

## REVIEW ARTICLE

# The role of tRNA synthetases in neurological and neuromuscular disorders

Veronika Boczonadi, Matthew J. Jennings and Rita Horvath

Wellcome Centre for Mitochondrial Research, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK

**Correspondence**

R. Horvath, Institute of Genetic Medicine,  
Newcastle University, Central Parkway, NE1  
3BZ, Newcastle upon Tyne, UK  
Fax: +44 (0)191 2418855  
Tel: +44 (0)191 2418855  
E-mail: rita.horvath@ncl.ac.uk

(Received 27 October 2017, revised 6  
December 2017, accepted 21 December  
2017, available online 1 February 2018)

doi:10.1002/1873-3468.12962

Edited by Wilhelm Just

**Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed enzymes responsible for charging tRNAs with their cognate amino acids, therefore essential for the first step in protein synthesis. Although the majority of protein synthesis happens in the cytosol, an additional translation apparatus is required to translate the 13 mitochondrial DNA-encoded proteins important for oxidative phosphorylation. Most ARS genes in these cellular compartments are distinct, but two genes are common, encoding aminoacyl-tRNA synthetases of glycine (*GARS*) and lysine (*KARS*) in both mitochondria and the cytosol. Mutations in the majority of the 37 nuclear-encoded human ARS genes have been linked to a variety of recessive and dominant tissue-specific disorders. Current data indicate that impaired enzyme function could explain the pathogenicity, however not all pathogenic ARSs mutations result in deficient catalytic function; thus, the consequences of mutations may arise from other molecular mechanisms. The peripheral nerves are frequently affected, as illustrated by the high number of mutations in cytosolic and bifunctional tRNA synthetases causing Charcot-Marie-Tooth disease (CMT). Here we provide insights on the pathomechanisms of CMT-causing tRNA synthetases with specific focus on the two bifunctional tRNA synthetases (*GARS*, *KARS*).**

**Keywords:** aminoacyl-tRNA synthetases; cytosolic and mitochondrial translation; Charcot-Marie-Tooth disease

Aminoacyl-tRNA synthetase proteins (ARS) are a family of nuclear-encoded enzymes that ensure correct translation of the genetic code by conjugating each of the 20 amino acids to their cognate tRNA molecule [1–3]. This aminoacylation reaction provides the substrate for the protein translation process. There are two groups of ARS enzymes: the cytosolic ARS, which are responsible for supplying aminoacyl-tRNA conjugates for general protein translation; mitochondrial ARSs, which are imported into the mitochondrial matrix and charge amino acids to their mitochondrial genome-encoded tRNA molecules (mt-tRNA). For most amino acids, there are dedicated isoforms encoded in the

genome for each of these compartments, but in the case of glycyl-ARS (*GARS*) and lysyl-ARS (*KARS*), the proteins are bifunctional and localised to both the cytosol and the mitochondria [4]. Furthermore, no mitochondrial glutamyl-tRNA synthetase (*QARS*) has been identified; Q-tRNA is instead suggested to be formed by postconjugation modification of glutamic acid [5].

## Mitochondrial tRNA synthetases (*ARS2* genes)

All mt-ARSs are synthesised in the cytosol; addressed to, and imported into, the mitochondria due to the

**Abbreviations**

ARSAL, Autosomal Recessive Spastic Ataxia with Leukoencephalopathy; ARSs, Aminoacyl-tRNA synthetases; CMT, Charcot-Marie-Tooth disease; *GARS*, glycyl-ARS; MLASA, mitochondrial myopathy, lactic acidosis and sideroblastic anaemia; MSC, multi-tRNA synthetase complex; NMJs, neuromuscular junctions; VEGF, vascular endothelial growth factor.

presence of an N-terminal presequence (mitochondrial targeting sequence), which is cleaved upon entry to the mitochondria [6]. Pathogenic variants of mt-ARSs show a variety of phenotypes involving tissues with high energy demand. Despite their crucial housekeeping function and ubiquitous expression, mutations in mt-ARSs have been implicated in a variety of paediatric and adult onset human neurological disorders of the brain, spinal cord and motor neurons in addition to disorders predominantly affecting other tissues manifesting as cardiomyopathy, myopathy, sensorineural hearing loss and endocrine symptoms [1–3,7]. A large number of autosomal recessive disorders specifically affect the brain, and result in lesions of certain neuronal cell types. The most typical clinical presentations are leukoencephalopathy with brainstem and spinal cord involvement and high lactate (LBSL) due to *DARS2* mutations [8], leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL) caused by *EARS2* mutations [9], but other mt-ARS mutations may also cause white matter lesions. Mutations within the *AARS2* gene result in two different phenotypes: late onset ovarian failure and leukodystrophy and infantile mitochondrial cardiomyopathy [10]. *MARS2* mutations have been previously linked to Autosomal Recessive Spastic Ataxia with Leukoencephalopathy (ARSAL) [11], presentations similar to that observed in Alpers–Huttenlocher syndrome have been reported in patients with mutations in *CARS2* [12], *FARS2* [13], *PARS2* [14], *TARS2* [15], *VARS2* [16] *NARS2* [16], *RARS2* [17] and *WARS2* [18]. These diseases display a broad clinical spectrum and may be further complicated with other symptoms such as developmental delay (*NARS2*, *PARS2*), pontocerebellar hypoplasia (*RARS2*), visual impairment (*FARS2*) psychomotor delay (*TARS2*) and microcephaly (*VARS2*). Other ARS2 diseases show characteristic tissue distribution, isolated sensorineural hearing loss (*MARS2* [19], *NARS2* [20]), hearing loss with premature ovarian failure (Perrault syndrome, *HARS2* [21] and *LARS2* [22]), mitochondrial myopathy, lactic acidosis and sideroblastic anaemia (MLASA syndrome (*YARS2* [23]), or hyperuricaemia, pulmonary hypertension renal failure and alkalosis (HUPRA syndrome, *SARS2* [24]). To date, no ARS2 mutations have been reported in human disease with an autosomal dominant mode of inheritance, all mutations are either homozygous or compound heterozygous (Fig. 1).

### Bifunctional and cytosolic tRNA synthetases

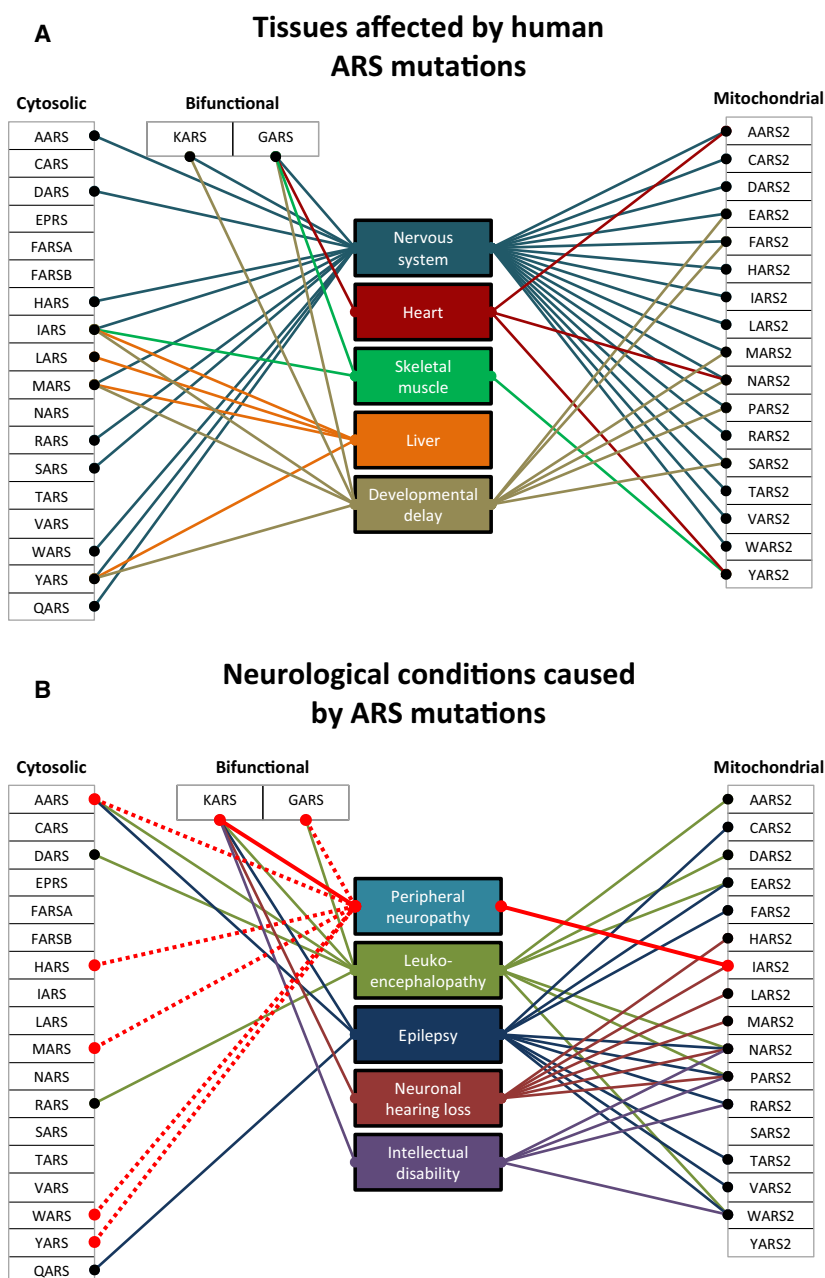
While autosomal dominant pathogenic variants of the cytosolic ARSs were originally reported in peripheral

neuropathies, newly emerging data show a spectrum of recessive disorders. Autosomal dominant neuropathies have been associated with mutations in *AARS* [25,26], *HARS* [27], *YARS* [28], *GARS* [29] and *WARS* [30]. Autosomal recessive ARS mutations have been recently reported in a range of conditions often affecting the central nervous system (microcephaly *AARS*, *QARS* [31,32]), epileptic encephalopathy (*AARS*, *QARS* [31–34]), sensorineural hearing loss (*HARS*, *KARS* [35,36]), developmental delay (*IARS*, *KARS*, *QARS*, *YARS* [34,37–39]), or causing liver dysfunction (*IARS*, *MARS*, *YARS* [39–41]) and lung disease (*MARS* [39,41]). The large variability of the clinical presentations caused by mutations even in a single ARS gene is remarkable and needs further investigations (Fig. 1).

