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GARS axonopathy: not every neuron's cup of tRNA

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Abstract

Charcot-Marie-Tooth disease type 2D, a hereditary axonal neuropathy, is caused by mutations in glycyl-tRNA synthetase (GARS). The mutations are distributed throughout the protein in multiple functional domains. In biochemical and cell culture experiments, some mutant forms of GARS have been indistinguishable from wild-type protein, suggesting that these *in vitro* tests may not adequately assess the aberrant activity responsible for axonal degeneration. Recently, mouse and fly models have offered new insight into the disease mechanism. There are still gaps in our understanding of how mutations in a ubiquitously expressed component of the translation machinery result in axonal neuropathy. Here we review recent reports, weigh the evidence for and against possible mechanisms, and suggest areas of focus for future work.

Keywords

glycyl-tRNA synthetase; tRNA synthetase; Charcot-Marie-Tooth Disease; protein translation; neurodegeneration

Charcot-Marie-Tooth disease (CMT), one of the most common inherited neurological disorders, includes a genetically diverse group of peripheral neuropathies characterized by progressive weakness and atrophy in the hands and feet 1. Pathophysiological studies categorize CMT as type I (demyelinating) or type 2 (axonal) 2. There is overlap between axonal forms of CMT and a similar group of clinical syndromes classified as distal hereditary motor neuropathy (dHMN) or distal spinal muscular atrophy (dSMA), which are distinguished from CMT2 only by the absence of sensory loss 3. CMT type 2D often causes more weakness in the distal upper extremities, a finding that is unusual among the CMTs, which usually present with length-dependent axonal degeneration affecting the feet more than the hands 3.

In 2003, the glycyl-tRNA synthetase gene *GARS* was found to be mutated in CMT2D⁴. *GARS* is responsible for covalently linking glycine with corresponding tRNAs in an ATP-dependent reaction. Charged tRNA^{gly} is then used in protein synthesis in the ribosome. *GARS* is thus ubiquitously expressed and absolutely necessary for protein translation in all cells. Since the CMT2D gene discovery, the challenge has been to determine how missense mutations in this critical and widely expressed protein which causes selective degeneration of axons in

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peripheral nerves, a common conundrum with hereditary neurodegenerative diseases linked to ubiquitous proteins and presenting selective neuronal vulnerability. Here we review work done to determine a mechanism for axonal degeneration in CMT2D and critically assess the likely mechanisms and suggest avenues for future investigation.

Genetics of CMT2D

GARS is one of thirty seven tRNA synthetase genes. It encodes both cytosolic and mitochondrial isoforms of the protein, which differ by a 54 amino acid N-terminal mitochondrial targeting sequence (Figure 1). In the protein region common to both isoforms, there are four functional domains: a WHEP-TRS domain that is highly conserved across tRNA synthetases (amino acids 13-63), two domains that form the catalytic core (amino acids 92-168 and 241-324) and an anticodon binding domain (amino acids 557-655). To date, 11 mutations in *GARS* have been reported (Table 1). There are varying degrees of genetic evidence to indicate that the mutations are pathogenic. The mutations E71G, L129P, and G240R, which segregate in pedigrees with lod scores over 5, are the most tightly linked to the disease⁴⁻⁷, while the L129P mutation is incompletely penetrant. Variants identified by sequencing *GARS* in small families and isolated patients with peripheral neuropathy, including A57V, P244L, I280F, D500N, G526R, S581L, G598A and H418R (which also has reduced penetrance), are less clearly implicated^{4, 8-12}.

Cell culture studies of GARS function and localization

In determining the functional consequences of *GARS* mutations, the initial focus was on the ability of the mutant enzyme to charge tRNA^{gly}. tRNA charging can be assayed in two ways: (1) aminoacylation as measured by quantitative capture of tritiated glycine, and (2) radioactively labeled inorganic phosphate release as a proxy for charging activity. These approaches were used to evaluate the activity of mutant *GARS* compared to wild-type enzyme. Of the seven mutations studied in aminoacylation assays with tritiated glycine, E71G, P234KY, D500N, and S581L were all active, while L129P, G240R, and G526R were inactive¹³. The inactivity of G526R was confirmed with the phosphate release assay¹⁴. These assays suggest that there is no direct correlation between disease pathogenesis and the *in vitro* charging capacity of the mutant proteins. Moving forward, it may be useful to determine whether these mutant enzymes can charge tRNAs in neurons and specifically in the axon.

