Human Molecular Genetics, 2017, Vol. 26, No. R2 R114–R127

doi: 10.1093/hmg/ddx231
Advance Access Publication Date: 15 June 2017
Invited Review

INVITED REVIEW

Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease

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Abstract

Aminoacyl-tRNA synthetases (ARSs) are responsible for charging amino acids to cognate tRNA molecules, which is the essential first step of protein translation. Interestingly, mutations in genes encoding ARS enzymes have been implicated in a broad spectrum of human inherited diseases. Bi-allelic mutations in ARSs typically cause severe, early-onset, recessive diseases that affect a wide range of tissues. The vast majority of these mutations show loss-of-function effects and impair protein translation. However, it is not clear how a subset cause tissue-specific phenotypes. In contrast, dominant ARS-mediated diseases specifically affect the peripheral nervous system—most commonly causing axonal peripheral neuropathy—and usually manifest later in life. These neuropathies are linked to heterozygosity for missense mutations in five ARS genes, which points to a shared mechanism of disease. However, it is not clear if a loss-of-function mechanism or a toxic gain-of-function mechanism is responsible for ARS-mediated neuropathy, or if a combination of these mechanisms operate on a mutation-specific basis. Here, we review our current understanding of recessive and dominant ARS-mediated disease. We also propose future directions for defining the molecular mechanisms of ARS mutations toward designing therapies for affected patient populations.

An Introduction to Aminoacyl-tRNA Synthetases

The conjugation of tRNA to cognate amino acids is an essential prerequisite for the translation of the genetic code into proteins. This conjugation is performed by a group of enzymes, aminoacyl-tRNA synthetases (ARSs), which are ubiquitously expressed and highly evolutionarily conserved. Each amino acid has a designated ARS enzyme to catalyze a bond with a cognate tRNA. There are 37 members of the ARS gene family—17 encode an enzyme that functions in the cytoplasm, 17 encode an enzyme that functions in the mitochondria, and three encode bifunctional proteins that charge tRNA in both cellular locations (1). The nomenclature for ARS genes and proteins is the single-letter code of the amino acid that the enzyme recognizes followed by 'ARS', with mitochondrial enzymes followed by a '2' (*e.g.* HARS for cytoplasmic histidyl-tRNA synthetase and HARS2 for mitochondrial histidyl-tRNA synthetase) (1).

Despite the essential canonical function and ubiquitous expression of ARS enzymes, mutations in ARS genes have been implicated in a variety of human diseases with both recessive and dominant inheritance patterns. Interestingly, these phenotypes range from later onset peripheral neuropathy to severe, multi-system developmental syndromes. Defining the mechanisms underlying the heterogeneity of ARS-related disease phenotypes is an important first step towards developing treatments for these disorders. Here, we review our current understanding of the molecular pathologies of ARS-related disease in the context of both recessive and dominant phenotypes.

The Role of ARS Mutations in Recessive Disease Phenotypes

Mutations in 31 ARS genes have been implicated in recessive disorders that display a wide range of clinical manifestations

Received: June 9, 2017. **Revised:** June 9, 2017. **Accepted:** June 12, 2017

(Table 1 and 104). Not surprisingly, pathogenic variants in ARS genes encoding a mitochondrial enzyme tend to cause phenotypes in tissues with a high metabolic demand, particularly in the central nervous system. Leukoencephalopathies (9–12,19–30,70), myopathies (14,93,98–102), and liver disease (33,36) are all common features of mitochondrial ARS disease phenotypes. Additionally, epilepsy (33–37,72,85,86,91), developmental delay, intellectual disability (69,72,73,87), ovarian failure (10–12,51,61), and sensorineural hearing loss (51,54,55,61,62,69,74) are frequently observed in patients with mitochondrial ARS mutations.

Mutations in ARS genes encoding cytoplasmic enzymes also cause a spectrum of recessive disorders, which often affect a wider array of tissues but that also typically include a neurological component. The recessive neurological phenotypes associated with cytoplasmic ARSs include hypomyelination (77), microcephaly (3,39,75,92), seizures (2,3,75,76,92), sensorineural hearing loss (48,56), and developmental delay (52,57,64,76,92,95). Some multisystem, cytoplasmic ARS-linked disorders also include liver dysfunction (53,64,65,105) and lung disease (64–66,105).

Although mutations in different ARS genes can cause overlapping recessive phenotypes, some tissues appear to be uniquely sensitive to mutations in a specific ARS. For example, retinitis pigmentosa (a component of Usher syndrome) has only been described in patients with bi-allelic *HARS* mutations (48). Defining the mechanisms that underlie tissue-specific effects of ARS mutations is a significant challenge moving forward (see below).