### Charcot–Marie–Tooth disease caused by defects of ARS mutations

Charcot–Marie–Tooth disease (CMT) was the first human disorder to be linked to ARSs mutations. CMT is the most common inherited neurological disease in European populations, with an estimated prevalence of around 1–4 per 10 000 individuals [42,43], and is characterised by symmetric atrophy and weakness in the distal muscles associated with sensory impairment caused by progressive degeneration of the peripheral nerves. CMT is broadly divided into two forms: demyelinating (CMT1) primarily affecting the myelin sheaths of peripheral neurons or axonal form (CMT2) primarily affecting the axons. These can be differentiated based on the electrophysiological and pathological presentation, however intermediate phenotypes exist [44]. Five of the 20 genes encoding cytosolic ARSs (*AARS*, *GARS*, *WARS*, *YARS* and *HARS*) have been reported to cause dominant inherited CMT, with the number expected to increase, and remarkably, no other dominantly inherited disease has been linked with mutations (Fig. 1). Furthermore, two additional ARS genes have also been associated with CMT. Heterozygous missense mutations in *MARS* have been found in two families causing CMT2U [45,46] and peripheral neuropathy was reported in two patients with compound heterozygous mutations in *KARS* [38].

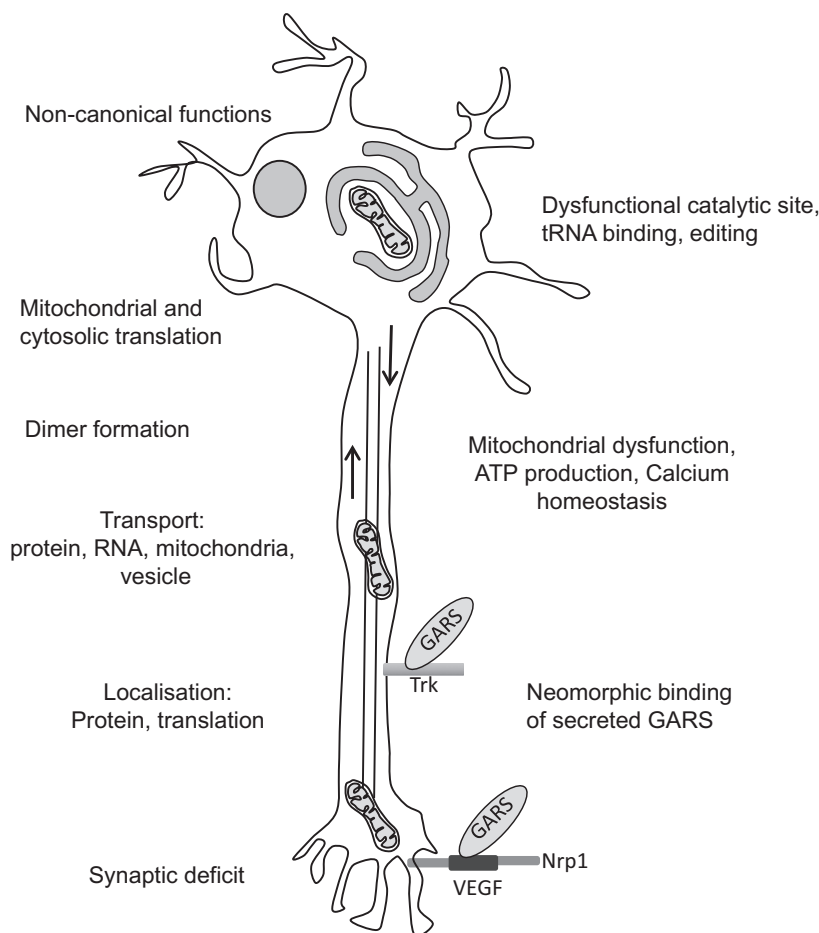
Besides the fact that new mutations are continuously discovered, neither the cause of the selective vulnerability, nor the molecular mechanisms leading to the disease, are well understood. Currently, there are no disease modifying therapies for CMT and clinical care focuses on managing symptoms with physical therapy and orthopaedic surgery. The similarity of clinical presentation resulting from dominant inherited ARS



**Fig. 1.** Clinical variability of diseases caused by ARSs mutations. (A) Tissues commonly affected by mutations in cytosolic, bifunctional and mitochondrial ARS genes. (B) Common neurological presentations reported in cytosolic, bifunctional and mitochondrial ARS genes, with peripheral neuropathy highlighted. Solid line indicates dominant mode of inheritance, dashed line indicates recessive mode of inheritance. References: *AARS* [33,48,50,96], *DARS* [97], *HARS* [27], *IARS* [37,40], *MARS* [45,98], *RARS* [99], *SARS* [18], *WARS* [30], *ARS* [28,39], *QARS* [34], *GARS* [7,29,71], *KARS* [38], *AARS2* [10,67], *CARS2* [12], *DARS2* [8], *EARS2* [9], *FARS2* [13,100], *HARS2* [21], *IARS2* [101,102], *LARS2* [22], *MARS2* [11,19], *NARS2* [20,103,104], *PARS2* [14,103], *RARS2* [17,105], *SARS2* [24], *TARS2* [15], *VARS2* [15,16], *WARS2* [18,106], *YARS2* [23,107].

mutations implies a shared mechanism of disease, though no mechanism so far has been proven. The major mechanisms proposed consist of: reduced aminoacylation activity, altered dimerisation or

localisation, gain-of-function pathogenic interactions and loss of noncanonical function (Fig. 2). We examine the evidence supporting, contradicting and lacking for each mechanism.



**Fig. 2.** Possible pathological pathways of axonal degeneration in tRNA synthetase mutations.

## Evidence of impaired catalytic function

The central housekeeping role of ARS proteins in cellular physiology implies that defects in their function would cause cellular dysfunction. Reduction in both the aminoacylation reaction rate and cognate accuracy of the enzyme present possible pathogenic mechanisms. ARS proteins act via a two-step process to facilitate tRNA-amino acid conjugation and the activity of mutant ARS enzymes can be determined *in vitro* using recombinant human protein [47]. These enzymatic assays have generally found pathogenic variants of cytosolic ARS to cause at least some degree of reduced enzymatic activity, with the notable exception of the *YARS* p.Glu196Lys variant (Table 1). Yeast complementation studies on the translational function of the mutant ARS and constructs using CMT-associated variants have been shown to cause either lethality or reduced growth in most, but not all, cytosolic ARS enzymes (Table 1). Although there is strong evidence of impaired catalytic function in CMT-

associated cytosolic ARS variants, the potential mechanism is less well characterised; with possibilities including dysfunction of the catalytic site, abnormal protein dimer formation and altered localisation.

By mapping ARS mutations onto the 3D protein structures determined by X-ray crystallography, it is possible to determine whether they are clustered around particular functional sites. The p.Asp71Tyr and p.Gly102Arg *AARS* mutations are predicted to lie within the catalytic domain, whereas the common p.Arg329His mutation is predicted to be at the tRNA-binding domain and the p.Asp893Asn and p.Glu688Gly mutations are reported within the editing domain [48–50]. Moving to *YARS* and *HARS*, most reported mutations are predicted to lie on the catalytic domain, though importantly, unlike *AARS*, these proteins do not contain editing domains [28,46,51–53]. Though no crystal structure has been determined for *MARS*, computational prediction maps one mutation, p.Arg6128Cys, between the catalytic and tRNA-binding domains, and another mutation, p.Pro800Thr, to the tRNA-binding domain [45,46], while the recently

**Table 1.** Functional evidence on pathogenicity of CMT-causing tRNA synthetase mutations. CMT, Charcot–Marie–Tooth disease; dHMN, distal hereditary motor neuropathy; AARS, alanyl-tRNA synthetase; YARS, tyrosine-tRNA synthetase; HARS, histidyl-tRNA synthetase; MARS, methionyl-tRNA synthetase; WARS, tryptophanyl-tRNA synthetase; GARS, glycyl-tRNA synthetase; KARS, lysyl-tRNA synthetase; NRP1, Neuropilin-1 precursor; VEGF, Vascular endothelial growth factor; TrkA/B/C, tropomyosin receptor kinase A/B/C.