Recent structural studies of *GARS* indicated that a majority of disease causing mutations lie on the dimer interface of the protein^{14, 15}. *GARS* normally forms a homodimer, and the effects of *GARS* mutations on dimerization have been examined by co-immunoprecipitation of endogenous mouse *Gars* with V5-tagged human *GARS*¹³. In this study, L129P and G240R had weaker dimer associations, P234KY and H418R were similar to wild-type, and D500N, G526R and S581L had stronger dimer interactions¹³. Disease-associated mutations thus have a range of effects on dimer interactions. More investigation is required to examine whether and how these effects on dimer interactions are relevant to the pathogenesis.

A number of studies have used cell-culture models to study how *GARS* mutations lead to CMT2D. Antonellis *et al.* found punctate structures by immunohistochemistry in normal human tissue sections and neuroblastoma cells, but they were unable to identify other proteins that colocalize with these *GARS* granules. Notably, the antibody stained granules did not colocalize with survival motor neuron (SMN, a nucleolar RNA binding protein), alpha-tubulin or oxidative phosphorylation complex IV, a marker of mitochondria¹⁶. As they are observed in spinal cord, root, and nerve sections of human autopsy tissue, these puncta indicate that *GARS* is transported into peripheral axons, where it has a distinctive distribution¹⁶. The *GARS* antibody also stains the nucleolus, indicating a possible role in RNA processing in this organelle¹⁶. The key to confirming the relevance of *GARS* granules to the pathogenesis of axonal

degeneration is to determine whether there are different distributions in CMT2D patients and animal models and to identify binding partners that colocalize with GARS in neurons. Other studies have used recombinant GARS with fluorescent tags to determine its subcellular distribution in transfected SH-SY5Y cells. The mitochondrial isoform of GARS tagged with eGFP on the C-terminus forms puncta in the cell, which interestingly do not form when eGFP-tagged GARS contains L129P, G240R, and H418R mutations¹⁶. It is, however, unclear whether these GARS-eGFP granules are analogous to endogenous GARS puncta. It is also unexpected that these wild-type GARS-eGFP puncta (seen with overexpression of the mitochondrial isoform) do not colocalize with mitochondria.

Another study compared the distribution of transfected epitope-tagged GARS to the distribution of endogenous GARS in the projections of N2a cells. It was found that wild-type transfected GARS had the same distribution as endogenous, but transfected mutant GARS failed to localize to sprouting neurites¹³.

Research into the subcellular localization is important partly because studies of tRNA synthetases in cell-free systems have shed little light on the CMT2D disease mechanism. The identification of GARS mutations in peripheral nerve disease has naturally shifted the focus of investigation to understanding how the wild type and mutant proteins function in a cellular context. The negative biochemical results have also raised the possibility that GARS may have a unique function in neurons, which is disrupted in the disease. Other tRNA synthetases have been shown to have functions other than charging, and this possibility is worth exploring with GARS in cell culture and animal models.

Animal Models of GARS-Linked Axonopathy

Animal models can help to explain the neuronal specificity of the effects of GARS mutations. In addition to the reports of human mutations described above, mutations have been identified in mice and flies. As biochemical and cell culture studies point to possible pathogenic mechanisms, these animal models play an important role in the confirmation and extension of the findings.

