Shared mechanisms and pathological phenotypes underlying aminoacyl-tRNA synthetase-related neuropathies

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Charcot-Marie-Tooth disease (CMT) is a heterogeneous group of inherited peripheral neuropathies; it is characterized by muscle weakness and wasting, as well as sensory dysfunction, that typically begins during adolescence and ultimately leads to lifelong disability. Occurring in ~1 in 2500 individuals, CMT is the most common hereditary neuromuscular condition and results from mutations in > 100 different genes. CMT is grouped into type 1 (CMT1), where demyelination and loss of nerve conduction velocity occur, type 2 (CMT2), where motor and sensory axons degenerate without loss of myelination/nerve conduction velocity, and intermediate CMT, where both demyelination and axon loss present alongside intermediate nerve conduction velocities.

Mutations in eight different genes encoding aminoacyl-tRNA synthetases (aaRSs) have been associated with peripheral neuropathy, making them the largest CMT-linked gene family. aaRS enzymes charge amino acids to cognate tRNAs, meaning they play a fundamental role in protein synthesis and are, consequently, ubiquitously expressed. As such, mutant aaRS proteins are found throughout the body in CMT patients, yet only peripheral neurons are impacted by the disease.

Several key commonalities have been observed in models of different aaRS-related neuropathies. Here, we briefly describe shared mechanisms and pathological phenotypes across different forms of aaRS-CMT (**Figure 1**), and highlight how these contribute to the selective peripheral neurodegeneration typical of CMT.

The severity of aaRS-related CMTs does not clearly correlate with loss of capacity for aminoacylation. Rather, a toxic gain-of-function linked to conformational relaxation appears to be a recurrent feature of CMT-aaRS mutant proteins. In keeping with this, recessive aaRS mutations that impair aminoacylation notably do not lead to peripheral neuropathy, instead driving severe multisystem developmental disorders (Meyer-Schuman and Antonellis, 2017).

CMT2D is a dominant axonal CMT caused by mutations in *GARS1*, which encodes glycyl-tRNA synthetase (GlyRS). > 20 different CMT-causing *GARS1* mutations have been identified, dispersed throughout the gene. Like all CMT-linked aaRS proteins, GlyRS charges tRNA as a dimer; the majority of CMT-causing GlyRS mutations lie within the dimer interface, yet their effect on dimerization is variable. A direct comparison of five spatially diverse mutations using hydrogen-deuterium exchange revealed that all mutations cause a conformational opening of GlyRS (He et al., 2011). This exposes consensus regions that remain buried in GlyRS^{WT}, providing neomorphic surfaces on the mutant protein.

Expression of CMT2W-causing mutant histidyl-tRNA synthetase (HisRS^{P134H}) in a haploid-yeast complementation assay completely blocked cell survival, suggesting loss of enzymatic activity. However, the single wild-type allele present in CMT2W patients is sufficient to support the survival of patient-derived lymphocytes. Like mutant GlyRS, the investigation of four different CMT2W and CMT2N mutant proteins in solution revealed an increase in hydrogen-deuterium exchange compared to HisRS^{WT} and AlaRS^{WT}, respectively (Blocquel et al., 2019; Sun et al., 2021). Similarly, dominant intermediate CMT subtype C (DI-CMTC)-causing mutations in *YARS1*, which encodes tyrosyl-tRNA synthetase (TyrRS), cause a conformational opening in a solution of both TyrRS^{G41R} and TyrRS^{E196K} (Blocquel et al., 2017). Structural relaxation and the opportunity for aberrant protein–protein interaction are therefore common factors in aaRS-CMT.

Animal models have provided further insights into the consequences of disease-linked conformational changes in aaRS proteins. By expressing CMT-associated mutant *GARS1* and *YARS1* in *Drosophila*, Ermanoska et al. (2014, 2023) were able to induce human-relevant phenotypes. For example, neuronal mutant *GARS1* expression caused flies to develop progressive motor impairment, while retinal expression of either mutant *GARS1* or *YARS1* induced a mild rough-eye phenotype, which is a marker for neurotoxicity (Ermanoska et al., 2014); this is perhaps linked to the impairement in actin cytoskeleton organization recently identified in fly models of both CMT2D and DI-CMTC (Ermanoska et al., 2023) and demonstrates similar cell autonomous effects of mutant aaRS proteins.

