

Evidence for a dominant-negative mechanism in *HARS1*-mediated peripheral neuropathy

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The pathogenic mechanism of neuropathy-associated aminoacyl-tRNA synthetase (ARS) gene variants is poorly defined. Mullen *et al.* generate new models of pathogenic, dominant *HARS1* mutations and show that they increase eIF2 α phosphorylation and decrease protein translation in neurons. These results are consistent with a dominant-negative mechanism of ARS-mediated peripheral neuropathy.

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To synthesize proteins, cells require a pool of tRNAs charged with the corresponding amino acid. This reaction is catalyzed by the aminoacyl-tRNA synthetase (ARS) family of enzymes, which can function in either the cytoplasm or the mitochondria to allow protein translation in each compartment [1]. Mutations in ARS-encoding genes can cause recessive and dominant human phenotypes [2,3]. Although recessive ARS-mediated disease severely affects the development and function of a broad array of tissues, dominant ARS mutations primarily cause a late-onset peripheral neuropathy with clinical heterogeneity including variable severity and age of onset. The mechanism of ARS-associated peripheral neuropathy is poorly understood and the primary mechanism is frequently debated in

the literature [2]. One possible explanation is that heterozygosity for neuropathy-associated ARS mutations, which are primarily loss-of-function mutations but do not significantly compromise protein stability, results in a dominant-negative effect where the mutant protein represses the ability of the remaining wild-type ARS protein to function. This argument is strengthened by the fact that all five ARS enzymes implicated in peripheral neuropathy function as homodimers [2]. An alternate argument is that pathogenic mutations cause conformational changes that allow the mutant ARS enzyme to aberrantly interact with other cellular proteins, which then affect neuronal pathways required for neuron development or health [4]. In this issue of *The FEBS Journal*, Mullen and colleagues report a

Abbreviations

ARS, aminoacyl-tRNA synthetases; eIF2 α , eukaryotic initiation factor 2 subunit α ; *HARS1*, cytoplasmic histidyl-tRNA synthetase; PC12, rat pheochromocytoma cells; tRNA, transfer ribonucleic acid.

detailed study of pathogenic histidyl-tRNA synthetase (*HARS1*) mutations and find molecular and cellular phenotypes that are consistent with a dominant-negative mechanism of disease [5].

Mullen *et al.* studied three independent *HARS1* variants that have been linked to peripheral neuropathy. Two (V155G and Y330C) were previously found to segregate with disease in small pedigrees, displayed loss-of-function characteristics in yeast complementation assays, and were functionally impaired in enzymatic assays [6]. The third (R137Q) was found in a single patient with a late-onset dominant peripheral neuropathy, caused reduced yeast growth, and caused aberrant axon morphologies when overexpressed in *C. elegans* [7]. Although these variants were interpreted as loss-of-function mutations, it was unclear how they impacted protein synthesis and how they might damage neurons.

To address the above questions, the authors paired two overexpression models: PC12 cells (which can be differentiated to generate axon-like projections) and zebrafish embryos. PC12 cells expressing the mutant *HARS1* proteins showed an increase in phosphorylated eIF2 α , a marker of the integrated stress response, and an indication of accumulating uncharged tRNA [8], consistent with significantly reduced *HARS1* function. This was accompanied by an approximate 20% reduction in global protein synthesis, as measured by OP-Puro incorporation. This downregulation in protein synthesis did not dramatically affect PC12 morphology, viability, or number of neurites formed; however, it did modestly decrease the length of the longest neurite in each cell. These findings suggest that the ability to form or maintain long neuronal processes, such as the long axons of the peripheral nerve, is dependent on protein synthesis.

To complement this approach with an *in vivo* model, the authors injected wild-type zebrafish embryos with V155G or Y330C human *HARS1* mRNA. Similar to what was observed when overexpressing R137Q *HARS1* in worm [7], by 48 hours postfertilization, zebrafish neurons showed improper guidance. Neuronal processes in mutant-expressing fish were also shorter than those of fish expressing wild-type human *HARS1* protein. Unsurprisingly, for such severe morphological defects, the fish also displayed motor deficits in behavioral assays. This latter phenotype indicates that, at least in the context of overexpression, loss-of-function *HARS1* mutations are sufficient to impair proper neuronal development in fish.