Mouse Models of CMT2D

The two mouse models that have been reported with *Gars* mutations have motor and sensory deficits of different severity. Phenotypic features and the results of informative crosses are summarized in Table 2. The first CMT2D model, *Nmf249*, is a spontaneous mutation identified at the Jackson Laboratory in a line with a dominantly inherited neurological phenotype and decreased lifespan. The defect was mapped to a 1.9 Mb critical region containing *Gars*, the mouse ortholog of *GARS*. The causal mutation, a CC → AAATA replacement, results in an in-frame change of a proline to a lysine and tyrosine at residue 278 in the mouse *Gars* gene, equivalent to P234KY in the human protein (the annotation of the mouse protein starts at the beginning of the mitochondrial targeting sequence, while the human protein does not include those residues in the count)¹⁷. The affected mice have sensory and motor deficits and survive for about 6-8 weeks. Marked loss of integrity of the neuromuscular junctions (NMJ) is evident at post-natal day 36. Motor and sensory nerves in affected mice showed decreased evoked potential amplitude and decreased nerve conduction velocity with histological and ultrastructural evidence of a decrease in large nerve fibers with normal myelin thickness around the remaining fibers¹⁷. These observations indicate a severe, dominantly inherited motor and sensory neuropathy.

Total *Gars* mRNA levels were equivalent to those found in wild-type mice, and in *in vitro* aminoacylation assays the P234KY mutant GARS protein was fully active. To investigate this further, a hypomorphic allele was examined. This *XM256* allele contains an insertion in the

second intron of the *Gars* gene and results in a 50% reduction in *Gars* mRNA levels¹⁷. These heterozygous mice have a normal lifespan and no NMJ pathology¹⁷. These results indicate that a simple loss of GARS function is not responsible for the disease.

Another *Gars* allele was recently identified in an ENU mutagenesis screen for neurological phenotypes at the MRC Mammalian Genetics Unit, Harwell UK¹⁸. Two littermates were found with deficits in rotarod and grip strength testing. Positional cloning and candidate gene sequencing showed a missense mutation in *Gars*, which results in a cysteine to arginine change at position 201 (C201R), equivalent to C157R in the human protein. The phenotype of the *Gars*^{C201R/+} mouse is considerably less severe than the *Gars*^{Nmf249/+} mouse. Heterozygotes have a normal lifespan with a 50% decrease in grip strength detectable in one month old mice; homozygotes are viable, albeit with a severe deficit. Most of the characterization was performed on the C3H background, which has a less severe pathology. No abnormal histology was observed in spinal cord sections of affected animals, but a 50% decrease in large diameter motor axons was seen in the sciatic nerve in 17-month-old *Gars*^{C201R/+} mice. Brain lysates from *Gars*^{C201R/+} mice and from *Gars*^{C201R/C201R} mice were evaluated for aminoacylation. While no significant difference was seen in the charging activity of lysates from heterozygotes, the lysates from homozygotes had a 60% reduction in enzyme activity. GARS protein levels were elevated in both heterozygotes and homozygotes at P15, perhaps indicating compensatory upregulation; in adult heterozygotes no difference in protein levels was observed in comparison to wild-type mice¹⁸.

Both mutant alleles have been crossed to the hypomorphic allele *Gars*^{XM256}. In both cases, no compound heterozygous offspring were produced, confirming by non-complementation that *Gars* mutations cause the motor phenotypes in the *Gars*^{C201R/+} and *Gars*^{NMF249/+} mice¹⁸. Why these *Gars*^{C201R/XM256} and *Gars*^{NMF249/XM256} mice do not survive is unclear, given the retained enzymatic activity in the heterozygous mutant mice, but it may indicate that both Gars P278KY and C201R require the presence of some wild-type enzyme for functionality. Alternatively, the toxicity of the mutant protein may be mitigated by the presence of wild-type enzyme, or the mutant proteins lack some other, unknown activity.

The histopathology and electrophysiology done on these mice show that both are good models for the disease phenotype. While the milder *Gars*^{C201R/+} mouse phenotype is more consistent with the disease seen in humans, with late onset, grip strength deficit, and no reduction in lifespan, the observations from the *Gars*^{Nmf249/+} mouse provide an indication of the effects of a more rapidly progressive pathology, potentially facilitating short-duration interventional studies.