Although recessive ARS-mediated disorders involve a wide range of phenotypes, they are all, by definition, caused by bi-allelic mutations. Typically, patients with recessive ARS-related disease are homozygous for missense mutations, compound heterozygous for missense mutations, or compound heterozygous for one missense mutation and one null allele. Homozygosity or compound heterozygosity for ARS null alleles would likely be lethal due to the essential nature of the encoded enzymes; this effect has been demonstrated in animal models (106). The genotypes identified in patients with recessive ARS-related disease strongly suggest a loss-of-function mechanism for disease pathogenesis. In support of this, functional studies on disease-causing mutations show a reduction of ARS protein levels (3,16,27,55,63,73,74,78,91,93,100,107,108), and/or severe decreases in mutant enzyme activity (33,36,48,51,57,63,64,75,99). The effect of ARS mutations on enzyme kinetics is often measured directly with *in vitro* aminoacylation assays. In addition, enzyme function can be inferred by measuring the amount of charged tRNA in patient cells (16,73,79,89,91,108), or by assessing the ability of the mutated gene to support cellular growth in yeast complementation assays (2,12,51,52,57,53,61,66,78,91,98,102). For mutations in mitochondrial ARS enzymes, defects in tRNA charging explain the impaired protein translation of oxidative phosphorylation complexes and respiratory defects observed in patient cells (12,14,16,27,33,34,37,63,69–71,74,79,80,86,91,98–100,108). Furthermore, studies of *IARS* (53) and *QARS* in zebrafish (75), and those of *HARS2* (51) and *LARS2* (61) in *C. elegans* indicate that knocking down the ARS gene phenocopies key elements of the disorders linked to bi-allelic ARS mutations. This further supports the conclusion that disease-associated mutations lead to a loss of ARS activity, which may ultimately result in impaired protein translation by one of two mechanisms (Fig. 1). First, impaired translation could occur directly due to ribosomal stalling in the environment of reduced aminoacylated tRNA (109–111). Second, reduced translation could occur indirectly due to the recognition of uncharged tRNA molecules by GCN2, and the subsequent phosphorylation of eIF2α and global repression of translation (112).

Moving forward, it will be important to understand the degree to which specific ARS mutations impair translation and how this contributes to phenotypic and tissue-type variability. There is evidence to suggest a link between the severity of the translation defect and the severity of the phenotype. For example, in siblings with *RARS2* mutations, the sibling with the greater reduction in mitochondrial OXPHOS protein complex levels presented with lactic acidosis and neurological symptoms, whereas the sibling with a milder OXPHOS reduction had lactic acidosis but no neurological symptoms (86). Further work is needed to elucidate this relationship, particularly with mutations in cytoplasmic and bifunctional ARS enzymes that cause recessive disease, which have not yet been directly linked to protein translation defects. Additionally, there is currently no explanation for the observation that certain ARS mutations cause tissue-specific phenotypes, despite knowledge that ARS enzymes are required for all living cells. One possibility is that mutations in a specific ARS affect the expression of cell type-specific proteins, possibly in a codon-dependent manner. Alternatively, the expression profile of a given tRNA—which can vary between tissues (110)—may modify the cell-specific impact of deficiencies in tRNA charging. Ribosomal profiling to detect stalling at vulnerable codons and tissue-specific tRNA expression studies will be useful approaches for addressing these questions.

The Role of ARS Mutations in Dominant Disease Phenotypes

Although there is a broad spectrum of phenotypes seen in ARS-mediated recessive diseases, dominant ARS-mediated disorders have, to date, a limited phenotypic range. Mutations in five ARS loci have been implicated in dominant Charcot-Marie-Tooth (CMT) disease and related neuropathic phenotypes; these include glycyl-(*GARS*), tyrosyl-(*YARS*), alanyl-(*AARS*), histidyl-(*HARS*), and tryptophanyl-tRNA synthetase (*WARS*) (4,40,49,94,96). CMT disease is an inherited peripheral neuropathy characterized by progressive loss of motor and sensory function. The phenotype manifests in an axon-length dependent manner (*i.e.*, symptoms first arise in distal motor and sensory structures innervated by long axons, which can progress proximally over time). Although CMT disease can be caused by a defect in the myelin sheath surrounding the axon (CMT Type 1), ARS-mediated CMT disease is predominantly caused by a defect in peripheral nerve axons (CMT Type 2).

The genetic evidence for the involvement of *GARS*, *YARS*, *AARS*, and *HARS* mutations in CMT disease is abundant, with numerous pathogenic mutations identified in each gene, many of which were identified in large pedigrees via linkage analysis (4,40,96). In addition, two variants in methionyl-tRNA synthetase (*MARS*) have been reported in patients with dominant CMT (113,114), but the role of *MARS* in CMT disease is less clear; although one of the reported *MARS* variants was shown to cause a loss-of-function effect in yeast complementation assays, it was identified in a small pedigree and was also detected in an unaffected relative (113). This suggests that the *MARS* variant is either not causal, or that it leads to a phenotype with decreased penetrance; additional research is needed to determine the role of *MARS* mutations in dominant CMT disease. Recently, a tryptophanyl-tRNA synthetase variant—H257R *WARS*—was identified in three unrelated families with autosomal dominant distal hereditary motor neuropathy (dHMN) (94). dHMN is phenotypically similar to CMT disease; it is marked by progressive distal limb muscle atrophy, without sensory involvement. This makes *WARS* the fifth *ARS* gene to be convincingly implicated in