Experimental method	Effect of mutations
<b>Cytosolic tRNA synthetase</b>	
<i>AARS</i>	Recombinant enzyme activity: Reduced: p.Asn71Tyr [50]; p.Arg329His [50]; Yeast growth: Lethal: p.Asn71Tyr [50]; Gly102Arg [108] p.Arg329His [50] Mouse: Purkinje cells loss, ataxia, no effect on NMJ morphology: Ala734Glu [109] (mutation in mouse <i>Aars</i> )
<i>HARS</i>	Yeast growth: Lethal: p.Thr132Ile [27]; p.Pro134His [27]; p.Arg137Gln [52]; p.Asp364Tyr [27] Reduced: p.Asp175Glu [27] <i>Caenorhabditis elegans</i> : Motor neuron toxicity: p.Arg137Gln (targeted to 4-aminobutyrate neurons) [52]
<i>MARS</i>	Yeast growth: Lethal: p.Arg618Cys (mutation has incomplete penetrance) [45] p.Pro800Thr lacks functional evidence
<i>WARS</i>	Recombinant enzyme activity: Abnormal: His257Arg [30] Yeast growth: Abnormal: Hist257Arg [30]
<i>YARS</i>	Recombinant enzyme activity: Reduced: p.Gly41Arg [110]; p.153-156delValLysGlnVal [110] Conflicting (reduced and normal): p.Glu196Lys [28,110] Yeast growth: Lethal: p.Gly41Arg [28] Reduced: p.Glu196Gln [51]; p.Glu196Lys (yeast orthologue, human is normal) [28] Human N2a neuroblastoma cells: Reduced axonal distribution: p.Gly41Arg [28],
<b>Bifunctional tRNA synthetase</b>	
<i>GARS</i>	Recombinant enzyme activity: Reduced: p.Ala57Val (minor reduction) [61]; p.Leu129Pro [111]; p.Asp146Asn [61]; p.Ser211Phe [61]; p.Gly240Arg [61]; p.Pro244Leu [61]; p.Ile280Phe [61]; p.His418Arg [61]; p.Gly526Arg [61]; p.Gly598Ala [61] Normal: p.Glu71Gly [54]; p.Asp500Asn [54]; p.Ser581Leu [54] Yeast growth: Lethal: p.Pro244Leu; p.His418Arg [111]; p.Gly526Arg [111]; Reduced: p.Leu129Pro [111]; p.Asp146Asn [61] Normal: p.Glu71Gly [111]; p.Gly240Arg [60,111]; p.Ile280Phe [61]; p.Gly598Ala [60] <i>Drosophila</i> : Abnormal dendritic morphology, altered mitochondrial translation, and progressive NMJ degeneration: p.Glu71Gly [89]; p.Gly240Arg [89,112]; p.Gly526Arg [89]; p.Pro234Lys,Tyr [112] Mouse: Secreted mutant GARS binds to NRP1, interfering with VEGF NMJ dysfunction, aberrant binding to TrkA, TrkB, and TrkC, receptors in sensory neurons: p.Pro278Lys [75,76]; p.Cys201Arg [75,76]
<i>KARS</i>	Recombinant enzyme activity: Reduced: p.Leu133His [38] Yeast growth: Lethal: p.Tyr173Serfs*7 [38] Normal: p.Leu133His [38]

reported *WARS* mutation, p.His257Arg, is in the catalytic domain [30]. The diversity of mutations between the different major domains reported for cytosolic ARS rules out dysfunction at either the catalytic site or in tRNA-binding as the sole mode of pathogenicity. Furthermore, the lack of clustering within any domain suggests impairment of canonical domain function may not be relevant to the disease pathophysiology, with secondary roles of the affected residues potentially being important.

Questions remain as to how the reduction in catalytic capacity in dominant mutations causes peripheral neuropathy specifically, without affecting other tissues with high demand for protein synthesis. A possibility is that similar biochemical impairment is seen as in recessive

phenotypes but milder due to the presence of some wild-type protein and therefore specifically effecting the unusual proteomic demands of peripheral nerves. Alternatively, dimerisation, localisation or more complex elements specific for peripheral nerves such as neuromuscular junction, neuronal receptors (neuropilin 1) may be involved in conjunction with the catalytic deficit to cause peripheral nerve-specific presentation. The question about aminoacylation deficiency and disease causality is controversial and still open in the literature, although we think that it may be causative at least in some mutations. However, other pathological mechanisms may have an additive effect and for certain mutations aminoacylation is not altered and different factors may explain the disease phenotype.



## Dimerisation

All CMT-associated ARS enzymes (AARS, GARS, WARS, YARS, HARS) catalytically function as dimers, and disruption to the dimer functionality has been suggested as a mechanism of pathogenicity in ARS-related CMT. Clustering of mutations of GARS has been reported around the homodimer interface, and mutations affecting the dimer interface have been shown to cause subtle conformational alterations to mutant wild-type dimers [54,55]. In addition, Dewan *et al.* [56] demonstrated that due to a H7 helix located in motif 1 of human KARS (member of the multi-synthetase complex), it is also capable of dimerisation. The effects of mutations on dimerisation have not been reported for either AARS or WARS, and some YARS mutations have been found to have no effect on monomer–dimer equilibrium [57–59]. A role of dimerisation presents several mechanistic possibilities, namely that (a) CMT-associated variants cannot form dimers and are therefore inactive, (b) CMT-associated variants form unstable dimers which are subsequently either aggregated or degraded or that (c) CMT-associated variants do form stable dimers which are either deficient in aminoacylation activity, deficient in other noncanonical functions or have a toxic gain-of-function.

Addressing the first possibility, several *GARS* and *YARS* variants have been shown to have binding affinities equivalent to, or exceeding that of, the wild-type protein [54,57], thus ruling out decreased dimerisation. Concerning dimer stability, structural studies of CMT-associated *YARS* variants show both increased and decreased protein stability, suggesting protein instability and aggregation *per se* is not a common mechanism. Animal studies of mutant and wild-type *YARS* and *GARS* have shown equivalent expression of the protein in the cell body [54,60,61], and furthermore, specific quantification of the transfected mutant *GARS* is equivalent at the cell body to wild-type [54], showing that the expression level of the protein is unaffected and therefore degradation is not a factor. It must therefore be asked: what evidence exists for the role of stable toxic ARS dimers? *In vitro* enzymatic assays have already shown that although aminoacylation is often affected in mutant homodimers, it is not a common feature of all CMT-associated mutations and therefore, considering the dominant mode of inheritance, it should be examined whether the aminoacylation activity of tRNA heterodimers may have a role. A study, looking at CMT-associated mutations in zebrafish showed that overexpression of the homologue of the nondimerising p.Thr130Lys mutation reduced NMJ toxicity of the CMT-associated

p.Gly526Arg mutation when alongside normal expression of wild-type *gars*, suggesting that dimerisation of the mutant protein with the wild-type is necessary for pathogenicity [62]. Supporting the potential for a stable dimer engaging in toxic neomorphic binding, a recent study examined the structural and catalytic properties of three CMT-associated *YARS* variants and showed that, while other properties such as conformational alteration of dimer structure were not common, exposure of an internal site on the dimers was, and this facilitates increased binding to TRIM28 [57]. How this or other neomorphic-binding partners are involved in the pathogenesis of remains to be elucidated, but it provides a principle along which variants in other genes could be examined.

## Cellular localisation

Alteration to the cellular distribution of cytosolic enzymes presents another pathogenic mechanism. If mutations disrupt the distribution of ARS proteins within the cell, and cause a deficit of protein activity in the axons of peripheral neurons, which are often much longer than axons of the central nervous system, this may plausibly explain why mutations cause length-dependent pathology in the long axons. However, the few studies that have investigated distribution both *in vitro* and *in vivo* have shown mixed results in alterations resulting from CMT-causing mutations. In the case of *YARS*, a clustering of expression can be observed around neuronal projections, which was abolished in N2a cells transfected with CMT-causing *YARS* mutations (p.Gly45Arg, p.Gln196Lys) [28]. A *HARS* mutation, p.Arg137G [52], modelled in *Caenorhabditis elegans* has shown a normal pattern of axonal expression alongside morphological abnormalities and three different *YARS* variants (p.Gly41Arg, p.153-156delValLysGlnVal, p.Glu196Lys) modelled in *Drosophila* showed no change in axonal localisation. Also the distribution of the mutant *Gars* in a CMT2D mouse model indicated unaltered localisation in the sciatic nerve fibres [41]. Therefore, despite evidence of altered localisation in some ARS mutations, it is currently unknown whether it is a common mechanism of disease.

## Noncanonical functions

The lack of clear mechanistic evidence supporting deficits in aminoacylation (catalytic function) has led the suggestion that cytosolic ARS disease may relate to entirely different ‘noncanonical’ ARS functions. This could include the loss of a noncanonical physiological

ARS function, or alternatively can be a gain of pathological function. During the evolutionary process, cytosolic ARS proteins, or complexes formed from them, have accrued additional functions to their core 'canonical' role in aminoacylating tRNA molecules with roles as diverse as mRNA splicing [63], modulation of angiogenesis [64] as well as roles in injury repair in the peripheral nervous system [65,66]. Whether disease-associated mutations in cytosolic ARSs cause disruption to a noncanonical function in the peripheral nervous system, remains to be confirmed, and if any alterations are found it is important to show that this is replicated across different mutations and critically across the disease-associated genes, which are not known to share many noncanonical functions. Investigation of loss of noncanonical function is further complicated as known functions are often not present in lower organisms, therefore limiting the relevance of more simple biological models such as yeast or *C. elegans*.