Although the three *HARS1* mutations studied were previously shown to decrease enzyme activity, it is possible that they also gain aberrant neomorphic properties that contribute to pathogenicity. Therefore, it is

critical to determine whether inhibiting *HARS1* activity or protein synthesis is sufficient to reproduce the cellular and morphological phenotypes caused by the *HARS1* mutations. When Mullen and colleagues treated differentiating PC12 cells with histidinol—a small-molecule inhibitor of *HARS1*—the neurites showed increased eIF2 α phosphorylation, reduced global protein translation, and a reduced length of the longest neurite in each cell; all of these findings are comparable to the effects observed upon overexpression of each *HARS1* mutant protein. Similarly, treatment with cycloheximide—a protein synthesis inhibitor—reduced the length of the longest neurite in each cell. Notably, cycloheximide also phenocopied the *HARS1* mutations in zebrafish, shortening the length of the neuronal processes in the dorsal root ganglia. These data demonstrate that chemically inhibiting protein synthesis will mimic the phenotype of *HARS1* mutations, supporting the hypothesis that reduced protein synthesis is part of the ARS-associated neuropathy disease mechanism.

As previous work has shown that haploinsufficiency is not a possible explanation for these peripheral neuropathies [2], reduced protein synthesis would have to be a result of a dominant-negative mechanism. This requires mutant ARS subunits to dimerize with, and repress, the function of the wild-type subunit. Previously, V155G and Y330C were shown to have sedimentation velocities almost identical to those of wild-type *HARS1* [6]—here, Mullen *et al.* show that R137Q behaves similarly, consistent with all three mutations retaining their ability to dimerize. Of note, R137Q affects a highly conserved residue at the dimer interface, underscoring that the effects of pathogenic mutations on dimerization cannot be predicted from structural assessments alone.

The severity of the phenotypes observed by Mullen *et al.* may be a result of exogenous *HARS1* expression; it is unclear if endogenous levels of mutant *HARS1* would have the same effect. Endogenous levels of mutant *HARS1* could have a milder dominant-negative effect, which may not suppress protein synthesis to the extent that impairs neuronal health. Moving forward, it will be important to confirm the effects of endogenous dominant ARS mutations on neuronal protein translation. Additionally, although Mullen *et al.* demonstrate that mutant *HARS1* proteins maintain their ability to dimerize with wild-type *HARS1*, this does not directly test whether dimerization is required for pathogenicity. This will be a critical experiment for fully demonstrating a dominant-negative mechanism of ARS-mediated peripheral neuropathy.