Table 1. ARS loci implicated in dominant and recessive human disease phenotypes

Gene	Locus	Location of Protein Function	Mode of Inheritance	Disease Phenotype(s)	Unique variants	References
AARS	16q22	Cytoplasm	Autosomal Recessive	Early-onset epileptic encephalopathy with myelination defect	2	(2)
				Microcephaly with hypomyelination, epileptic encephalopathy, and spasticity	2	(3)
			Autosomal Dominant	Charcot-Marie-Tooth disease type 2N	3	(4,5–7)
				Distal hereditary motor neuropathy	1	(8)
AARS2	6p21.1	Mitochondria	Autosomal Recessive	Leukoencephalopathy with ovarian failure	19	(9–13)
				Cardiomyopathy	2	(14)
				Multiple respiratory chain complex defects	4	(15)
CARS2	13q34	Mitochondria	Autosomal Recessive	Epileptic encephalopathy	2	(16)
				Progressive myoclonic epilepsy	2	(17)
DARS	2q21.3	Cytoplasm	Autosomal Recessive	Hypomyelination with brain stem and spinal cord involvement and leg spasticity	2	(18)
DARS2	1q25.1	Mitochondria	Autosomal Recessive	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation	11	(19–24)
EARS2	16p12.2	Mitochondria	Autosomal Recessive	Leukoencephalopathy with thalamus and brainstem involvement and high lactate	20	(25–30)
				Neonatal lactic acidosis, recurrent hypoglycemia, agenesis of corpus callosum	2	(31)
				Multiple respiratory chain complex defects	3	(15)
FARS2	6p25.1	Mitochondria	Autosomal Recessive	Hereditary spastic paraplegia	1	(32)
				Alpers syndrome	5	(33,34)
				Early onset epilepsy	5	(35–37)
				Global delay, dysarthria and tremor	2	(38)
GARS	7p15	Mitochondria and Cytoplasm	Autosomal Recessive	Systemic mitochondrial disease	2	(39)
				Cardiomyopathy	1	(15)
			Autosomal Dominant	Charcot-Marie-Tooth disease type 2D	4	(40–43)
				Distal hereditary motor neuropathy	10	(40,41,43–47)
HARS	5q31.3	Cytoplasm	Autosomal Recessive	Usher syndrome	1	(48)
			Autosomal Dominant	Charcot-Marie-Tooth disease type 2W	5	(49,50)
				Distal hereditary motor neuropathy	2	(50)
HARS2	5q31.3	Mitochondria	Autosomal Recessive	Perrault syndrome	2	(51)
IARS	9q22.31	Cytoplasm	Autosomal Recessive	Prenatal growth retardation, neonatal cholestasis, muscular hypotonia, intellectual disability, infantile hepatopathy	8	(52,53)
IARS2	1q41	Mitochondria	Autosomal Recessive	Cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss, skeletal dysplasia syndrome; Leigh syndrome	4	(54,55)
KARS	16q23.1	Mitochondria and Cytoplasm	Autosomal Recessive	Nonsyndromic hearing loss (DFNB89)	2	(56)
				Recessive intermediate Charcot-Marie-Tooth disease type B, dysmorphic features, developmental delay, self-abusive behavior, vestibular Schwannoma	2	(57)
				Visual impairment, microcephaly, developmental delay, seizures	2	(58)
LARS	5q32	Cytoplasm	Autosomal Recessive	Infantile hepatopathy	4	(59,60)
LARS2	3p21.31	Mitochondria	Autosomal Recessive	Perrault syndrome	5	(61,62)
				Hydrops, lactic acidosis, sideroblastic anemia, multisystem failure	2	(63)
MARS	12q13.3	Cytoplasm	Autosomal Recessive	Interstitial lung disease and liver disease	7	(64–66)
			Autosomal Dominant	Charcot-Marie-Tooth disease type 2U	2	(67,68)
MARS2	2q33.1	Cytoplasm	Autosomal Recessive	Developmental delay, sensorineural hearing loss	2	(69)
				Autosomal recessive spastic ataxia with leukoencephalopathy	2	(70)
NARS2	11q14.1	Mitochondria	Autosomal Recessive	Alpers syndrome	1	(71)

(continued)

Table 1. (continued)