Although the findings in the mice argue against a simple loss of function mechanism for the GARS mutations, there is still some ambiguity because of the lack of viability of the compound heterozygotes and because of the reduced enzymatic activity in the *Gars*^{C201R} homozygote. If the mutations did not cause a loss of aminoacylation function, then deleting the other copy of *Gars* should not result in lethality. In fact, one could argue that the phenotype should not have been worse because the compound heterozygotes have the same dose of mutant protein as the heterozygous mutants. The differences in severity of both models on different backgrounds show that the GARS phenotype is easily modified. This is consistent with the range of onset and incomplete penetrance seen in patients. Important questions could be answered with transgenic mice overexpressing GARS. If robust overexpression of a mutant transgene replicates the phenotype, then we could conclude that axons degenerate because of a dominant negative or toxic gain of function in GARS. Similarly, if robust expression of wild-type protein is able to rescue the mutant phenotypes, then we could conclude that a loss of GARS function, perhaps a non-canonical function, is the cause of disease.

***Drosophila* Models of GARS deficiency**

The *aats-gly* gene in *Drosophila* has 60 percent homology with human GARS, and 10 of the mutations identified in humans are missense changes in amino acids that are conserved in fly. In 2007 the Luo group identified a fly mutation in a random mutagenesis screen for genes necessary for dendrite and axon formation and arborization in olfactory projection neurons. The screen was performed with the Mosaic Analysis with a Repressible Cell Marker (MARCM) technique, which allows individual cells deficient in a protein to be studied on a heterozygous background¹⁹.

The *Drosophila* mutation that was identified caused a missense change equivalent to P98L in the human protein²⁰. Three additional experiments supported the conclusion that the homozygous mutations probably cause a loss of translation as deletion of *aats-gly*, two other tRNA synthetases, and the locus *Df(3L)mito*, which contains two genes involved in the mitochondrial translation machinery all replicated the phenotype²⁰. It is particularly noteworthy that removal of the mitochondrial translation machinery resembled the phenotype in severity and penetrance. It is also interesting that the neuronal cell bodies appeared healthy in all of these mutants, indicating that axons and dendrites have a greater sensitivity to reduced protein translation²⁰. This study also attempted to connect the fly phenotype to the human disease through a series of rescue experiments. It was demonstrated that the wild-type human GARS transgene fully rescued the phenotype, while the human mutants GARS^{E71G} and GARS^{L129P} did not²⁰.

The *Drosophila* work underscores the importance of GARS, and tRNA synthetases in general, in neurite growth and arborization. Other non-neuronal tissues were not examined to determine the effects of deficiency in GARS, so it is unclear if neurons are particularly vulnerable to loss of housekeeping function. The most remarkable observation from this study was that human GARS^{E71G} was unable to rescue the arborization defect. This is the only assay in which GARS^{E71G} is distinguishable from wild type. This finding questions the sensitivity or relevance of other functional assays that have been reported. In subcellular localization, *in vitro* aminoacylation activity, and yeast rescue, GARS^{E71G} (which is strongly implicated genetically) behaves like wild-type, but in *Drosophila* olfactory axons, GARS^{E71G} is not equivalent to wild-type GARS.

Of particular interest is that in the Chihara *et al.* study, overexpression of several GARS and *aats-gly* wild-type and mutant transgenes did not cause any overt phenotype or problems with projection neuron arborization, suggesting that the mutant protein does not have dominant negative or toxic effects in *Drosophila*. Overexpression was not, however, the focus of this study. It is difficult to evaluate the relevance of this invertebrate model that has recessive inheritance and where effects are only viewed in individual neurons, when there are mouse models that point to toxicity rather than simple loss of function of the mutant protein.

Diseases Caused by Mutations in other tRNA Synthetase Genes

GARS was the first tRNA synthetase gene to be linked to a disease. Since then mutations in 4 of the 36 other tRNA synthetase genes have been linked to neurological phenotypes in humans or mice, indicating that tRNA synthetases play a particularly important role in nervous system development and maintenance.