### Neuromuscular diseases caused by defects of bifunctional ARS mutations

Reported mutations in bifunctional enzymes, GARS and KARS show either recessive or dominant inheritance, with strikingly different tissue specific clinical manifestations. Dominant *GARS* mutations have been identified in patients with axonal peripheral neuropathy (CMT2D or distal spinal muscular atrophy type: dSMA-V) [29,55] and recessive mutations were reported with patients with predominant cardiomyopathy leading to death in an infant or cardiomyopathy and mitochondrial myopathy with exercise intolerance [7,67,68]. Mutations in *KARS* have been so far associated with various phenotypes: autosomal recessive CMT [38], nonsyndromic hearing loss [36], childhood-onset visual impairment with progressive microcephaly with combined mitochondrial respiratory chain defect [68], and a severe cardiomyopathy associated with myopathy, intellectual disability and lactic acidosis [69], and recently with early-onset, profound sensorineural hearing loss and leukoencephalopathy [70], making clear genotype–phenotype correlations difficult for *KARS*.

Despite of many efforts it is not clear how variants of bifunctional ARSs impact the mitochondrial function and whether or not the mitochondrial impairment is solely recessive, similar to mutations in other nuclear-encoded genes affecting mitochondrial translation, while neuropathy is caused by the defect of another, probably cytosolic, function.

With the exceptions of one patient with recessive *KARS* mutations developed CMT, recessive *GARS* or

*KARS* mutations are not associated with peripheral neuropathy, rather with prominent heart and skeletal muscle dysfunction. As the majority of patients with recessive *GARS* and *KARS* mutations are children, it is possible that neuropathy will develop in a later stage; however, no reliable clinical data support this hypothesis. There is only limited clinical information available on parents of children with recessive disease, who carry heterozygous *GARS* or *KARS* mutations. In one case, the father presented with mild neuropathy detected on electrophysiology, but the mother showed no clinical or electrophysiological alterations [71]. These data suggest that the mechanism of disease is different for dominant and recessive mutations. It is possible that recessive mutations have a detrimental effect on mitochondrial translation only in homozygous state or in combination with another heterozygous pathogenic mutation, leading to tissue-specific manifestations in the heart, skeletal muscle, brain or inner ears potentially by loss-of-function, whereas for dominant mutations, another mechanism leads to peripheral nerve lesion, one similar to other cytosolic CMT-causing ARS mutations (see above) (Fig. 2, Table 1).

### Gain-of-function through neomorphic binding

Among various ARSs, the secretion of wild-type KARS (colon cancer cells, macrophages) and GARS (immune cells, mouse motor neurons and differentiated myotubes) to extracellular space via exosomes has been confirmed by several studies [72,73]. Since this observation, it has been investigated whether pathogenic *GARS* mutations introduce abnormal conformational changes leading to aberrant protein interactions. Supporting a toxic gain-of-function, recent findings in mice carrying pathogenic *Gars* mutations show that the mutant Gars protein acquires a neomorphic-binding activity that directly antagonises an essential signalling pathway for motor neuron survival [73]. CMT-causing mutations alter the conformation of Gars, enabling it to bind the neuropilin 1 (Nrp1) receptor. This aberrant interaction competitively interferes with the binding of the cognate ligand vascular endothelial growth factor (VEGF) to Nrp1. Genetic reduction of Nrp1 in mice worsens the neuropathy, whereas enhanced expression of VEGF improves motor function. These findings link the selective neuronal pathology to the neomorphic-binding activity of mutant GARS that antagonises the VEGF–Nrp1 interaction, and indicate that the VEGF–Nrp1 signalling axis is an actionable target for treating CMT2D. While VEGF–

NRP1 signalling has been implicated to play a role in the nervous system [74] supporting its role in neuronal migration and axonal guidance, this pathway does not fully explain the late onset disease progression. This pathway was also linked to developmental cardiovascular defects and vascular homeostasis which was not observed in *Gars* mouse models [75]. Furthermore, abnormal interference of mutant GARS with Trk receptors has been shown to have a role in the development of sensory neurons [76]. Exploration of the interacting partners of several mutant ARS variants may shed light on a common pathological mechanism explaining the neuromuscular phenotype.

### Mitochondrial function

Several genetic forms of CMT are caused by mutations in proteins affecting mitochondrial function such as mitofusin 2 (MFN2), ganglioside-induced differentiation-associated-protein 1 (GDAP1), heat shock protein beta 8 (HSPB8), and heat shock protein beta 1 (HSPB1) [77], suggesting that mitochondria are important in motor neurons. As mutations in other cytosolic ARSs cause similar neuropathies, it is possible that combination of several mechanisms including both cytoplasmic and mitochondrial function might play a role in the disease phenotype, or a so far unknown noncanonical function specifically targeting peripheral nerves explains why peripheral neuropathy is seen in isolation.

Investigation of mitochondrial function of bifunctional ARS has been reported in several studies. A mitochondrial isoform of KARS have been reported to interact with SOD1 in the mitochondria of the nervous system [78], which was suggested to contribute to mitochondrial dysfunction in ALS. More extensive studies showed loss-of-function effect of *GARS* mutations in *Drosophila*, which leads to progressive defects of dendritic morphology due to altered mitochondrial translation [79]. Spaulding and her colleagues also found an interesting link by identifying significantly fewer mitochondria at nerve terminals in two different mouse models of *GARS* mutations [80]. This observation highlights that mitochondrial function may be altered in *GARS* mutations and a variety of mechanisms may underlie the mitochondrial dysfunction, including deficits in ATP production, failure of axonal transport, or changes in intracellular calcium dynamics. Keeping in line with this, antioxidants including ascorbic acid and forms of glutathione were reduced in mutant *Gars*<sup>Nmf249/+</sup> mice spinal cord and sciatic nerve, suggesting changes in oxidative pathways [81]. Therefore, mitochondrial dysfunction, whether primary

or secondary, can be suggested as contributing factor and further studies on animal models in combination with patient-derived motor neurons are warranted. Our preliminary data in *GARS*-related neuropathy suggest that mitochondrial dysfunction involving very specific pathways is essential for the motor nerve dysfunction (unpublished data).

### Synaptic dysfunction

As neurons and muscle fibres are highly metabolically active, it is rational to hypothesise that the neuromuscular junctions (NMJs) are affected by even minor mitochondrial dysfunction. Recent data showed synaptic maturation abnormalities with specific, progressive NMJ degeneration in *Drosophila* where ubiquitous mutant *gars* was expressed [82]. In addition to this, abnormal axonal transport was also suggested as potential disease mechanisms, which have been reported in a range of CMT2 models [60]. To further strengthen this theory, both *Gars* mutant mouse models show muscle atrophy associated with compromised development of the NMJ prior to synaptic degeneration and highlight the neuromuscular synapse as an important site of early, selective pathology in CMT2D mice [83,84].

### Abnormal axonal translation

The housekeeping function of the ARSs is to catalyse the aminoacylation of tRNA with their cognate amino acid, which is the first step of protein synthesis. Data from *in vitro* aminoacylation assay and yeast complementation studies showed that some CMT mutations do not affect the enzymatic activity, indicating that loss of aminoacylation activity alone is not required to cause peripheral neuropathy [85]. Furthermore, in both CMT2D mouse models (*Gars*<sup>Nmf249/+</sup> and *Gars*<sup>C201R/+</sup> mice), mutations in *Gars* do not reduce tRNA<sup>Gly</sup> aminoacylation activity [54,83,86,87]. On the other hand, the long axons of the peripheral nervous system could be particularly sensitive to defective protein translation. This idea, that mutant ARSs could affect protein translation within the axons, was investigated in a *Drosophila* model using a new method based on noncanonical amino acid tagging, which allows to cell-type-specifically monitor translation *in vivo* [88,89]. Direct evaluation of protein translation rates in sensory and motor neurons expressing CMT-associated mutant GARS (both cytoplasmic and mitochondrial forms) and YARS showed global translational slowdown which was not attributed to the aminoacylation activity. It is therefore possible that defective protein



synthesis locally in the axons act as a common pathogenic mechanism underlying mutant tRNA synthetase-associated CMT [89]. With the focus on bifunctional ARSs more in-depth study required to investigate how the mutant GARS and KARS affect cytosolic and mitochondrial protein synthesis in isolation and how the variants influence the interaction between two translational systems.

## Noncanonical functions of bifunctional ARSs

It is well documented that several cytoplasmic ARSs acquired unique signal mediators during evolution, which facilitate numerous noncanonical biological processes [90]. Among the various functions for ARSs includes inflammation, transcriptional regulation, translational regulation, apoptosis, rRNA transcription, angiogenesis, cell signalling, autoimmune response, tRNA maturation and mitochondrial RNA splicing [91]. Also, several cytosolic and bifunctional ARSs have been linked to biological processes besides protein translation. Detection of GARS in the serum of normal human subjects and mouse [72] as well as in patients with cancer [92] indicates a role in immune defence system, and its therapeutic potential against tumorigenesis has been suggested [72]. Other studies showed a chaperone-like function for GARS whereby GARS interacts with ubiquitin-like proteins and facilitates neddylation, which critically regulates cell cycle progression by degrading key regulators of the cell cycle and hence play a crucial role in selective protein degradation [93].