Gene	Locus	Location of Protein Function	Mode of Inheritance	Disease Phenotype(s)	Unique variants	References
				Developmental delay, intellectual disability, epilepsy, myopathy	6	(72,73)
				Nonsyndromic deafness	1	(74)
				Leigh syndrome	2	(74)
PARS2	3p21.31	Mitochondria	Autosomal Recessive	Alpers syndrome	2	(71)
				Infantile-onset developmental delay and epilepsy	2	(72)
QARS	3p21.31	Cytoplasm and Mitochondria	Autosomal Recessive	Progressive microcephaly, cerebral-cerebellar atrophy, hypomyelination, intractable seizures, developmental delay	6	(75,76)
RARS	5q34	Cytoplasm	Autosomal Recessive	Hypomyelination	5	(77)
RARS2	6q16.1	Mitochondria	Autosomal Recessive	Pontocerebellar hypoplasia	14	(78–84)
				Early onset epileptic encephalopathy	2	(85)
				Lactic acidosis with or without neurological symptoms (microcephaly, seizures, developmental delay)	1	(86)
				Intellectual disability	1	(87)
SARS	1p13.3	Cytoplasm	Autosomal Recessive	Intellectual disability, ataxia, microcephaly, speech impairment, aggressive behavior	1	(88)
SARS2	19q13.2	Mitochondria	Autosomal Recessive	Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis	2	(89,90)
TARS2	1q21.2	Mitochondria	Autosomal Recessive	Axial hypotonia and severe psychomotor delay	2	(91)
VARS	6p21.33	Cytoplasm	Autosomal Recessive	Severe developmental delay, microcephaly, seizures	2	(92)
VARS2	6p21.33	Mitochondria	Autosomal Recessive	Microcephaly and epilepsy	1	(91)
				Encephalocardiomyopathy	2	(93)
				Multiple respiratory chain complex defects	2	(15)
WARS	14q32.2	Cytoplasm	Autosomal Dominant	Distal hereditary motor neuropathy	1	(94)
WARS2	1p12	Mitochondria	Autosomal Recessive	Intellectual disability, ataxia, microcephaly, speech impairment, aggressive behavior	2	(88)
YARS	1p35.1	Cytoplasm	Autosomal Recessive	Multisystem disease, developmental delay, failure to thrive	2	(95)
			Autosomal Dominant	Dominant intermediate Charcot-Marie-Tooth disease type C	5	(96,97)
YARS2	12p11.21	Mitochondria	Autosomal Recessive	Myopathy, lactic acidosis, sideroblastic anemia, cardiomyopathy, respiratory insufficiency	7	(98–103)
				Multiple respiratory chain complex defects	1	(15)

dominant axonal neuropathy. Combined, the genetic analyses implicating five ARS loci in dominant axonal neuropathy strongly suggest that ARS enzymes are particularly important for peripheral nerve axons.

Patients with ARS-mediated axonal neuropathy are typically heterozygous for missense mutations; however, one in-frame deletion has been described (96). A major question moving forward is how these mutations, in the heterozygous state, cause a late-onset, tissue-specific phenotype. It is worth noting that, to date, none of the parents of patients with recessive diseases associated with *GARS*, *YARS*, *HARS*, or *AARS* mutations have been shown to be affected with a dominant axonal neuropathy. However, interpreting this is complicated by the decreased penetrance and later onset of ARS-related neuropathy (44). This may suggest that some variants only cause recessive disease, while other variants may also cause dominant disease. Further evaluation of the functional differences between these two sets of variants will yield important insights into disease mechanisms.

The Molecular Mechanisms of ARS-Related Human Inherited Disease

Current genetic and functional evidence clearly point to a loss of enzymatic function as the molecular mechanism of ARS-mediated recessive disease. As noted above, causal genotypes correspond to functional data showing reduced gene function. Thus, patient mutations lead to a loss of ARS activity, which in turn results in impaired protein translation and the associated recessive disease phenotype.

In contrast to the recessive phenotypes, the molecular mechanism of ARS-related dominant axonal neuropathy is less clear. The fact that mutations in five genes encoding an aminoacyl-tRNA synthetase (*GARS*, *YARS*, *AARS*, *HARS*, and *WARS*) cause a similar dominant phenotype points to a common disease mechanism. In support of this, over-expression of neuropathy-associated *GARS* and *YARS* mutants in a *Drosophila* model cause a strikingly similar phenotype (115). Although there is a growing body of work defining various non-canonical functions for the above five ARS enzymes

