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Ubiquitously expressed proteins and restricted phenotypes: exploring cell-specific sensitivities to impaired tRNA charging

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Abstract

Aminoacyl-tRNA synthetases (ARS) are ubiquitously expressed, essential enzymes that charge tRNA with cognate amino acids. Variants in genes encoding ARS enzymes lead to myriad human inherited diseases. First, missense, nonsense, and frameshift alleles cause recessive multi-system disorders that differentially affect tissues depending on which ARS is mutated. Second, missense alleles cause dominant peripheral neuropathy. A preponderance of evidence has shown that both phenotypic classes are associated with loss-of-function alleles, suggesting that tRNA charging plays a central role in disease pathogenesis. However, it is currently unclear how perturbation in the function of these ubiquitously expressed enzymes leads to tissue-specific or tissue-predominant phenotypes. Here, we review our current understanding of ARS-associated disease phenotypes and explore potential explanations for the observed tissue specificity.

Keywords

aminoacyl-tRNA synthetases; tRNA charging; protein translation; peripheral neuropathy; developmental delay; recessive disease

Ubiquitously expressed proteins and tissue-specific effects

Ubiquitously expressed genes typically encode proteins that are required for all cell types [1]. Interestingly, variants in ubiquitously expressed, essential genes sometimes result in tissue-specific or tissue-predominant phenotypes in affected patients. For example, variants in 20 ubiquitously expressed ribosomal protein genes have been implicated in the blood disorder Diamond-Blackfan anemia (MIM 105650), which is a defect of erythroid progenitors [2, 3]. Similarly, variants in the ubiquitously expressed tRNA nucleotidyl

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transferase 1 locus (*TRNT1* [MIM 612907]) [4] have been implicated in phenotypes affecting erythrocytes including sideroblastic anemia [5] and erythrocytic microcytosis [6]. These observations suggest that some cells are more sensitive to defects in housekeeping processes, or that some essential genes have secondary functions that are critical for a subset of tissues. Recent findings have shown that variants in aminoacyl-tRNA synthetases—a family of enzymes critical for protein translation in all cells—cause myriad clinical phenotypes that vary depending on the affected enzyme. Here, we explore tissue-specific phenotypes associated with aminoacyl-tRNA synthetase gene variants and provide plausible mechanisms to explain these observations.

Aminoacyl-tRNA synthetases and human inherited disease

Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed, essential enzymes that are responsible for charging tRNA with cognate amino acids, a crucial step in translating genetic information into proteins [7]. ARSs charge tRNA through a two-step reaction that involves: (i) activating the amino acid with ATP to form an aminoacyl adenylate intermediate; and (ii) transferring the aminoacyl group to the tRNA and releasing the charged tRNA (Figure 1) [7]. Each ARS enzyme contains peptide sequences that are important for activating the amino acid (*e.g.*, a catalytic domain) as well as sequences important for recognizing the appropriate tRNA molecules (*e.g.*, an anticodon binding domain) [8]. ARS activity is specific to amino acid and tRNA pairings, and there are multiple mechanisms that contribute to this specificity including recognition of amino acids by the ARS active site and molecular signals in tRNAs that trigger aminoacylation [9, 10]. In addition to tRNA charging, secondary functions have been described for ARS enzymes [11, 12]. For example, ARSs participate in inflammation, angiogenesis, apoptosis, and tumorigenesis [11, 12]. This expansion of functions has been linked to the addition of appended domains [13] and to protein isoforms arising from alternative splicing [14, 15]. In total, there are 37 ARS genes encoded in the human nuclear genome: 17 encode cytoplasmic enzymes; 17 encode mitochondrial enzymes; and three encode enzymes that function in both compartments [7]. ARSs are abbreviated using the single-letter code for the amino acid followed by ‘ARS1’ for cytoplasmic enzymes or ‘ARS2’ for mitochondrial enzymes [7]. For example, cytoplasmic cysteinyl-tRNA synthetase (MIM 123859) is abbreviated ‘CARS1’, and mitochondrial cysteinyl-tRNA synthetase (MIM 612800) is abbreviated ‘CARS2’.

Over the past 15 years, variants in ARS loci have been associated with a variety of human inherited disease phenotypes (Table S1). In 2003, variants in glycyl-tRNA synthetase 1 (*GARS1* [MIM 600287]) were reported in patients with dominant peripheral neuropathies (also referred to as Charcot-Marie-Tooth [CMT] disease) [16]. Since then, variants in four additional ARS loci have been implicated in CMT disease [17–20]. The mechanism by which ARS variants affect the peripheral nervous system has remained elusive. Additionally, variants in 34 of the 37 ARS loci have been implicated in early-onset, multi-system recessive disease phenotypes [21]. In these cases, most patients present with overlapping clinical phenotypes; however, some tissues appear to be more sensitive to the reduced function of a particular ARS and the mechanisms behind this tissue specificity are not defined. Here, we review our current understanding of ARS-related inherited diseases and

propose a path forward toward defining the mechanisms that lead to tissue-specific or tissue-predominant phenotypes.