The cytosolic form of KARS is part of the multi-tRNA synthetase complex (MSC) and involved in the housekeeping role; however, N-terminal cleavage of KARS by caspase-8 and an interaction with syntenin through its C-terminal end leads to dissociation from the MSC complex and translocation to the plasma membrane where it associates with and stabilises a 67-kDa laminin receptor (p67LR) [94]. A study on HCT116 colon cancer cells showed that KARS localised to the plasma membrane is involved in cell–cell adhesion and more importantly suppression of KARS leads to impaired migratory abilities [95]. Migration defects in CMT-associated mutations may be involved in peripheral nerve lesions. Our group identified delayed cellular migration in patient-derived cells carrying pathogenic dominant *GARS* mutations (unpublished data).

Recently, new investigations using a metabolomics analysis in a mouse model of CMT2D, (*Gars*<sup>Nmf249/+</sup>) attempted to identify changes in metabolite abundance that may be indicative of the pathophysiology [81]. Despite the fact that the metabolomics analysis of spinal

cord from a CMT2D mouse model revealed distinct metabolite fingerprints, associated with the disease (ascorbic acid, carnitine, glycine), none of the studied candidates proved to be specific to the disease development or were confirmed in CMT patient samples. Still the potential promise of metabolite profiling to understand disease mechanisms cannot be disregarded.

## Conclusions and perspectives

In this review, we summarised the research on the pathomechanisms of ARS mutations causing peripheral neuropathy; however, what remains particularly unclear is the cause of the high degree of tissue specificity. Some excellent *in vivo* (mice, *Drosophila*) and *in vitro* (human fibroblasts, iPSCs, neuronal cells) model systems have been developed to study ARSs in different tissues, which will hopefully provide further insights into the disease mechanism. Various non-canonical functions of ARSs have become increasingly interesting, and by being secreted could have widespread effects or could act as potential biomarkers. To date, there is no good biomarker available to study the progression of neuropathy in these slowly progressive diseases. Understanding why peripheral nerves are predominantly affected will open potential therapeutic targets for a larger group of CMT patients; however, further research is still needed.

## Acknowledgements

RH is a Wellcome Trust Investigator (109915/Z/15/Z), who receives support from the Wellcome Centre for Mitochondrial Research (203105/Z/16/Z), Medical Research Council (UK) (MR/N025431/1) and the European Research Council (309548).

## References

- 1 Meyer-Schuman R and Antonellis A (2017) Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease. *Hum Mol Genet* **26**, R114–R127.
- 2 Sissler M, Gonzalez-Serrano LE and Westhof E (2017) Recent advances in mitochondrial aminoacyl-tRNA synthetases and disease. *Trends Mol Med* **23**, 693–708.
- 3 Ognjenovic J and Simonovic M (2017) Human aminoacyl-tRNA synthetases in diseases of the nervous system. *RNA Biol*, doi: 10.1080/15476286.2017.1330245.
- 4 Bonnefond L, Fender A, Rudinger-Thirion J, Giege R, Florentz C and Sissler M (2005) Toward the full set of human mitochondrial aminoacyl-tRNA synthetases: characterization of AspRS and TyrRS. *Biochemistry* **44**, 4805–4816.

- 5 Echevarria L, Clemente P, Hernandez-Sierra R, Gallardo ME, Fernandez-Moreno MA and Garesse R (2014) Glutamyl-tRNA<sup>Gln</sup> amidotransferase is essential for mammalian mitochondrial translation in vivo. *Biochem J* **460**, 91–101.
- 6 Carapito C, Kuhn L, Karim L, Rompais M, Rabilloud T, Schwenzer H and Sissler M (2017) Two proteomic methodologies for defining N-termini of mature human mitochondrial aminoacyl-tRNA synthetases. *Methods* **113**, 111–119.
- 7 Opreacu SN, Chepa-Lotrea X, Takase R, Golas G, Markello TC, Adams DR, Toro C, Gropman AL, Hou YM, Malicdan MCV *et al.* (2017) Compound heterozygosity for loss-of-function GARS variants results in a multisystem developmental syndrome that includes severe growth retardation. *Hum Mutat* **38**, 1412–1420.
- 8 Miyake N, Yamashita S, Kurosawa K, Miyatake S, Tsurusaki Y, Doi H, Saito H and Matsumoto N (2011) A novel homozygous mutation of DARS2 may cause a severe LBSL variant. *Clin Genet* **80**, 293–296.
- 9 Sahin S, Cansu A, Kalay E, Dincer T, Kul S, Cakir IM, Kamasak T and Budak GY (2016) Leukoencephalopathy with thalamus and brainstem involvement and high lactate caused by novel mutations in the EARS2 gene in two siblings. *J Neurol Sci* **365**, 54–58.
- 10 Dallabona C, Diodato D, Kevelam SH, Haack TB, Wong LJ, Salomons GS, Baruffini E, Melchionda L, Mariotti C, Strom TM *et al.* (2014) Novel (ovario) leukodystrophy related to AARS2 mutations. *Neurology* **82**, 2063–2071.
- 11 Bayat V, Thiffault I, Jaiswal M, Tétreault M, Donti T, Sasarman F, Bernard G, Demers-Lamarche J, Dicaire MJ, Mathieu J *et al.* (2012) Mutations in the mitochondrial methionyl-tRNA synthetase cause a neurodegenerative phenotype in flies and a recessive ataxia (ARSAL) in humans. *PLoS Biol* **10**, e1001288.
- 12 Coughlin CR 2nd, Scharer GH, Friederich MW, Yu HC, Geiger EA, Creadon-Swindell G, Collins AE, Vanlander AV, Coster RV, Powell CA *et al.* (2015) Mutations in the mitochondrial cysteinyl-tRNA synthase gene, CARS2, lead to a severe epileptic encephalopathy and complex movement disorder. *J Med Genet* **52**, 532–540.
- 13 Almalki A, Alston CL, Parker A, Simonic I, Mehta SG, He L, Reza M, Oliveira JM, Lightowlers RN, McFarland R *et al.* (2014) Mutation of the human mitochondrial phenylalanine-tRNA synthetase causes infantile-onset epilepsy and cytochrome c oxidase deficiency. *Biochim Biophys Acta* **1842**, 56–64.
- 14 Mizuguchi T, Nakashima M, Kato M, Yamada K, Okanishi T, Ekhilevitch N, Mandel H, Eran A, Toyono M, Sawaishi Y *et al.* (2017) PARS2 and NARS2 mutations in infantile-onset neurodegenerative disorder. *J Hum Genet* **62**, 525–529.
- 15 Diodato D, Melchionda L, Haack TB, Dallabona C, Baruffini E, Donnini C, Granata T, Ragona F, Balestri P, Margollicci M *et al.* (2014) VARS2 and TARS2 mutations in patients with mitochondrial encephalomyopathies. *Hum Mutat* **35**, 983–989.
- 16 Baertling F, Alhaddad B, Seibt A, Budaeus S, Meitinger T, Strom TM, Mayatepek E, Schaper J, Prokisch H, Haack TB *et al.* (2017) Neonatal encephalocardiomyopathy caused by mutations in VARS2. *Metab Brain Dis* **32**, 267–270.
- 17 Luhl S, Bode H, Schlötzer W, Bartsakoulia M, Horvath R, Abicht A, Stenzel M, Kirschner J and Grünert SC (2016) Novel homozygous RARS2 mutation in two siblings without pontocerebellar hypoplasia - further expansion of the phenotypic spectrum. *Orphanet J Rare Dis* **11**, 140.
- 18 Musante L, Püttmann L, Kahrizi K, Garshasbi M, Hu H, Stehr H, Lipkowitz B, Otto S, Jensen LR, Tzschach A *et al.* (2017) Mutations of the aminoacyl-tRNA-synthetases SARS and WARS2 are implicated in the etiology of autosomal recessive intellectual disability. *Hum Mutat* **38**, 621–636.
- 19 Webb BD, Wheeler PG, Hagen JJ, Cohen N, Linderman MD, Diaz GA, Naidich TP, Rodenburg RJ, Houten SM and Schadt EE (2015) Novel, compound heterozygous, single-nucleotide variants in MARS2 associated with developmental delay, poor growth, and sensorineural hearing loss. *Hum Mutat* **36**, 587–592.
- 20 Simon M, Richard EM, Wang X, Shahzad M, Huang VH, Qaiser TA, Potluri P, Mahl SE, Davila A, Nazli S *et al.* (2015) Mutations of human NARS2, encoding the mitochondrial asparaginyl-tRNA synthetase, cause nonsyndromic deafness and Leigh syndrome. *PLoS Genet* **11**, e1005097.
- 21 Pierce SB, Chisholm KM, Lynch ED, Lee MK, Walsh T, Opitz JM, Li W, Klevit RE and King MC (2011) Mutations in mitochondrial histidyl tRNA synthetase HARS2 cause ovarian dysgenesis and sensorineural hearing loss of Perrault syndrome. *Proc Natl Acad Sci U S A* **108**, 6543–6548.
- 22 Pierce SB, Gersak K, Michaelson-Cohen R, Walsh T, Lee MK, Malach D, Klevit RE, King MC and Levy-Lahad E (2013) Mutations in LARS2, encoding mitochondrial leucyl-tRNA synthetase, lead to premature ovarian failure and hearing loss in Perrault syndrome. *Am J Hum Genet* **92**, 614–620.
- 23 Riley LG, Gersak K, Michaelson-Cohen R, Walsh T, Lee MK, Malach D, Klevit RE, King MC and Levy-Lahad E (2013) Phenotypic variability and identification of novel YARS2 mutations in YARS2 mitochondrial myopathy, lactic acidosis and sideroblastic anaemia. *Orphanet J Rare Dis* **8**, 193.