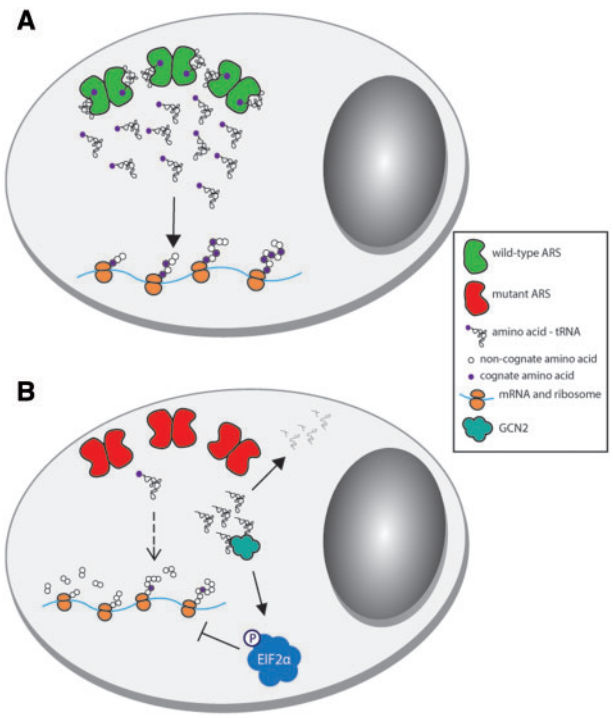

Figure 1. Potential mechanisms of ARS-related recessive disease. (**A**) Two wild-type ARS alleles supply cells with the requisite charged tRNA for protein translation. (**B**) Two loss-of-function ARS alleles severely reduce the amount of charged tRNA available for translation, which impairs protein production. Uncharged tRNA is either degraded or binds to GCN2, which phosphorylates eIF2α and inhibits global translation. In both panels, dimeric enzymes functioning in the cytoplasm are shown for simplicity; however, please note that some ARS enzymes act as monomers and that some effects apply to mitochondrial translation.

(Table 2), none thus far are common to all five nor do they relate to neuron (or axon) function. Thus, it is currently challenging to investigate if a loss of some non-canonical function is responsible for ARS-related neuropathy. Also, given that most neuropathy-associated ARS mutations impair rather than enhance enzyme function (104), it is unlikely that a gain of canonical function is responsible for disease. As a result, there are currently two mechanisms being explored: impaired ARS activity and toxic gain-of-function effects (Fig. 2). It should be emphasized that these pathogenic mechanisms may not be mutually exclusive—for example, impaired tRNA charging may be a prerequisite for a gain-of-function effect, or the two molecular mechanisms may work in concert to modulate phenotypic severity.

Impaired ARS function in dominant axonal neuropathy

Data from *in vitro* aminoacylation and yeast complementation assays indicate that neuropathy-associated ARS mutations cause deficits in tRNA charging (104). Additionally, data from three animal models suggest that the mutant proteins are subfunctional. First, in *Drosophila* projection neurons, the morphological defects caused by neuron-specific homozygosity for a null *gars* allele were fully rescued by a wild-type human *GARS* transgene, partially rescued by the neuropathy-associated E71G allele, and not rescued by the neuropathy-associated L129P allele (134). Second, whereas over-expressing wild-type *gars* rescues the neuromuscular phenotype of zebrafish homozygous for a loss-of-function allele, over-expressing the neuropathy-associated G526R allele showed no rescue (135). Finally, whereas mice heterozygous for P234KY or C201R *Gars* display a dominant neuropathy, homozygosity for these mutations results in reduced viability (136). In sum, there is an abundance of data showing that neuropathy-associated ARS missense mutations have a deleterious effect on gene function, indicating that this molecular consequence is a component of disease pathogenesis.

In contrast, three lines of evidence argue against a simple loss-of-function effect as the underlying mechanism of ARS-related neuropathy. First, mice heterozygous for a *Gars* null allele have a wild-type phenotype (106). Second, examination of ARS alleles in human populations reveals null alleles in the heterozygous state. For example, the Genome Aggregation Database (137) annotates many ARS null alleles in the heterozygous state (23 in *GARS*, 52 in *HARS*, 97 in *AARS*, 32 in *YARS*, and 15 in *WARS*). Combined, these data rule out haploinsufficiency as the primary mechanism for a penetrant, ARS-related neuropathy. Finally, over-expression of wild-type human GARS in mice heterozygous for either P234KY or C201R *Gars* did not rescue the phenotype, indicating that the neuropathy caused by these mutations does not arise from a simple loss-of-function effect (136).

One likely explanation for the role of loss-of-function missense mutations in dominant neuropathy is a dominant-negative effect. Prerequisites for a dominant-negative effect include: (1) the mutant protein should be stably expressed; (2) the mutant protein should have reduced or ablated function; and (3) the affected protein should normally dimerize (or oligomerize) and mutant subunits should retain the ability to interact with wild-type subunits. Indeed, AARS, YARS, GARS, HARS, and WARS all charge tRNA as dimers; if an inactive mutant subunit dimerizes with a wild-type subunit, it could result in a dramatic reduction in tRNA charging compared to the haploinsufficient state. This would shift the burden of tRNA charging onto the reduced population (*i.e.*, 25%) of wild-type:wild-type dimers.