Dominant peripheral neuropathies: tRNA charging deficits and axon function

Inherited or *de novo* variants in five ARS genes (*GARS1*, *YARS1* [MIM 603623], *AARS1* [MIM 601065], *HARS1* [MIM 142810], and *WARS1* [MIM 191050]) have been implicated in dominant Charcot-Marie-Tooth disease (CMT), an inherited neurodegenerative peripheral neuropathy that impairs motor and sensory function in the distal extremities [16-20]; please note that while variants in *MARS1* (MIM 156560) have been reported in patients with dominant CMT [22-26], a genetic argument for pathogenicity has not been established [22-24]. Neuropathy-associated ARS variants are uniformly missense or in-frame deletion variants [17]. The majority of these variants show loss-of-function effects in functional assays including *in vitro* aminoacylation assays and yeast complementation assays [21, 27]. Though data indicate loss-of-function effects for dominant-neuropathy-associated alleles, haploinsufficiency is unlikely to be the disease mechanism based on: (i) studies showing that heterozygous null *Gars1* mice do not have a neuropathic phenotype while mice heterozygous for pathogenic missense variants do [28, 29]; and (ii) the frequency of null alleles in the heterozygous state in gnomAD for each of the five ARS genes implicated in dominant neuropathy [30]. Indeed, the probability of loss of function intolerance (pLI) has been calculated for these five genes based on the number of loss-of-function variants identified in gnomAD (pLI is 0 for *YARS1*, *AARS1*, and *HARS1*; 0.3 for *WARS1*; and 0.31 for *GARS1*), which predicts that these genes are tolerant to the loss of one allele (the closer the pLI is to one, the more intolerant the gene is to the loss of one allele), further indicating that haploinsufficiency is unlikely [30].

Two non-mutually-exclusive hypotheses have been proposed to explain the mechanism of ARS variants in dominant neuropathy (Figure 2). First, since all five implicated enzymes function as homodimers and since the variants show loss-of-function effects, a dominant-negative mechanism may explain the dominant phenotype (Figure 2A) [21]. Non-functional mutant subunits may dimerize with wild-type subunits in a heterozygous patient cell and drastically reduce tRNA charging. An impairment in ARS function—and therefore protein translation—may be particularly detrimental to long peripheral nerve axons, potentially explaining the tissue-specificity [21]. Further work investigating if dimerization is necessary for pathogenicity and if variants in monomeric enzymes can cause dominant neuropathy will help assess the role of dominant-negative effects in disease pathogenesis.

A second hypothesis states that gain-of-function effects contribute to disease pathogenesis (Figure 2B) [21, 31]. Variants in *GARS1* [32] and *YARS1* [33] induce conformational openings that permit aberrant interactions with other proteins [31]. Mutant *GARS1* has been reported to aberrantly interact with neuropilin 1 (NRP1 [MIM 602069]) [34], tropomyosin receptor kinase (TRK) receptors [35], and histone deacetylase 6 (HDAC6 [MIM 300272]) [36, 37]. Similarly, mutant *YARS1* has been reported to aberrantly interact with tripartite motif-containing protein 28 (TRIM28 [MIM 602742]) [33]. These interactions have been

proposed to explain the sensitivity of the peripheral nervous system to *GARS1* and *YARS1* variants via neuron-specific pathways [31]; however, it should be noted that interactions between *GARS1* and *NRP1* do not explain early-onset, severe, *GARS*-associated neuropathy [38]. Regardless of the molecular mechanism, it is clear that the pathogenic alleles are dominantly toxic. In support of this notion, allele-specific RNAi rescues aspects of dominant neuropathy in a mouse model of *GARS1* variants [38], which is consistent with dominant-negative and gain-of-function mechanisms.

Variants in tRNA synthetase genes cause a spectrum of recessive disease phenotypes

To date, genetic variants in 34 of the 37 *ARS* loci have been implicated in human recessive disease phenotypes [21]; these 34 loci include all 17 genes encoding an *ARS* enzyme that functions only in the mitochondria [21]. Unsurprisingly, disease-associated variants affecting mitochondrial *ARS* enzymes typically cause clinical manifestations in highly metabolic tissues. These include, but are not limited to, the central nervous system (CNS) (*CARS2* [39], *FARS2* [MIM 611592] [40], *NARS2* [MIM 612803] [41, 42], *PARS2* [MIM 612036] [41, 42], *RARS2* [MIM 611524] [43], *TARS2* [MIM 612805] [44], *DARS2* [MIM 610956] [45], *EARS2* [MIM 612799] [46–49], *MARS2* [MIM 609728] [50, 51], and *WARS2* [MIM 604733] [52]), muscle (*YARS2* [MIM 610957] [53]), ovaries (*HARS2* [MIM 600783] [54], *LARS2* [MIM 604544] [55], and *AARS2* [MIM 612035] [56]) and the skeletal system (*IARS2* [MIM 612801] [57]) [21, 58]. Diseases associated with mitochondrial *ARS*s have been classified based on whether variants cause clinical manifestations in the CNS and/or other tissues, highlighting the spectrum of tissues involved [58]. It is currently unclear why variants in mitochondrial *ARS*s cause phenotypes affecting different tissues depending on which enzyme is affected. Two models for explaining this phenomenon have been presented [58]. First, since mitochondrial protein translation is important for oxidative phosphorylation, the tissue-specific phenotypes may arise due to temporal and spatial differences in energy requirements [58]. Second, mitochondrial *ARS*s may have secondary functions that have yet to be discovered and that may be affected by the pathogenic variants [58]. In addition, amino-acid concentrations and mitochondrial tRNA expression may be variable in different tissues [59], which would convey differential susceptibility to impaired mitochondrial *ARS* function. Further work investigating tissue-specific energy requirements, secondary functions of mitochondrial *ARS* enzymes, and the basic biology of tRNA expression and amino-acid availability in mitochondria is needed to address these questions.