Two recessive diseases of the central nervous system are associated with mutations in mitochondrial tRNA synthetases: a leukoencephalopathy caused by mutations in the mitochondrial aspartyl-tRNA synthetase gene (*DARS2*)²¹, and an infantile encephalopathy most severely affecting the cerebellum caused by mutations in the mitochondrial arginyl-tRNA synthetase gene (*RARS2*)²². In mice, mutations in the editing domain of alanyl-tRNA

synthetase (Aars) cause Purkinje cell degeneration, ataxia, and hair follicle dystrophy (*sticky*)²³. This study reported that the *sticky Aars* mutant mischarges tRNA^{ala} with serine, and hypothesized that this leads to accumulation of misfolded proteins due to a resulting decrease in the fidelity of protein translation²³.

Two missense mutations and a four amino acid deletion in the tyrosyl-tRNA synthetase gene (YARS) have been identified in dominantly inherited intermediate CMT (DI-CMT)²⁴. Of the diseases that have been connected to tRNA synthetase genes, this disease is most similar to GARS-linked CMT2D in inheritance pattern and phenotype. The differences in the disease manifestations are that DI-CMT has both axonal and demyelinating features and lower limb predominance. It was initially demonstrated that neither of the YARS missense mutants release pyrophosphate as quickly²⁴. More recent data suggest that one of them, E196K, is fully active in an aminoacylation assay, in yeast experiments, and in *Drosophila* genetic complementation assays (these results are more consistent with the observations with GARS)²⁵.

A *Drosophila* model of DI-CMT has also been generated through transgenic overexpression of mutant YARS and *aats-tyr*, the fly ortholog. While wild-type overexpression did not result in an abnormal phenotype, overexpression of transgenes with disease causing mutations produced expression-level-dependent phenotypes. High levels of expression of some of the mutant transgenes in all tissues caused lethality, while lower levels of ubiquitous expression and neuronal expression of mutant transgenes resulted in motor performance defects as measured by negative geotaxis, jumping, and flying²⁵. This work nicely demonstrates that there is some toxicity of the mutant protein in neurons and that this toxicity is conserved in invertebrates. This is the first report of toxicity of a mutant tRNA synthetase and should guide future work on GARS.

The similarities between the results of the functional assays that have been performed seem to increase the likelihood that the mechanism of YARS and GARS-linked CMT is the same. With this in mind, it will be interesting to see how research in each area will advance our understanding of both diseases. The dosage-dependent effects of mutant YARS overexpression suggest that perhaps expression levels of mutant GARS were simply not high enough to generate a phenotype in the experiments reported by Chihara *et al*[HK1].

Possible Mechanisms for GARS Axonopathy

There are at least nine possible mechanisms for axonal degeneration with GARS mutations. None are mutually exclusive; some have more support than others, and some are unlikely but difficult to rule out completely (Figure 2).

Loss of charging function

Some of the reported mutations cause loss of function as assayed by *in vitro* aminoacylation or yeast viability. These results are consistent with a disease mechanism of haploinsufficiency in charging function. However, other mutations that segregate with the disease do not alter enzyme activity in these assays, making it unlikely that the disease is due to a simple loss of this function. The finding that the mouse that is heterozygous for the hypomorphic allele (XM256/+) has no phenotype also presents strong evidence that the disease is not caused by a simple loss of function. There is, however, evidence from mouse models that a loss of function may contribute to the pathogenesis of the disease. The lethality observed in compound heterozygous mice (*Gars*^{Nmf249/XM256}, *Gars*^{C201R/XM256}) suggests that despite the activity of both the C157R and P234KY mutant enzymes *in vitro*, neither can restore viability to a mouse deficient in wild-type *Gars*. Finally, the E71G mutant protein was unable to fully rescue the arborization defects in *Aats-gly*-deficient olfactory projection neurons in *Drosophila*, suggesting that this mutation may also cause some loss of function that is not detectable with

other assays. While toxic-gain or dominant-negative mechanisms may be more likely, the loss of function seen with these mutations may still be a part of the pathogenesis.

