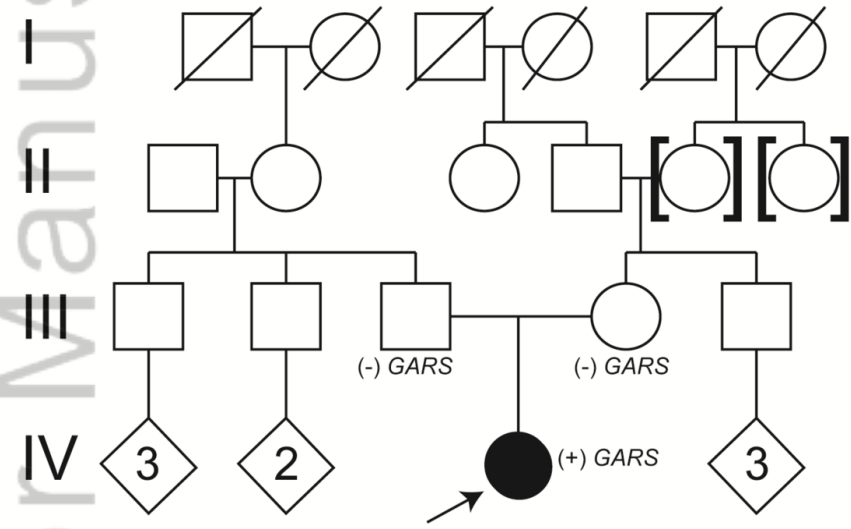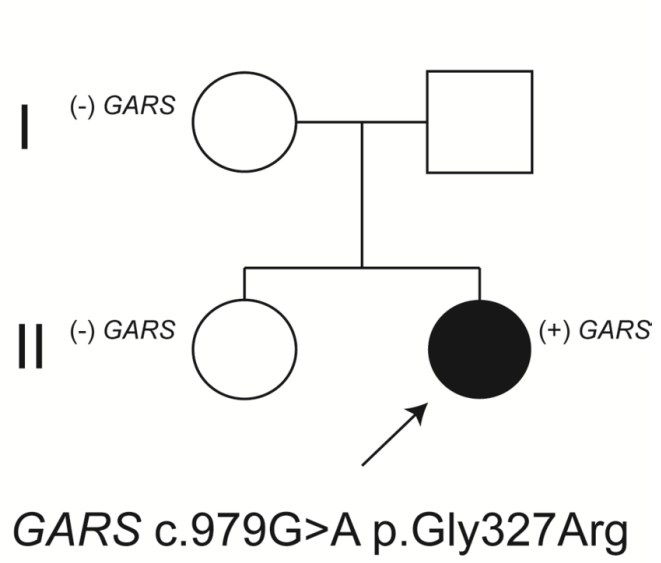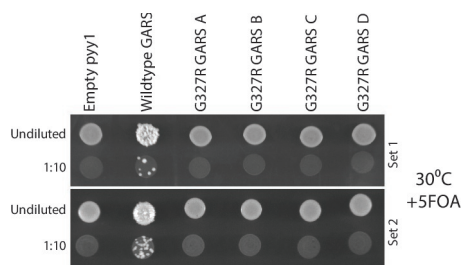
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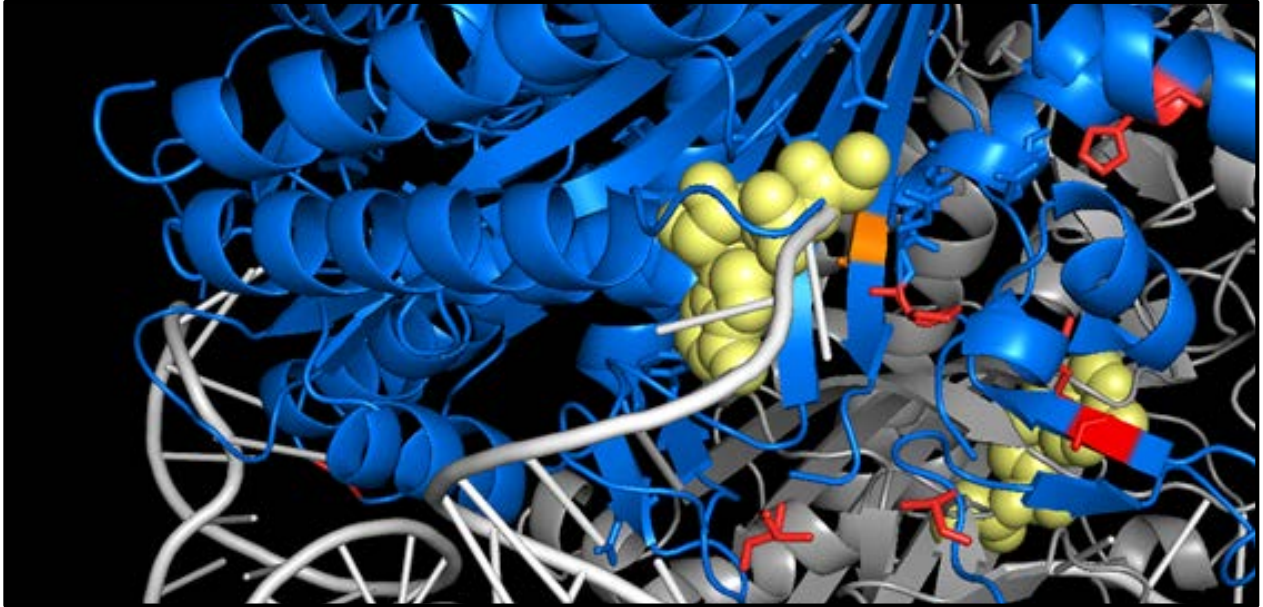
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P38088 SYG_YEAST	260	FNKLLEFNNSKTPFASASE	G	KSFRNEISPRAGLLRVREFLMAEIEHFVDPLDKSHPKFNE	319
Q9CZD3 GARS_MOUSE	298	FKRLLEFNQGKLPFAAAQE	G	NSFRNEISPRSGLIRVREFTMAEIEHFVDPTEKDHPKFQS	357
Q10039 GARS_CAEEL	318	FKRLLEFNQGKLPFAAAQ	G	LGFRNEISPRQGLIRVREFTMCEIEHFVDPEDKSLAKFAK	377
Q5I0G4 GARS_RAT	206	FKRLLEFNQGKLPFAAAQ	G	NSFRNEISPRSGLIRVREFTMAEIEHFVDPTEKDHPKFPS	265
Q5RBL1 GARS_PONAB	308	FKRLLEFNQGKLPFAAAQ	G	NSFRNEISPRSGLIRVREFTMAEIEHFVDPSEKDHPKFQN	367

Supplemental Figure 1: Local alignment of the GARS protein across different species. Human, yeast, mouse, C. elegans (CAEEL), rat, and orangutan (PONAB) are shown. The amino acid position that corresponds to 327 of human GARS is boxed in red and is completely conserved across these species. Shaded regions show other conserved regions. "*" indicates an exact match, ":" indicates a high match, and "." indicates a partial match.

Supplemental Figure 2: Crystal structure of the human GARS protein (*Zhang, et al., 2002*).

The orange highlighted portion represents the p.Gly327Arg variant site. Amino acids in red represent known pathogenic variants and the catalytic core is represented by light gray.