Drosophila models of DI-CMTC have also been used to elucidate potential mechanisms by which aberrant interactions of mutant TyrRS drive disease. The interaction of wild-type TyrRS with the transcriptional repressor, tripartite motif-containing 28 (TRIM28), in the nucleus is strengthened by CMT-causing mutations. TRIM28-TyrRS forms a complex with histone deacetylase 1 to regulate acetylation and activation of transcription factors, such as E2 promoter binding factor 1 (E2F1) (Bervoets et al., 2019). In HEK-293T cells and patient lymphocytes, expression of TyrRS^{G41R} or TyrRS^{E196K} weakens the interaction between TRIM28 and E2F1. This results in reduced E2F1 de-acetylation and therefore E2F1 hyperactivation, causing upregulation of E2F1 transcriptional targets. Identification of mutant TyrRS interactors offers potentially druggable targets. For instance, hyperactivation of E2F1 can be reversed using the histone deacetylase 1 activator, dexamethasone.

However, dexamethasone administration in *Drosophila* expressing TyrRS^{E196K} failed to rescue locomotor deficits, suggesting that mutant TyrRS has a broader neurotoxic effect. Indeed, transcriptomic analyses in *Drosophila* expressing TyrRS^{WT} or TyrRS^{E196K} revealed 830 differentially expressed genes, including increased expression of 39 transcription factors specifically linked to TyrRS^{E196K} (Bervoets et al., 2019). Accordingly, preventing nuclear access of TyrRS^{E196K} by mutating its nuclear localization signal rescued climbing deficits and restored neuromuscular junction (NMJ) morphology in the DI-CMTC flies. Therefore, inhibiting the interaction of mutant aaRS proteins with select transcriptional regulators in the nucleus may provide therapeutic benefits.

In addition to transcriptional dysregulation, translation impairment has also been identified in animal models of both DI-CMTC and CMT2D. Motor-neuronal expression of three CMT-linked *GARS1* mutants – GlyRS^{E71G}, GlyRS^{G240R}, and GlyRS^{G526R} – leads to NMJ pathology and motor performance deficits (Niehues et al., 2015). Using fluorescent noncanonical amino-acid tagging, where fluorescence intensity is proportional to the rate of protein synthesis, clear reductions in neuronal translation were observed upon mutant *GARS1* expression, independent from aminoacylation defects. Importantly, the same group observed translational slow-down following the expression of three different *YARS1* mutants, suggesting impaired protein synthesis is a critical feature of aaRS-CMT.

Mutant GlyRS has also been shown to aberrantly bind and sequester tRNA^{Gly}, resulting in insufficient codon delivery to the ribosome, restricting translation elongation (Zuko et al., 2021). Codon sequestration and ribosomal stalling were confirmed in tissue, with ~60% more tRNA^{Gly} co-immunoprecipitating with GlyRS from the brains of *Gars*$^{C201R/+}$, compared to wild-type mice (Zuko et al., 2021). Furthermore, ribosomal profiling identified more frequent Gly codons in the ribosomal A-site for