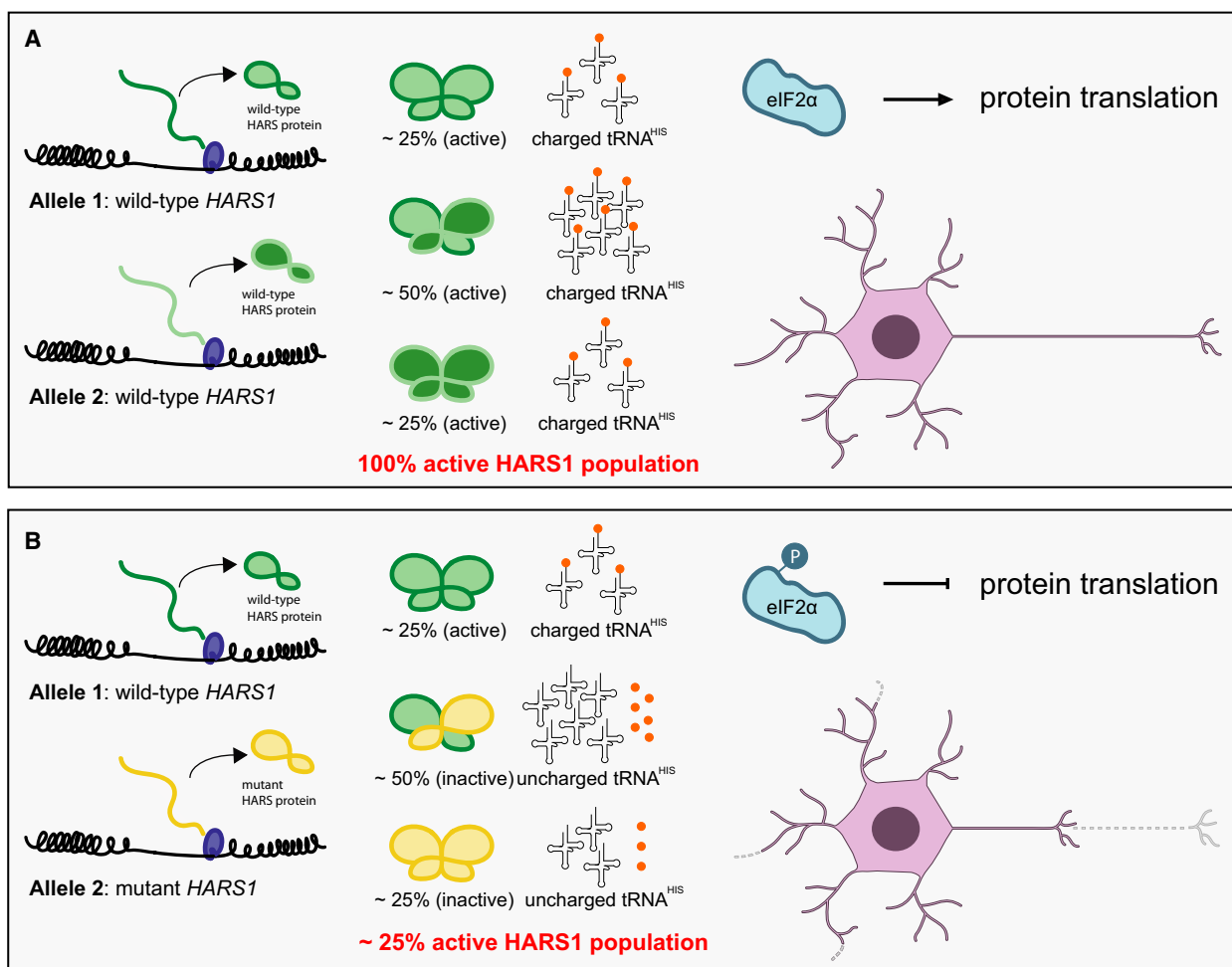


Fig. 1. Proposed mechanism for *HARS1*-mediated peripheral neuropathy. (A) In an unaffected individual, two wild-type *HARS1* alleles produce a fully active population of *HARS1* enzymes and a sufficiently charged population of tRNA^{HIS} for global protein translation to proceed. Long axons can develop properly and can be maintained for neuronal function. (B) In a patient with dominant neuropathy, heterozygosity for a dominant-negative *HARS1* mutation leads to a heterogeneous *HARS1* population. The majority of *HARS1* homodimers comprise either two mutant subunits or one mutant and one wild-type subunit, all of which have reduced activity. There is an abundance of uncharged tRNA^{HIS} in the neuron, which leads to eIF2 α phosphorylation, a global reduction in protein synthesis, and degeneration of the long axons required for a healthy peripheral nervous system.

As a whole, this study provides strong evidence for dominant, loss-of-function *HARS1* mutations impairing protein translation and neuronal health, consistent with a dominant-negative effect (see Figure). Moving forward, it will be important to test whether reducing dimerization of the mutant allele can improve these phenotypes. Additionally, by phenocopying these mutations with chemical inhibitors of *HARS1* and protein synthesis, Mullen *et al.* weaken the argument that *ARS* mutations act through neomorphic gain-of-function interactions, unrelated to protein translation. These results also indicate that therapeutic efforts for *ARS*-mediated peripheral neuropathy should be aimed

at restoring *ARS* function and improving protein translation.

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Author contributions

RMS and AA wrote the paper.

Conflict of interest

The authors declare no conflict of interest.

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