- 24 Belostotsky R, Ben-Shalom E, Rinat C, Becker-Cohen R, Feinstein S, Zeligson S, Segel R, Elpeleg O, Nassar S and Frishberg Y (2011) Mutations in the mitochondrial seryl-tRNA synthetase cause hyperuricemia, pulmonary hypertension, renal failure in infancy and alkalosis, HUPRA syndrome. *Am J Hum Genet* **88**, 193–200.
- 25 Bansagi B, Antoniadis T, Burton-Jones S, Murphy SM, McHugh J, Alexander M, Wells R, Davies J, Hilton-Jones D, Lochmüller H *et al.* (2015) Genotype/phenotype correlations in AARS-related neuropathy in a cohort of patients from the United Kingdom and Ireland. *J Neurol* **262**, 1899–1908.
- 26 Bansagi B, Griffin H, Whittaker RG, Antoniadis T, Evangelista T, Miller J, Greenslade M, Forester N, Duff J, Bradshaw A *et al.* (2017) Genetic heterogeneity of motor neuropathies. *Neurology* **88**, 1226–1234.
- 27 Safka Brozkova D, Deconinck T, Griffin LB, Ferbert A, Haberlova J, Mazanec R, Lassuthova P, Roth C, Pilunthanakul T, Rautenstrauss B *et al.* (2015) Loss of function mutations in HARS cause a spectrum of inherited peripheral neuropathies. *Brain* **138**, 2161–2172.
- 28 Jordanova A, Irobi J, Thomas FP, Van Dijck P, Meerschaert K, Dewil M, Dierick I, Jacobs A, De Vriendt E, Guerguelcheva V *et al.* (2006) Disrupted function and axonal distribution of mutant tyrosyl-tRNA synthetase in dominant intermediate Charcot-Marie-Tooth neuropathy. *Nat Genet* **38**, 197–202.
- 29 Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin SQ, Jordanova A, Kremensky I, Christodoulou K, Middleton LT *et al.* (2003) Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. *Am J Hum Genet* **72**, 1293–1299.
- 30 Tsai PC, Soong BW, Mademan I, Huang YH, Liu CR, Hsiao CT, Wu HT, Liu TT, Liu YT, Tseng YT *et al.* (2017) A recurrent WARS mutation is a novel cause of autosomal dominant distal hereditary motor neuropathy. *Brain* **140**, 1252–1266.
- 31 Nakayama T, Wu J, Galvin-Parton P, Weiss J, Andriola MR, Hill RS, Vaughan DJ, El-Quessny M, Barry BJ, Partlow JN *et al.* (2017) Deficient activity of alanyl-tRNA synthetase underlies an autosomal recessive syndrome of progressive microcephaly, hypomyelination, and epileptic encephalopathy. *Hum Mutat* **38**, 1348–1354.
- 32 Zhang X, Ling J, Barcia G, Jing L, Wu J, Barry BJ, Mochida GH, Hill RS, Weimer JM, Stein Q *et al.* (2014) Mutations in QARS, encoding glutaminyl-tRNA synthetase, cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures. *Am J Hum Genet* **94**, 547–558.
- 33 Simons C, Griffin LB, Helman G, Golas G, Pizzino A, Bloom M, Murphy JL, Crawford J, Evans SH, Topper S *et al.* (2015) Loss-of-function alanyl-tRNA synthetase mutations cause an autosomal-recessive early-onset epileptic encephalopathy with persistent myelination defect. *Am J Hum Genet* **96**, 675–681.
- 34 Salvarinova R, Ye CX, Rossi A, Biancheri R, Roland EH, Pavlidis P, Ross CJ, Tarailo-Graovac M, Wasserman WW and van Karnebeek CD (2015) Expansion of the QARS deficiency phenotype with report of a family with isolated supratentorial brain abnormalities. *Neurogenetics* **16**, 145–149.
- 35 Puffenberger EG, Jinks RN, Sougnéz C, Cibulskis K, Willert RA, Achilly NP, Cassidy RP, Fiorentini CJ, Heiken KF, Lawrence JJ *et al.* (2012) Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One* **7**, e28936.
- 36 Santos-Cortez RL, Lee K, Azeem Z, Antonellis PJ, Pollock LM, Khan S, Irfanullah, Andrade-Elizondo PB, Chiu I, Adams MD *et al.* (2013) Mutations in KARS, encoding lysyl-tRNA synthetase, cause autosomal-recessive nonsyndromic hearing impairment DFN89. *Am J Hum Genet* **93**, 132–140.
- 37 Orenstein N, Weiss K, Oprescu SN, Shapira R, Kidron D, Vanagaite-Basel L, Antonellis A and Muenke M (2017) Bi-allelic IARS mutations in a child with intra-uterine growth retardation, neonatal cholestasis, and mild developmental delay. *Clin Genet* **91**, 913–917.
- 38 McLaughlin HM, Sakaguchi R, Liu C, Igarashi T, Pehlivan D, Chu K, Iyer R, Cruz P, Cherukuri PF, Hansen NF *et al.* (2010) Compound heterozygosity for loss-of-function lysyl-tRNA synthetase mutations in a patient with peripheral neuropathy. *Am J Hum Genet* **87**, 560–566.
- 39 Nowaczyk MJ, Huang L, Tarnopolsky M, Schwartzentruber J, Majewski J, Bulman DE, Hartley T and Boycott KM (2017) A novel multisystem disease associated with recessive mutations in the tyrosyl-tRNA synthetase (YARS) gene. *Am J Med Genet A* **173**, 126–134.
- 40 Kopajtich R, Murayama K, Janecke AR, Haack TB, Breuer M, Knisely AS, Harting I, Ohashi T, Okazaki Y, Watanabe D *et al.* (2016) Biallelic IARS mutations cause growth retardation with prenatal onset, intellectual disability, muscular hypotonia, and infantile hepatopathy. *Am J Hum Genet* **99**, 414–422.
- 41 Sun Y, Hu G, Luo J, Fang D, Yu Y, Wang X, Chen J and Qiu W (2017) Mutations in methionyl-tRNA synthetase gene in a Chinese family with interstitial lung and liver disease, postnatal growth failure and anemia. *J Hum Genet* **62**, 647–651.
- 42 Skre H (1974) Genetic and clinical aspects of Charcot-Marie-Tooth's disease. *Clin Genet* **6**, 98–118.
- 43 Bargiela D, Yu-Wai-Man P, Keogh M, Horvath R and Chinnery PF (2015) Prevalence of neurogenetic