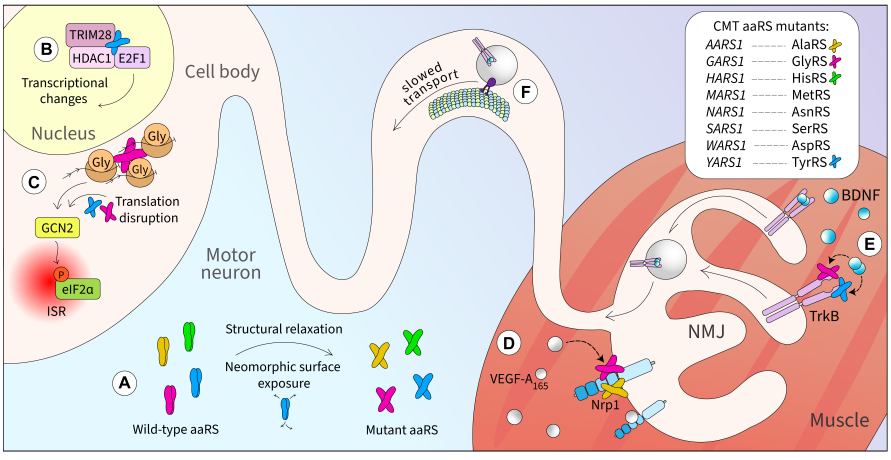

Figure 1 | **Structural relaxation of mutant aaRS proteins drives aberrant interactions underlying CMT.**
(A) Charcot-Marie-Tooth disease (CMT)-causing mutant aminoacyl-tRNA synthetase (aaRS) proteins display conformational opening, mediating aberrant interactions. (B) Mutant tyrosyl-tRNA synthetase (TyrRS) binds more tightly to tripartite motif-containing 28 (TRIM28) in the nucleus, which weakens the TRIM28-E2 promoter binding factor 1 (E2F1) interaction and upregulates transcriptional targets of E2F1. (C) Mutant glycyl-tRNA synthetase (GlyRS) sequesters tRNA^{Gly}, triggering ribosomal stalling. This restricts protein synthesis and activates the integrated stress response (ISR), which are both also caused by mutant TyrRS. (D) GlyRS and alanyl-tRNA synthetase (AlaRS) mutants bind the vascular endothelial growth factor-A$_{165}$ (VEGF-A$_{165}$) receptor, neuropilin 1 (Nrp1), interfering with neuronal survival signaling. (E) Mis-interactions between mutant GlyRS/TyrRS and the receptor for brain-derived neurotrophic factor (BDNF), tropomyosin receptor kinase B (TrkB), impede neurotrophin signaling at the neuromuscular junction (NMJ). (F) This impairs retrograde axonal transport of neurotrophin-containing signaling endosomes to the soma. These common mechanisms contribute to CMT pathogenesis, and their prevention is a promising approach for CMT treatment. eIF2α: eukaryotic translation initiation factor 2α ("P" = phosphorylation); GCN2: general control nondepreprepressible 2; Gly: stalled ribosomes translating tRNA^{Gly}; HDAC1: histone deacetylase 1.

mutant GlyRS compared to wild-type. Accordingly, overexpression of $tRNA^{Gly}$ (GCC codon) in CMT2D mutant *Drosophila* partially rescued translation defects and peripheral neuropathy-like phenotypes. Moreover, the expression of 27 copies of $tRNA^{Gly}$ in CMT2D mice fully restored body weight, muscle mass, motor function, and nerve conduction velocity.

Stalled ribosomes can activate the integrated stress response (ISR) through general control nonderepressible 2 (GCN2), and the ISR has been implicated in aaRS-linked CMT (Spaulding et al., 2021). ISR activation was shown to be increased in spinal motor neurons of CMT2D mice ($Gars^{\Delta ETAQ/+}$, $Gars^{C201R/+}$, and $Gars^{P278KY/+}$) via immunolabeling of phosphorylated eukaryotic translation initiation factor 2α (phospho-eIF2α). These findings were replicated in $Yars^{E196K}$ mice modeling DI-CMTC. To assess the involvement of GCN2 in CMT pathogenesis, $Gars^{P278KY/+}$ mice were crossed with $Gcn2^{-/-}$ knockout mice. Disease onset in $Gars^{P278KY/+}$ mice typically occurs by 2 weeks of age; however, homozygous removal of *Gcn2* prevented the onset of neuropathy, with body weight and motor performance restored to near wild-type levels at 16 weeks of age. Similar improvements were seen using pharmacological inhibitors of GCN2 in $Gars^{\Delta ETAQ/+}$ mice at disease onset. Furthermore, in $Gars^{\Delta ETAQ/+}$ mice, overexpression of $tRNA^{Gly}$ restored phosphorylation of eIF2α to normal levels (Zuko et al., 2021). This suggests that the ISR is activated due to protein translation defects resulting from $tRNA^{Gly}$ sequestration and ribosomal stalling in aaRS-linked CMT.

Additionally, mutant aaRS proteins have been shown to mis-interact and interfere with neuronal survival signaling, which may partly explain the selective vulnerability of neurons to degeneration in CMT. *In vitro* pull-down assays identified strong binding interactions between neuronal transmembrane protein neuropilin 1 (Nrp1) and mutant GlyRS, but not $GlyRS^{WT}$ (He et al., 2015). Furthermore, $GlyRS^{P234KY}$ co-immunoprecipitated with Nrp1 from neural tissue to a greater extent than $GlyRS^{WT}$. Interestingly, the interaction site of mutant GlyRS maps to the binding site of vascular endothelial growth factor-A_{165} (VEGF-A_{165}), which signals through Nrp1 to promote neuroprotective effects. Direct antagonism of VEGF-A_{165}-Nrp1 signaling by mutant GlyRS was shown *in vitro* by the enhanced displacement of VEGF-A_{165} with increasing concentrations of mutant GlyRS, and *vice versa*.