Three lines of evidence support a dominant-negative effect for ARS mutations that cause dominant neuropathy. First, yeast cells expressing one wild-type and one mutant copy of tyrosyl-tRNA synthetase showed depleted growth compared to yeast cells expressing only the wild-type enzyme (96). Second, zebrafish homozygous for a loss-of-function *gars* missense allele show a severe neuromuscular defect, and zebrafish heterozygous for this allele have no phenotype (135). Importantly, the missense mutation (T209K) was shown to ablate dimerization. When T209K was over-expressed in either $gars^{T209K/+}$ or $gars^{+/+}$ zebrafish, the fish had no phenotype. However, over-expression of G526R *gars*, which dimerizes (135) and is non-functional (138), caused enhanced neuromuscular junction defects. Notably, over-expression of T209K in *cis* with G526R improved the neuromuscular junction phenotype, suggesting that dimerization is required for the toxicity of G526R *gars*. Finally, the H257R *WARS* mutation decreases enzyme activity *in vitro* but does not impact dimerization (94). To measure the potential downstream impact on protein synthesis, cultured cells were co-transfected with a construct to express wild-type or H257R *WARS* (or an empty vector) and a β-Gal or luciferase construct as a proxy for protein translation. Whereas wild-type WARS increased reporter activity above that of the empty vector, H257R WARS decreased reporter activity below that of the empty vector, indicating that H257R WARS suppressed endogenous levels of protein synthesis. However, a dominant-negative effect may not apply to all neuropathy-associated ARS mutations; as noted above, the effects of P234KY and C201R *Gars* were not rescued by over-expression of human GARS in affected mice. Thus, more research is needed to explore a dominant-negative mechanism and to determine if it can be alleviated by supplying the wild-type enzyme.