- disorders in the North of England. *Neurology* **85**, 1195–1201.
- 44 Rossor AM, Evans MR and Reilly MM (2015) A practical approach to the genetic neuropathies. *Pract Neurol* **15**, 187–198.
  - 45 Gonzalez M, McLaughlin H, Houlden H, Guo M, Yo-Tsen L, Hadjivassiliou M, Speziani F, Yang XL, Antonellis A, Reilly MM *et al.* (2013) Exome sequencing identifies a significant variant in methionyl-tRNA synthetase (MARS) in a family with late-onset CMT2. *J Neurol Neurosurg Psychiatry* **84**, 1247–1249.
  - 46 Hyun YS, Park HJ, Heo SH, Yoon BR, Nam SH, Kim SB, Park CI, Choi BO and Chung KW (2014) Rare variants in methionyl- and tyrosyl-tRNA synthetase genes in late-onset autosomal dominant Charcot-Marie-Tooth neuropathy. *Clin Genet* **86**, 592–594.
  - 47 Oprescu SN, Griffin LB, Beg AA and Antonellis A (2017) Predicting the pathogenicity of aminoacyl-tRNA synthetase mutations. *Methods* **113**, 139–151.
  - 48 Latour P, Thauvin-Robinet C, Baudelet-Méry C, Soichot P, Cusin V, Faivre L, Locatelli MC, Mayençon M, Sarcey A, Broussolle E *et al.* (2010) A major determinant for binding and aminoacylation of tRNA(Ala) in cytoplasmic Alanyl-tRNA synthetase is mutated in dominant axonal Charcot-Marie-Tooth disease. *Am J Hum Genet* **86**, 77–82.
  - 49 Ribas de Pouplana L, Buechter D, Sardesai NY and Schimmel P (1998) Functional analysis of peptide motif for RNA microhelix binding suggests new family of RNA-binding domains. *EMBO J* **17**, 5449–5457.
  - 50 McLaughlin HM, Sakaguchi R, Giblin W, Comparative Sequencing Program NISC, Wilson TE, Biesecker L, Lupski JR, Talbot K, Vance JM, Züchner S *et al.* (2012) A recurrent loss-of-function alanyl-tRNA synthetase (AARS) mutation in patients with Charcot-Marie-Tooth disease type 2N (CMT2N). *Hum Mutat* **33**, 244–253.
  - 51 Gonzaga-Jauregui C, Harel T, Gambin T, Kousi M, Griffin LB, Francescatto L, Ozes B, Karaca E, Jhangiani SN, Bainbridge MN *et al.* (2015) Exome sequence analysis suggests that genetic burden contributes to phenotypic variability and complex neuropathy. *Cell Rep* **12**, 1169–1183.
  - 52 Vester A, Velez-Ruiz G, McLaughlin HM, Comparative Sequencing Program NISC, Lupski JR, Talbot K, Vance JM, Züchner S, Roda RH, Fischbeck KH *et al.* (2013) A loss-of-function variant in the human histidyl-tRNA synthetase (HARS) gene is neurotoxic in vivo. *Hum Mutat* **34**, 191–199.
  - 53 Perona JJ and Gruic-Sovulj I (2014) Synthetic and editing mechanisms of aminoacyl-tRNA synthetases. *Top Curr Chem* **344**, 1–41.
  - 54 Nangle LA, Zhang W, Xie W, Yang XL and Schimmel P (2007) Charcot-Marie-Tooth disease-associated mutant tRNA synthetases linked to altered dimer interface and neurite distribution defect. *Proc Natl Acad Sci U S A* **104**, 11239–11244.
  - 55 Xie W, Nangle LA, Zhang W, Schimmel P and Yang XL (2007) Long-range structural effects of a Charcot-Marie-Tooth disease-causing mutation in human glycyl-tRNA synthetase. *Proc Natl Acad Sci U S A* **104**, 9976–9981.
  - 56 Dewan V, Wei M, Kleiman L and Musier-Forsyth K (2012) Dual role for motif 1 residues of human lysyl-tRNA synthetase in dimerization and packaging into HIV-1. *J Biol Chem* **287**, 41955–41962.
  - 57 Blocquel D, Li S, Wei N, Daub H, Sajish M, Erfurth ML, Kooi G, Zhou J, Bai G, Schimmel P *et al.* (2017) Alternative stable conformation capable of protein misinteraction links tRNA synthetase to peripheral neuropathy. *Nucleic Acids Res* **45**, 8091–8104.
  - 58 Naganuma M, Sekine S, Fukunaga R and Yokoyama S (2009) Unique protein architecture of alanyl-tRNA synthetase for aminoacylation, editing, and dimerization. *Proc Natl Acad Sci U S A* **106**, 8489–8494.
  - 59 Doublé S, Bricogne G, Gilmore C and Carter JCW (1995) Tryptophanyl-tRNA synthetase crystal structure reveals an unexpected homology to tyrosyl-tRNA synthetase. *Structure* **3**, 17–31.
  - 60 Stum M, McLaughlin HM, Kleinbrink EL, Miers KE, Ackerman SL, Seburn KL, Antonellis A and Burgess RW (2011) An assessment of mechanisms underlying peripheral axonal degeneration caused by aminoacyl-tRNA synthetase mutations. *Mol Cell Neurosci* **46**, 432–443.
  - 61 Griffin LB, Sakaguchi R, McGuigan D, Gonzalez MA, Searby C, Zuchner S, Hou YM and Antonellis A (2014) Impaired function is a common feature of neuropathy-associated glycyl-tRNA synthetase mutations. *Hum Mutat* **35**, 1363–1371.
  - 62 Malissov N, Griffin LB, Antonellis A and Beis D (2016) Dimerization is required for GARS-mediated neurotoxicity in dominant CMT disease. *Hum Mol Genet* **25**, 1528–1542.
  - 63 Li GY, Becam AM, Slonimski PP and Herbert CJ (1996) In vitro mutagenesis of the mitochondrial leucyl tRNA synthetase of *Saccharomyces cerevisiae* shows that the suppressor activity of the mutant proteins is related to the splicing function of the wild-type protein. *Mol Gen Genet* **252**, 667–675.
  - 64 Wakasugi K, Slike BM, Hood J, Otani A, Ewalt KL, Friedlander M, Cheresch DA and Schimmel P (2002) A human aminoacyl-tRNA synthetase as a regulator of angiogenesis. *Proc Natl Acad Sci U S A* **99**, 173–177.
  - 65 Guo M, Yang XL and Schimmel P (2010) New functions of aminoacyl-tRNA synthetases beyond translation. *Nat Rev Mol Cell Biol* **11**, 668–674.
  - 66 Park BS, Yeo SG, Jung J and Jeong NY (2015) A novel therapeutic target for peripheral nerve injury-



- related diseases: aminoacyl-tRNA synthetases. *Neural Regen Res* **10**, 1656–1662.
- 67 Taylor RW, Pyle A, Griffin H, Blakely EL, Duff J, He L, Smertenko T, Alston CL, Neeve VC, Best A *et al.* (2014) Use of whole-exome sequencing to determine the genetic basis of multiple mitochondrial respiratory chain complex deficiencies. *JAMA* **312**, 68–77.
- 68 McMillan HJ, Humphreys P, Smith A, Schwartzentruber J, Chakraborty P, Bulman DE, Beaulieu CL, FORGE Canada Consortium, Majewski J, Boycott KM *et al.* (2015) Congenital visual impairment and progressive microcephaly due to lysyl-transfer ribonucleic acid (RNA) synthetase (KARS) mutations: the expanding phenotype of aminoacyl-transfer RNA synthetase mutations in human disease. *J Child Neurol* **30**, 1037–1043.
- 69 Verrigni D, Diodato D, Di Nottia M, Torraco A, Bellacchio E, Rizza T, Tozzi G, Verardo M, Piemonte F, Tasca G *et al.* (2017) Novel mutations in KARS cause hypertrophic cardiomyopathy and combined mitochondrial respiratory chain defect. *Clin Genet* **91**, 918–923.
- 70 Zhou XL, He LX, Yu LJ, Wang Y, Wang XJ, Wang ED and Yang T (2017) Mutations in KARS cause early-onset hearing loss and leukoencephalopathy: Potential pathogenic mechanism. *Hum Mutat* **38**, 1740–1750.
- 71 McMillan HJ, Schwartzentruber J, Smith A, Lee S, Chakraborty P, Bulman DE, Beaulieu CL, Majewski J, Boycott KM and Geraghty MT (2014) Compound heterozygous mutations in glycyl-tRNA synthetase are a proposed cause of systemic mitochondrial disease. *BMC Med Genet* **15**, 36.
- 72 Park MC, Kang T, Jin D, Han JM, Kim SB, Park YJ, Cho K, Park YW, Guo M, He W *et al.* (2012) Secreted human glycyl-tRNA synthetase implicated in defense against ERK-activated tumorigenesis. *Proc Natl Acad Sci U S A* **109**, E640–E647.
- 73 He W, Bai G, Zhou H, Wei N, White NM, Lauer J, Liu H, Shi Y, Dumitru CD, Lettieri K *et al.* (2015) CMT2D neuropathy is linked to the neomorphic binding activity of glycyl-tRNA synthetase. *Nature* **526**, 710–714.
- 74 Huettl RE, Soellner H, Bianchi E, Novitch BG and Huber AB (2011) Npn-1 contributes to axon-axon interactions that differentially control sensory and motor innervation of the limb. *PLoS Biol* **9**, e1001020.
- 75 Sleight JN, Gomez-Martin A, Wei N, Bai G, Yang XL and Schiavo G (2017) Neuropilin 1 sequestration by neuropathogenic mutant glycyl-tRNA synthetase is permissive to vascular homeostasis. *Sci Rep* **7**, 9216.
- 76 Sleight JN, Dawes JM, West SJ, Wei N, Spaulding EL, Gómez-Martín A, Zhang Q, Burgess RW, Cader MZ, Talbot K *et al.* (2017) Trk receptor signaling and sensory neuron fate are perturbed in human neuropathy caused by Gars mutations. *Proc Natl Acad Sci U S A* **114**, E3324–E3333.
- 77 Banchs I, Casanovas C, Alberti A, De Jorge L, Povedano M, Montero J, Martinez-Matos JA and Volpini V (2009) Diagnosis of Charcot-Marie-Tooth disease. *J Biomed Biotechnol* **2009**, 985415.
- 78 Kawamata H, Magrane J, Kunst C, King MP and Manfredi G (2008) Lysyl-tRNA synthetase is a target for mutant SOD1 toxicity in mitochondria. *J Biol Chem* **283**, 28321–28328.
- 79 Chihara T, Luginbuhl D and Luo L (2007) Cytoplasmic and mitochondrial protein translation in axonal and dendritic terminal arborization. *Nat Neurosci* **10**, 828–837.
- 80 Spaulding EL, Sleight JN, Morelli KH, Pinter MJ, Burgess RW and Seburn KL (2016) Synaptic deficits at neuromuscular junctions in two mouse models of Charcot-Marie-Tooth Type 2d. *J Neurosci* **36**, 3254–3267.
- 81 Bais P, Beebe K, Morelli KH, Currie ME, Norberg SN, Evsikov AV, Miers KE, Seburn KL, Guergueltcheva V, Kremensky I *et al.* (2016) Metabolite profile of a mouse model of Charcot-Marie-Tooth type 2D neuropathy: implications for disease mechanisms and interventions. *Biol Open* **5**, 908–920.
- 82 Grice SJ, Sleight JN, Motley WW, Liu JL, Burgess RW, Talbot K and Cader MZ (2015) Dominant, toxic gain-of-function mutations in gars lead to non-cell autonomous neuropathology. *Hum Mol Genet* **24**, 4397–4406.
- 83 Sleight JN, Grice SJ, Burgess RW, Talbot K and Cader MZ (2014) Neuromuscular junction maturation defects precede impaired lower motor neuron connectivity in Charcot-Marie-Tooth type 2D mice. *Hum Mol Genet* **23**, 2639–2650.
- 84 Motley WW, Seburn KL, Nawaz MH, Miers KE, Cheng J, Antonellis A, Green ED, Talbot K, Yang XL, Fischbeck KH *et al.* (2011) Charcot-Marie-Tooth-linked mutant GARS is toxic to peripheral neurons independent of wild-type GARS levels. *PLoS Genet* **7**, e1002399.
- 85 Storkebaum E (2016) Peripheral neuropathy via mutant tRNA synthetases: inhibition of protein translation provides a possible explanation. *BioEssays* **38**, 818–829.
- 86 Seburn KL, Nangle LA, Cox GA, Schimmel P and Burgess RW (2006) An active dominant mutation of glycyl-tRNA synthetase causes neuropathy in a Charcot-Marie-Tooth 2D mouse model. *Neuron* **51**, 715–726.
- 87 Achilli F, Bros-Facer V, Williams HP, Banks GT, AlQatari M, Chia R, Tucci V, Groves M, Nickols CD, Seburn KL *et al.* (2009) An ENU-induced mutation in mouse glycyl-tRNA synthetase (GARS) causes