Deficient VEGF signaling drives selective degeneration of motor neurons in mice, promoting the enhancement of VEGF-Nrp1 signaling as a therapeutic approach in CMT. Indeed, unilateral lentiviral overexpression of VEGF-A_{165} in hindlimb muscles of $Gars^{P278KY/+}$ CMT2D mice reduced the muscle weakness observed in the GFP-control-treated contralateral limb. Importantly, overexpression of VEGF-A_{121}, which has overlapping functions with VEGF-A_{165}, but not through Nrp1, did not rescue motor deficits (He et al., 2015). These findings demonstrate the rescue of neuromuscular phenotypes by displacing the aberrantly bound mutant GlyRS from Nrp1, emphasizing the key role this mis-interaction plays in disease. CMT2N-causing mutant $AlaRS^{R329H}$ has also been shown to mis-interact with Nrp1 (Sun et al., 2021), providing evidence for common aberrant binding partners of CMT-mutant aaRS proteins.

A second neurotrophic factor signaling pathway has been implicated in aaRS-linked CMT. CMT2D mutant, but not wild-type, GlyRS aberrantly interacts with the extracellular domain of tropomyosin receptor kinase B (TrkB), the receptor for brain-derived neurotrophic factor (BDNF). Distal neuronal targets, such as muscle, secrete BDNF, which binds TrkB and aids neuronal survival through activation of critical downstream signaling cascades (e.g., MAPK-ERK1/2 and PI3K-AKT). Activated BDNF-TrkB complexes are internalized and sorted into specialized organelles called signaling endosomes, which are retrogradely transported from the axon terminal to the soma, where they relay these survival-promoting signals.

The long axons of peripheral neurons are vulnerable to alterations in axonal transport, which is known to be disturbed in many CMT subtypes. We, therefore, performed intravital imaging of fluorescently labeled signaling endosomes in the sciatic nerve and identified clear axonal transport deficits in $Gars^{C201R/+}$ and $Gars^{\Delta ETAQ/+}$ CMT2D mice. Intramuscular injection of recombinant $GlyRS^{L129P}$ or $GlyRS^{G240R}$, but not $GlyRS^{WT}$, acutely triggered axonal transport deficits in wild-type mice, demonstrating that mutant GlyRS within muscle is sufficient to induce a non-cell autonomous defect in axonal trafficking. Furthermore, increasing the availability of BDNF at the NMJ by intramuscular injection of recombinant protein or AAV8-tMCK-BDNF, rescued axonal transport deficits in CMT2D mice (Sleigh et al., 2023). Signaling endosome adaptor proteins were also restored by AAV8-tMCK-BDNF treatment in $Gars^{C201R/+}$ mice. These findings mirror the phenotypic rescue observed upon increasing muscular availability of VEGF-A_{165} in CMT2D mice, suggesting that excess BDNF displaces mutant GlyRS from TrkB.

We have recently also shown the binding of DI-CMT-mutant $TyrRS^{E196K}$, but not $TyrRS^{WT}$, to TrkB (Rhymes et al., 2024). Analogous to observations with mutant GlyRS, acute intramuscular injection of recombinant $TyrRS^{E196K}$, but not $TyrRS^{WT}$, produced axonal transport deficits in wild-type mice, replicating phenotypes observed in symptomatic $Yars^{E196K/E196K}$ mice. Increasing intramuscular BDNF availability through acute injection of recombinant protein or AAV8-tMCK-BDNF again restored axonal transport in $Yars^{E196K/E196K}$ mice. These findings confirm that several mutant aaRS proteins aberrantly interact with and perturb the function of neurotrophic factor receptors, which provides a potential rationale for the neuronal selectivity of aaRS-linked neuropathies.

In conclusion, it is apparent that mutant aaRS-linked CMTs result, not from a loss of enzymatic aminoacylation function, but from toxic gain(s)-of-function mediated by exposed neomorphic surfaces on mutant proteins driving aberrant mis-interactions. Several pathomechanisms are now known to be shared between different CMT subtypes associated with aaRSs, including mis-interactions with Nrp1 and TrkB, perturbed axonal transport of signaling endosomes and disrupted protein synthesis associated with ISR activation. Preventing the aberrant interactions or boosting the impeded pathways are therefore promising therapeutic strategies in the treatment of CMT subtypes resulting from aaRS mutations.

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References

Bervoets S, Wei N, Erfurth ML, Yusein-Myashkova S, Ermanoska B, Mateiu L, Asselbergh B, Blocquel D, Kakad P, Penserga T, Thomas FP, Guergueltcheva V, Tournev I, Godenschwege T, Jordanova A, Yang XL (2019) Transcriptional dysregulation by a nucleus-localized aminoacyl-tRNA synthetase associated with Charcot-Marie-Tooth neuropathy. Nature Commun 10:5045.