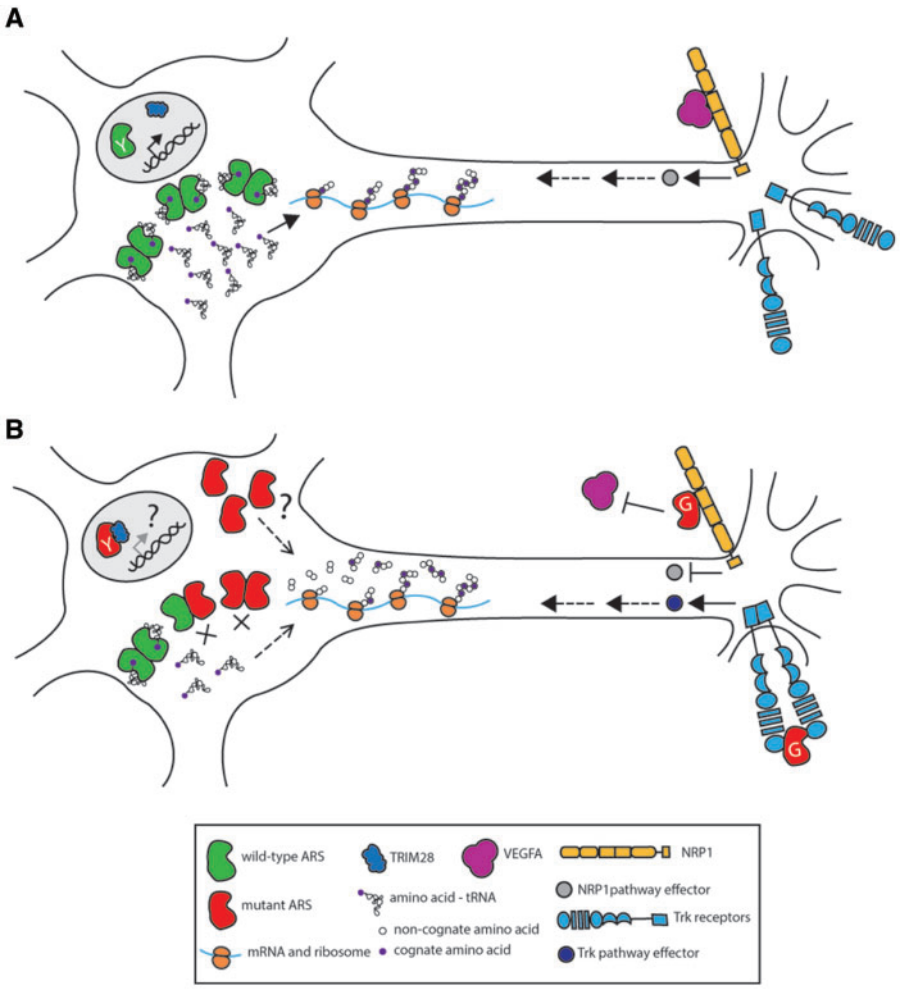

Figure 2. Potential mechanisms of ARS-related dominant axonal neuropathy. Neurons are illustrated with the cell body on the left and the axon extending to the right. A wild-type neuron (**A**) has functional ARS activity (green dimers) facilitating protein translation. There is appropriate NRP1 (orange transmembrane protein) and Trk signaling (blue transmembrane protein). YARS translocates to the nucleus upon oxidative stress and binds TRIM28 (blue), potentially changing the regulation of DNA damage response genes. Proposed mechanisms of ARS-mediated peripheral neuropathy are represented in (**B**); see text for details. Neuronal function may be compromised by impaired protein translation due to an unknown function of mutant ARS (red subunits) and/or a depletion in available charged tRNA from a significant reduction of aminoacylation activity. For peripheral neuropathy related to *GARS* mutations, mutant GARS may interfere with NRP1 signaling by preventing VEGFA (magenta) from binding to NRP1. In developing sensory neurons, mutant GARS may also act as a ligand for Trk receptors, aberrantly activating Trk signaling. For peripheral neuropathy related to *YARS* mutations, increased mutant YARS binding to TRIM28 (blue) may change the expression of DNA damage response genes.

Table 2. Non-canonical functions of ARS enzymes implicated in dominant neuropathy

ARS	Species	Non-canonical function
AARS	*Homo sapiens*	C-terminal splice variant binds DNA (116)
GARS	*Homo sapiens*	Chaperone in neddylation pathway (117)
	Homo sapiens	Tumorigenesis defense (118)
	Saccharomyces cerevisiae	mRNA 3' end formation (119)
HARS	*Homo sapiens*	Epitope for autoantibodies in inflammatory myositis (120–122)
WARS	*Homo sapiens*	Activates macrophages in immune response (123)
	Homo sapiens	Mini-WARS inhibits angiogenesis (124–128)
YARS	*Homo sapiens*	Locates to the nucleus and protects against DNA damage (129–131)
	Homo sapiens	Mini-YARS promotes angiogenesis (125,127,128,132,133)

It is important to consider how impaired ARS function (possibly exacerbated by a dominant-negative mechanism) would specifically affect peripheral nerve axons. One possibility is that the long axons of the peripheral nervous system are particularly sensitive to defects in housekeeping functions, such as protein translation. It is quite likely that these functions would have to be maintained throughout the axoplasm of long axons. Indeed, mutations in other ubiquitously expressed genes (*e.g. MFN2* and *RAB7*) have been implicated in axonal neuropathy (139,140).

The role of toxic gain-of-function effects in dominant axonal neuropathy

Another possibility is that neuropathy-associated ARS mutations cause the encoded enzymes to gain a novel, dominantly toxic function that specifically affects the peripheral nervous system. For example, there is evidence that *YARS* (141) and *GARS* (142,143) mutations change enzyme conformations and expose amino-acid residues. These structural changes were recently shown to facilitate increased binding of YARS to TRIM28 in the nucleus, which may alter the expression of DNA damage response genes (129); however, the role of this interaction in neuropathy is unclear. Conformational changes have also been described for neuropathy-associated *GARS* mutations (142,143). A series of pull-down assays showed that some mutant GARS proteins bind to the membrane receptor neuropilin-1 (NRP1), interfering with the binding of VEGF-A_{165} to an extracellular domain of NRP1 (143). This interaction relies on the secretion of GARS from the cell, which was detected by enriching for exosomes in cell culture medium and performing a western blot for wild-type GARS.

VEGF-NRP1 signaling is important for cardiovascular development, as well as motor neuron cell body migration and axon guidance (144,145). Interestingly, a closer examination of *Gars*$^{P234KY/+}$ prenatal mice found a defect in the migration of facial motor neurons similar to that in NRP1 and VEGF-A_{165} null animals (143). Furthermore, mice double heterozygous for *Gars*$^{P234KY/+}$ and *Nrp1*$^{+/-}$ developed an earlier and more severe neuromuscular phenotype, pointing to a genetic interaction between *Gars* and *Nrp1*. When *Gars*$^{P234KY/+}$ mice were treated with VEGF-A_{165}, their motor performance improved. Importantly, treating *Gars*$^{P234KY/+}$ with other neurotrophic factors or with other isoforms of VEGF-A that do not strongly bind NRP1 did not show the same improvements, pointing to the specificity of the VEGF-A_{165}/NRP1 interaction for improving the neuromuscular phenotype (143).

When considering the timing and location of VEGF-A_{165}/NRP1 signaling, several questions arise. The VEGF-A_{165}/NRP1 interaction is known to be important in cardiovascular development, which is unaffected in the *Gars*$^{C201R/+}$ mouse model (146) and in patients with ARS-related neuropathy. Additionally, NRP1 is primarily expressed developmentally, fitting with the migration defects in facial motor neurons of *Gars*$^{P234KY/+}$ mice. However, it is unclear how this translates to the human neuropathy phenotype, which is marked by degeneration, not developmental defects. It is also possible that developmental perturbations may play a role in disease later in life—recent work characterizing sensory deficits in *Gars* $^{C201R/+}$ mice showed that these mice had fewer large diameter sensory neurons and more small diameter sensory neurons than wild-type mice, and that this imbalance was present at birth (147). This was attributed to mutant GARS binding aberrantly to TrkA, TrkB, and TrkC, receptors that have a role in developmental fate switching in sensory neuron subtypes. *In vitro* immunoprecipitation experiments revealed that TrkA-C interact with P234KY and C201R GARS, but not wild-type GARS. To show the relevance of this interaction in cells, mutant (LI29P and G240R) and wild-type GARS was added to the media of neuroblastoma cells over-expressing TrkB. Mutant GARS caused a greater increase in the Trk signaling cascade compared to wild-type, suggesting that mutant GARS activates Trk signaling.