Aggregation

The GARS mutations in CMT2D resemble SOD1 missense mutations in familial amyotrophic lateral sclerosis (ALS), as in being distributed across different functional domains in the protein, and in the fact that the mutations do not have a consistent effect on the known function of the protein. As with other mutant proteins in neurodegenerative disease, mutant SOD1 forms aggregates. While there is still debate about whether mutant protein aggregates are a cause of toxicity or a result of a protective mechanism to sequester toxic protein, the aggregates generally correlate with toxicity²⁶. Aggregates of mutant GARS have not been observed in patient cells or in cell culture systems overexpressing mutant GARS 13-16. As this work has also used multiple antibodies, it is unlikely that antibody sites are masked in aggregates of mutant protein. Thus it is unlikely that aggregates are formed in cells expressing mutant GARS, and aggregation is probably not part of the pathogenesis of CMT due to GARS mutations. This does not, however, rule out the possibility that GARS is toxic to the motor neuron through aberrant protein interactions.

Mischarging

Unlike AARS, GARS does not have a known editing domain. It is worth testing whether mutations that cause CMT decrease the fidelity of tRNA charging, but with current data it is unlikely that this is the mechanism of toxicity. AARS mutations cause the *sticky* phenotype only when bred to homozygosity. While the recessive inheritance suggests a loss of function (unlike GARS), the fact that mutations in this member of the tRNA synthetase family cause neurodegeneration in mice may be relevant. It will be interesting to see whether a link between these two diseases can be found and whether there are any human diseases caused by mutations in tRNA synthetase editing domains.

Nucleolar dysfunction

The function of GARS in the nucleolus is unknown. Antonellis *et al.* showed that GARS colocalizes with nucleolar antigen in SH-SY5Y cells, but the reason for its presence there is unclear¹⁶. It is difficult to know whether mutants have impaired nuclear import, because recombinant GARS does not appear to be concentrated in the nucleolus. It is also possible that nucleolar staining is an artifact of the antibody. Methionyl-tRNA synthetase (MARS) has also been found in the nucleolus and has been shown to regulate rRNA synthesis in proliferative cells. MARS is, however, absent from the nucleolus in quiescent cells²⁷. If GARS has a similar function in the nucleolus, it seems unlikely that this function plays an important role in a disease manifested primarily in terminally differentiated motor and sensory neurons.

Dimerization

There are a number of ways that aberrant dimer formation could lead to GARS dysfunction or GARS toxicity in neurons. Dimerization may not be an independent mechanism, but rather a potential link between basic GARS biochemistry and the other pathogenic mechanisms listed here. For example GARS monomers may not be distributed to nerve terminals as well as dimers. Similarly, it may be that an accumulation of monomers is toxic to the cell.

Non-canonical functions

Essential housekeeping proteins commonly acquire additional functions through evolution, and various non-canonical functions have been ascribed to tRNA synthetases (as reviewed in 28-29). These range from anti-angiogenic properties observed with YARS and WARS, to regulation of transcription seen with KARS. Yeast GARS1 has been shown to participate in

the formation of mRNA 3'-ends. It could be that while the canonical charging function may be preserved with some mutations, all of the mutations alter a function of GARS that has not yet been identified.

Disease-associated mutations lead to new, toxic, protein-protein or protein-RNA interactions

If mutant GARS is toxic to motor neurons, it may be because of a protein-protein or protein-RNA interaction that is detrimental to axon health. The expression profile of this interacting protein could help explain the motor and sensory specificity of the phenotype. The neuronal specificity of GARS mutation effects in CMT2D could be due to the cell-specific expression of a key interacting factor, as has been proposed, for example, in Huntington's disease³⁰.

Mitochondrial toxicity or dysfunction

Mitochondrial defects have been reported in various neurodegenerative diseases, including axonal CMT. CMT2A is caused by mutations in mitofusin 2 (MFN2)³¹, a membrane protein that is responsible for the maintenance of mitochondrial morphology. Mutations in ganglioside-induced differentiation associated protein 1 (GDAP1) likely lead to CMT2K and CMT4A by a similar mechanism of altered mitochondrial structure³²⁻³³. GARS is not a membrane protein, and it is unlikely to have a similar function in mitochondrial architecture, but it is a mitochondrial protein, and the pathogenesis of the disease could be linked to mitochondrial toxicity or altered function in this organelle.