- peripheral sensory and motor phenotypes creating a model of Charcot-Marie-Tooth type 2D peripheral neuropathy. *Dis Model Mech* **2**, 359–373.
- 88 Erdmann I, Marter K, Kobler O, Niehues S, Abele J, Müller A, Bussmann J, Storkebaum E, Ziv T, Thomas U *et al.* (2015) Cell-selective labelling of proteomes in *Drosophila melanogaster*. *Nat Commun* **6**, 7521.
  - 89 Niehues S, Bussmann J, Steffes G, Erdmann I, Köhrer C, Sun L, Wagner M, Schäfer K, Wang G, Koerdet SN *et al.* (2015) Impaired protein translation in *Drosophila* models for Charcot-Marie-Tooth neuropathy caused by mutant tRNA synthetases. *Nat Commun* **6**, 7520.
  - 90 Guo M and Schimmel P (2013) Essential nontranslational functions of tRNA synthetases. *Nat Chem Biol* **9**, 145–153.
  - 91 Pang YL, Poruri K and Martinis SA (2014) tRNA synthetase: tRNA aminoacylation and beyond. *Wiley Interdiscip Rev RNA* **5**, 461–480.
  - 92 Mun J, Kim YH, Yu J, Bae J, Noh DY, Yu MH and Lee C (2010) A proteomic approach based on multiple parallel separation for the unambiguous identification of an antibody cognate antigen. *Electrophoresis* **31**, 3428–3436.
  - 93 Mo Z, Zhang Q, Liu Z, Lauer J, Shi Y, Sun L, Griffin PR and Yang XL (2016) Neddylation requires glycyl-tRNA synthetase to protect activated E2. *Nat Struct Mol Biol* **23**, 730–737.
  - 94 Kim DG, Choi JW, Lee JY, Kim H, Oh YS, Lee JW, Tak YK, Song JM, Razin E, Yun SH *et al.* (2012) Interaction of two translational components, lysyl-tRNA synthetase and p40/37LRP, in plasma membrane promotes laminin-dependent cell migration. *FASEB J* **26**, 4142–4159.
  - 95 Nam SH, Kang M, Ryu J, Kim HJ, Kim D, Kim DG, Kwon NH, Kim S and Lee JW (2016) Suppression of lysyl-tRNA synthetase, KRS, causes incomplete epithelial-mesenchymal transition and ineffective cell-extracellular matrix adhesion for migration. *Int J Oncol* **48**, 1553–1560.
  - 96 Zhao Z, Hashiguchi A, Hu J, Sakiyama Y, Okamoto Y, Tokunaga S, Zhu L, Shen H and Takashima H (2012) Alanyl-tRNA synthetase mutation in a family with dominant distal hereditary motor neuropathy. *Neurology* **78**, 1644–1649.
  - 97 Taft RJ, Vanderver A, Leventer RJ, Damiani SA, Simons C, Grimmond SM, Miller D, Schmidt J, Lockhart PJ, Pope K *et al.* (2013) Mutations in DARS cause hypomyelination with brain stem and spinal cord involvement and leg spasticity. *Am J Hum Genet* **92**, 774–780.
  - 98 van Meel E, Wegner DJ, Cliften P, Willing MC, White FV, Kornfeld S and Cole FS (2013) Rare recessive loss-of-function methionyl-tRNA synthetase mutations presenting as a multi-organ phenotype. *BMC Med Genet* **14**, 106.
  - 99 Wolf NI, Salomons GS, Rodenburg RJ, Pouwels PJ, Schieving JH, Derks TG, Fock JM, Rump P, van Beek DM, van der Knaap MS *et al.* (2014) Mutations in RARS cause hypomyelination. *Ann Neurol* **76**, 134–139.
  - 100 Elo JM, Yadavalli SS, Euro L, Isohanni P, Götz A, Carroll CJ, Valanne L, Alkuraya FS, Uusimaa J, Paetau A *et al.* (2012) Mitochondrial phenylalanyl-tRNA synthetase mutations underlie fatal infantile Alpers encephalopathy. *Hum Mol Genet* **21**, 4521–4529.
  - 101 Schwartztruber J, Buhas D, Majewski J, Sasarman F, Papillon-Cavanagh S, Thiffault I, Sheldon KM, Massicotte C, Patry L, Simon M *et al.* (2014) Mutation in the nuclear-encoded mitochondrial isoleucyl-tRNA synthetase IARS2 in patients with cataracts, growth hormone deficiency with short stature, partial sensorineural deafness, and peripheral neuropathy or with Leigh syndrome. *Hum Mutat* **35**, 1285–1289.
  - 102 Moosa S, Haagerup A, Gregersen PA, Petersen KK, Altmüller J, Thiele H, Nürnberg P, Cho TJ, Kim OH, Nishimura G *et al.* (2017) Confirmation of CAGSSS syndrome as a distinct entity in a Danish patient with a novel homozygous mutation in IARS2. *Am J Med Genet A* **173**, 1102–1108.
  - 103 Sofou K, Kollberg G, Holmström M, Dávila M, Darin N, Gustafsson CM, Holme E, Oldfors A, Tulinius M and Asin-Cayuela J (2015) Whole exome sequencing reveals mutations in NARS2 and PARS2, encoding the mitochondrial asparaginyl-tRNA synthetase and prolyl-tRNA synthetase, in patients with Alpers syndrome. *Mol Genet Genomic Med* **3**, 59–68.
  - 104 Vanlander AV, Menten B, Smet J, De Meirleir L, Sante T, De Paepe B, Seneca S, Pearce SF, Powell CA, Vergult S *et al.* (2015) Two siblings with homozygous pathogenic splice-site variant in mitochondrial asparaginyl-tRNA synthetase (NARS2). *Hum Mutat* **36**, 222–231.
  - 105 Edvardson S, Haag A, Kolesnikova O, Gomori JM, Tarassov I, Einbinder T, Saada A and Elpeleg O (2007) Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. *Am J Hum Genet* **81**, 857–862.
  - 106 Theisen BE, Rummyantseva A, Cohen JS, Alcaraz WA, Shinde DN, Tang S, Srivastava S, Pevsner J, Trifunovic A and Fatemi A (2017) Deficiency of WARS2, encoding mitochondrial tryptophanyl tRNA synthetase, causes severe infantile onset leukoencephalopathy. *Am J Med Genet A* **173**, 2505–2510.
  - 107 Riley LG, Cooper S, Hickey P, Rudinger-Thirion J, McKenzie M, Compton A, Lim SC, Thorburn D, Ryan

- MT, Giegé R *et al.* (2010) Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia–MLASA syndrome. *Am J Hum Genet* **87**, 52–59.
- 108 Motley WW, Griffin LB, Mademan I, Baets J, De Vriendt E, De Jonghe P, Antonellis A, Jordanova A and Scherer SS (2015) A novel AARS mutation in a family with dominant myeloneuropathy. *Neurology* **84**, 2040–2047.
- 109 Sarna JR and Hawkes R (2011) Patterned Purkinje cell loss in the ataxic sticky mouse. *Eur J Neurosci* **34**, 79–86.
- 110 Froelich CA and First EA (2011) Dominant Intermediate Charcot-Marie-Tooth disorder is not due to a catalytic defect in tyrosyl-tRNA synthetase. *Biochemistry* **50**, 7132–7145.
- 111 Antonellis A, Lee-Lin SQ, Wasterlain A, Leo P, Quezado M, Goldfarb LG, Myung K, Burgess S, Fischbeck KH and Green ED (2006) Functional analyses of glycyl-tRNA synthetase mutations suggest a key role for tRNA-charging enzymes in peripheral axons. *J Neurosci* **26**, 10397–10406.
- 112 Ermanoska B, Motley WW, Leitão-Gonçalves R, Asselbergh B, Lee LH, De Rijk P, Slegers K, Ooms T, Godenschwege TA, Timmerman V *et al.* (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.