Although the finding of novel binding interactions with NRP1 and Trk receptors yield new insights into the pathogenesis of some *GARS* mutations, it would be surprising if this mechanism was shared among neuropathy-associated mutations in different ARS loci. The structures of the five neuropathy-associated ARS enzymes differ significantly, so it is unlikely that they would all have the capacity to bind to the same membrane receptors when mutated. However, if different mutant ARS enzymes aberrantly bind to different proteins that act in a common pathway, it is possible that this may explain the shared pathogenic effect.

Such a common pathway may be related to neuronal signaling, as discussed above, or may be related to protein translation independent of deficits in aminoacylation. Interestingly, the latter possibility could provide an explanation for the translation defects observed in *Drosophila* models of *GARS* and *YARS* mutants. When several neuropathy-associated mutations in human *GARS* (E71G, G240R, and G526R) or *YARS* (G41R, 153-156delVKQV, and E196K) are over-expressed in *Drosophila* motor or sensory neurons, they reduce protein translation rates and cause muscle denervation and morphological defects (115). However, this study concluded that mutant GARS does not impair the endogenous activity of *Drosophila gars*, and that the reduced translation rate caused by over-expressing G240R human *GARS* cannot be rescued by over-expressing wild-type *Drosophila gars*. These findings are particularly interesting since, if the translation defects in flies caused by over-expressing mutant GARS are not a result of mutant GARS suppressing the endogenous protein via a dominant-negative effect, then it is possible that they are caused by aberrant interactions between GARS mutants and the translational machinery.

Future directions

There is currently evidence to support multiple proposed mechanisms of ARS-mediated peripheral neuropathy; however, additional research is needed to determine if either mechanism applies to all neuropathy-associated ARS mutations and loci. For the loss-of-function model, it will be key to determine if dimerization is required for pathogenicity. For example, it will be crucial to determine if monomeric enzymes, such as MARS, are implicated in dominant axonal neuropathy, since a dominant-negative mechanism would not be possible for monomeric enzymes. Identifying *MARS* variants that segregate with disease in large pedigrees or demonstrating that *MARS* variants cause neuropathy in animal models would be important steps toward resolving this issue.

For the gain-of-function model, it will be important to show that any novel protein-protein interactions—whether with NRP1, TRIM28, Trk receptors, or other proteins—are specific to mutations associated with neuropathy, and that these interactions do not occur with nonpathogenic protein variants. Additionally, showing that multiple mutant ARS enzymes can participate in the same aberrant interaction, or different aberrant interactions that lead to the same cellular effect, will add weight to this model. Finally, demonstrating that mutations in other components of these pathways also cause peripheral neuropathy would be a strong confirmation of this mechanism.

After refining the loss- and gain-of-function models, the next step will be to determine if there is any interplay between the two mechanisms that may affect phenotypic outcome. For example, some mutations, like G598A *GARS* (41), are linked to an early-onset, severe spinal muscular atrophy, which may be

due to the compound effects of loss-of-function and gain-of-function mechanisms.

Conclusions

ARSs are emerging as a significant cause of rare inherited diseases, particularly recessive mitochondrial disorders, recessive multisystem disorders, and dominant axonal neuropathies. A total of 31 out of the 37 human ARS enzymes have been implicated in a genetic disease phenotype. Identifying and characterizing the remaining ARS loci and alleles associated with disease will provide an important tool for clinicians, and will broaden our understanding of how these enzymes function and tolerate variation. Looking towards potential therapies, it will be necessary to determine the downstream effects of ARS mutations for both dominant and recessive disease-causing alleles, and to determine how certain ARS mutations lead to tissue-specific phenotypes. Finally, we should explore whether improving ARS function itself is a viable mode of therapy for both recessive and dominant phenotypes, and if mutant-allele repression is an effective strategy for alleviating ARS-associated dominant neuropathy.

Acknowledgements

We would like to thank all of the patients and their families for agreeing to participate in the studies reviewed here; and each of our colleagues for contributing to our current knowledge of ARS-associated disease. R.M. is supported by the Michigan Predoctoral Training in Genetics Program (GM007544). A.A. is supported by funding from the National Institute of General Medical Sciences (GM118647).

Conflict of Interest statement. None declared.

Funding

Michigan Pre-doctoral Training in Genetics Program (GM007544) and National Institute of General Medical Sciences (GM118647).

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