Impaired axonal transport

Peripheral motor axons are particularly long, highly terminally branched, and support nerve terminals that are much larger than elsewhere in the nervous system. With the distance and volume of proteins to travel along the narrow axon, active transport is critical to the health of the axon and its terminals. Impaired transport has been shown to cause motor neuron degeneration: kinesin mutations cause motor deficits in *Drosophila*³⁴, and mutations in dynactin p150 cause motor neuron phenotypes in humans, mice and flies³⁵⁻³⁷.

There is evidence of local protein translation in axons³⁸, and it is likely that the peripheral motor axon in particular is translationally active. GARS is likely among the cargo that is transported in both directions along microtubules between the soma and the nerve terminal. It is possible that axons degenerate because mutant GARS protein is not included in anterograde or retrograde transport cargo, and the axon is unable to clear the mutant protein.

BOX 1: Outstanding questions

- How do the variable effects on charging function and dimer formation relate to disease pathogenesis?
- Which GARS isoform (cytosolic or mitochondrial) is most relevant to disease?
- Is GARS transported down the axon?
- Does GARS have non-canonical functions? Are any of these functions unique to neurons?
- Do mutations in GARS cause it to associate with new RNAs or proteins?
- Do post-translational modifications of GARS impact its localization and binding partners.
- Will transgenic overexpression of mutant GARS model the disease?
- Will transgenic overexpression of wild-type GARS mitigate the pathology?

- Do YARS and GARS mutations cause CMT by the same mechanism?

Concluding Thoughts

Many mechanisms may contribute to the disease manifestations, but all the mutations likely share the same pathophysiology. A functional assay for GARS mutations that correlates with pathogenicity is needed. A specific focus must be placed on mutations that segregate with disease. Studying mutations with weak genetic evidence could confuse the search for a common mechanism. It is worthwhile to focus on mutations such as E71G, L129P, C157R, P234KY, and G240R that are most likely pathogenic because they segregate with disease in large human and mouse pedigrees. Of the mutations with the strongest supporting evidence, E71G continues to be an outlier. An assay that is able to identify a functional consequence of this mutation would likely point to the critical mechanism.

At this juncture, the most likely candidates for a unifying mechanism are impaired axonal transport, mitochondrial dysfunction, new protein associations that lead to toxicity and loss or gain of a non-canonical activity. Amino-acid mischarging, abnormal dimerization, aggregation, and a simple loss of charging function are unlikely to be primarily responsible for the pathogenesis of the disease.

A special focus should be placed on determining if the mutant protein is damaging to cells and tissues through a toxicity or dominant negative effect, and if overexpression of wild-type protein is protective. These experiments would help categorize the mechanism as either a loss-of-function or purely dominant effect. This determination will allow us to develop and test therapeutic measures. There is still much to be learned about normal and abnormal protein-protein and protein-RNA interactions of GARS and about its activity and toxicity in cells and especially neurons. As work in cell culture helps direct the search for a CMT2D mechanism, these observations need to be confirmed *in vivo* in animal models and ultimately in patients.

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Figure 1.

The GARS transcript produces two protein products, a cytosolic isoform and a mitochondrial isoform that has a 54 amino acid mitochondrial targeting sequence. The remaining 685 amino acids encoded by the transcript are shared by both isoforms. The mutations that have been identified in GARS (black, human; red, mouse) are distributed across the entire protein in all of the functional domains. Many of the mutations that have been identified lie in one of the three regions that form the dimer interface.

Figure 2.

Possible mechanisms by which mutations in GARS could be leading to axon degeneration in these uniquely shaped cells. Some of these are unlikely or have been ruled out by functional analysis ((1) Loss of charging function, (2) Aggregation, (3) Mischarging), others require further investigation ((4) Nucleolar dysfunction, (5) Dimerization, (6) Non-canonical functions, (7) novel interactions resulting from mutations, (8) Mitochondrial toxicity or dysfunction, (9) Impaired axonal transport that lead to deficits in local translation).