Blocquel D, Li S, Wei N, Daub H, Sajish M, Erfurth ML, Kooi G, Zhou J, Bai G, Schimmel P, Jordanova A, Yang XL (2017) Alternative stable conformation capable of protein misinteraction links tRNA synthetase to peripheral neuropathy. Nucleic Acids Res 45: 8091-8104.

Blocquel D, Sun L, Matuszek Z, Li S, Weber T, Kuhle B, Kooi G, Wei N, Baets J, Pan T, Schimmel P, Yang XL (2019) CMT disease severity correlates with mutation-induced open conformation of histidyl-tRNA synthetase, not aminoacylation loss, in patient cells. Proc Natl Acad Sci U S A 116: 19440-19448.

Ermanoska B, Motley WW, Leitão-Gonçalves R, Asselbergh B, Lee LTH, De Rijk P, Sleegers K, Ooms T, Godenschwege TA, Timmerman V, Fischbeck KH, Jordanova A (2014) CMT-associated mutations in glycyl- and tyrosyl-tRNA synthetases exhibit similar pattern of toxicity and share common genetic modifiers in *Drosophila*. Neurobiol Dis 68:180-189.

Ermanoska B, et al. (2023) Tyrosyl-tRNA synthetase has a noncanonical function in actin bundling. Nat Commun 14:999.

He W, Zhang HM, Chong YE, Guo M, Marshall AG, Yang XL (2011) Dispersed disease-causing neomorphic mutations on a single protein promote the same localized conformational opening. Proc Natl Acad Sci U S A 108:12307-12312.

He W, Bai G, Zhou H, Wei N, White NM, Lauer J, Liu H, Shi Y, Dumitru CD, Lettieri K, Shubayev V, Jordanova A, Guergueltcheva V, Griffin PR, Burgess RW, Pfaff SL, Yang XL (2015) CMT2D neuropathy is linked to the neomorphic binding activity of glycyl-tRNA synthetase. Nature 526:710-714.

Meyer-Schuman R, Antonellis A (2017). Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease. Hum Mol Genet 26(R2):R114-127.

Niehues S, Bussmann J, Steffes G, Erdmann I, Köhrer C, Sun L, Wagner M, Schäfer K, Wang G, Koerdt SN, Stum M, RajBhandary UL, Thomas U, Aberle H, Burgess RW, Yang XL, Dieterich D, Storkebaum E (2015) Impaired protein translation in *Drosophila* models for Charcot-Marie-Tooth neuropathy caused by mutant tRNA synthetases. Nature Commun 6:7520.

Rhymes ER, Simkin RL, Qu J, Villarroel-Campos D, Surana S, Tong Y, Shapiro R, Burgess RW, Yang XL, Schiavo G, Sleigh JN (2024) Boosting BDNF in muscle rescues impaired axonal transport in a mouse model of DI-CMTC peripheral neuropathy. Neurobiol Dis 195:106501.

Sleigh JN, Villarroel-Campos D, Surana S, Wickenden T, Tong Y, Simkin RL, Vargas JNS, Rhymes ER, Tosolini AP, West SJ, Zhang Q, Yang XL, Schiavo G (2023) Boosting peripheral BDNF rescues impaired in vivo axonal transport in CMT2D mice. JCI Insight 8:e157191.

Spaulding EL, et al. (2021) The integrated stress response contributes to tRNA synthetase-associated peripheral neuropathy. Science 373:1156-1161.

Sun L, Wei N, Kuhle B, Blocquel D, Novick S, Matuszek Z, Zhou H, He W, Zhang J, Weber T, Horvath R, Latour P, Pan T, Schimmel P, Griffin PR, Yang XL (2021) CMT2N-causing aminoacylation domain mutants enable Nrp1 interaction with AlaRS. Proc Natl Acad Sci U S A 118:e2012898118.

Zuko A, Mallik M, Thompson R, Spaulding EL, Wienand AR, Been M, Tadenev ALD, van Bakel N, Sijlmans C, Santos LA, Bussmann J, Catinozzi M, Das S, Kulshrestha D, Burgess RW, Ignatova Z, Storkebaum E (2021) tRNA overexpression rescues peripheral neuropathy caused by mutations in tRNA synthetase. Science 373:1161-1166.

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