Table 1

The genetics of GARS variants identified in patients

Mutation	Geographical Origins	Age of Onset	Disease Phenotype	Penetrance	Genetic Evidence	Reference
A57V	Ghana	12 years		NA	Found in a screen of 33 patients	Rohkamm <i>et al.</i> , 2007
E71G	Mongolia	18 years (average)	CMT2D/dSMA-V	Complete	Segregation in a large pedigree Lod score of 6.07	Antonellis <i>et al.</i> , 2003 Sambuughin <i>et al.</i> , 1998
L129P	Bulgaria	17 years (average)	dSMA-V	Incomplete	Segregation in a large pedigree Lod score of 5.99	Antonellis <i>et al.</i> , 2003 Christodoulou <i>et al.</i> , 1995
G240R	North America	23 years (average)	CMT2D	Complete	Segregation in large pedigree Lod score of 6.89	Antonellis <i>et al.</i> , 2003 Ionasescu <i>et al.</i> , 1996
P244L	Japan	Adolescence	CMT2D	NA	Found in a screen of 89 patients absent from 100 healthy controls	Abe <i>et al.</i> , 2006
I280F	UK	11 to 18 years	CMT2	Complete	Segregation in two generations of a small pedigree	James <i>et al.</i> , 2006
H418R	UK/Australia	26 years (average)	dSMA-V	Incomplete	Segregation in a large family. 13 individuals carry the mutation	Sivakumar <i>et al.</i> , 2005
D500N	Italy	6 years to >53 years	CMT2D/dSMA-V	Incomplete	Segregation in a 3 generation pedigree with 5 affected individuals	Del Bo <i>et al.</i> , 2006
G526R	France	20 years (average)	dSMA-V	Complete	Segregation in a two generations of a small pedigree	Antonellis <i>et al.</i> , 2003
S581L	UK	4 years	Lower-limb predominant CMT2	Complete	4 generation pedigree	James <i>et al.</i> , 2006
G598A	UK	6 months	severe HMN	NA	<i>de novo</i>	James <i>et al.</i> , 2006

Table 2
Mouse phenotypes with *Gars* alleles

<i>Gars</i> Genotype	Life Expectancy	Strain Differences	Phenotype	Reference
<i>Gars</i> ^{Nmf249/+} or <i>Gars</i> ^{P278KY/+}	6-8 weeks	Outcross to CAST/EiJ extended lifespan but did not alter onset or severity of motor phenotype	Sensory and motor deficits Abnormal NMJ morphology Impaired nerve impulse transmission Reduced NCVs Loss of large diameter peripheral axons	Seburn <i>et al.</i> 2006
<i>Gars</i> ^{Nmf249/Nmf249}	Embryonic lethal	NA	NA	Seburn <i>et al.</i> 2006
<i>Gars</i> ^{XM256/+}	Normal lifespan	NA	Reduced GARS RNA levels Normal NMJ morphology Normal NCVs	Seburn <i>et al.</i> 2006
<i>Gars</i> ^{XM256/XM256}	Embryonic lethal	NA	NA	Seburn <i>et al.</i> 2006
<i>Gars</i> ^{Nmf249/XM256}	Embryonic lethal	NA	NA	Seburn <i>et al.</i> 2006
<i>Gars</i> ^{C201R/+}	Normal lifespan	Motor phenotype is more severe on B6 background than on C3H	Decreased grip strength Poor skilled motor function Increased total GARS protein at p15 Reduction in large diameter axons in sciatic nerve	Achilli <i>et al.</i> 2009
<i>Gars</i> ^{C201R/C201R}	17 days (maximum)	Maximum life expectancy 15 days on C3H background	Reduced weight and viability Impaired limb movement	Achilli <i>et al.</i> 2009
<i>Gars</i> ^{C201R/XM256}	Embryonic lethal	NA	NA	Achilli <i>et al.</i> 2009