Variants in 17 of the 20 *ARS* loci that encode cytoplasmic enzymes have been implicated in recessive diseases that often affect a wide array of tissues [21]. Pathogenic alleles in these 17 *ARS* genes largely cause overlapping recessive clinical presentations, which often include neurodevelopmental phenotypes. Interestingly, some tissues appear to be more sensitive to defects in a particular *ARS* gene. Extensive genetic and functional data implicate a loss-of-function molecular pathology in *ARS*-associated recessive disease [21]. Given that all of the *ARS*s share the common function of tRNA charging, a logical notion would be that disease-associated *ARS* variants cause defects in protein translation, which then contribute to disease pathogenesis (Figure 3A, Key Figure). However, while a preponderance of evidence points

to reduced ARS function as the culprit in ARS-related recessive phenotypes, the role of secondary functions cannot be ruled out (Figure 3B).

While loss-of-function ARS alleles have been convincingly implicated in a range of recessive phenotypes, a critical question has not been addressed: Why do some tissues appear to be more sensitive to impairments of particular ARSs? We speculate that differing requirements across tissues to translate proteins enriched for specific amino acids may contribute to the tissue-specific phenotypes. That is, some tissues may have high demands for proteins rich in a certain amino acid and therefore may be more sensitive to defects in charging tRNA with that amino acid. In addition, it is known that different tissues have differing availabilities of specific tRNAs [59, 60] and amino acids [61, 62], which could also influence whether a tissue is more or less sensitive to defects in specific ARSs. Here, we explore potential mechanisms to explain tissue-specific features of ARS-related disease using examples of ARS loci that cause recessive phenotypes (Figure 4).

Brittle hair and nails: cysteine, threonine, and keratin biology

Hair is a keratinized tissue that contains two major structural components: (i) hair keratins that form keratin intermediate filaments; and (ii) keratin-associated proteins that are located in the matrix around—and form disulfide bonds with—the keratin intermediate filaments [63]. The nail plate is another keratinized structure that also has a high content of keratin proteins and keratin-associated proteins [64, 65]. Variants in genes encoding hair and nail keratins result in diseases affecting the structure of these tissues including monilethrix (MIM 158000) and ectodermal dysplasia of hair and nail type [66, 67]. Variants in two ARS loci, cysteinyl-tRNA synthetase 1 (*CARS1*) and threonyl-tRNA synthetase 1 (*TARS1*; MIM 187790), cause brittle hair and nails, suggesting that keratinocytes may be particularly sensitive to impaired tRNA charging with cysteine and threonine.

CARS1 encodes the enzyme that charges tRNA^{Cys} with cysteine in the cytoplasm [7] and bi-allelic variants in *CARS1* cause a multi-system, recessive disease [68]. *CARS1* variants were identified in four patients from three families, and each of the variants showed loss-of-function effects on CARS1 protein expression, tRNA charging, or cell viability in a yeast model [68]. The patients presented with features that overlap with other ARS-related recessive phenotypes including developmental delay, microcephaly, and liver dysfunction [68]. Interestingly, all patients also presented with brittle hair and nails [68], features that had not been reported in ARS-associated diseases until recently when patients with *TARS1* variants were reported with brittle hair [69]. In the latter study, two patients were diagnosed with trichothiodystrophy (TTD), a rare, recessive, neuroectodermal disease with characteristic brittle hair [69]. Each patient carried bi-allelic *TARS1* variants that cause loss-of-function effects on TARS1 protein levels and tRNA charging [69]. One of the patients additionally had ichthyosis, follicular keratosis, delayed physical development, recurrent respiratory tract infections, and acromandibular dysplasia [69]. The second patient was born encased in a tight membrane and had ichthyosis [69]. A nail phenotype was not reported in these patients [69].

How *CARS1* variants lead to brittle hair and nails, and how variants in *TARS1* lead to brittle hair is not understood. For *CARS1* variants, this phenotype may reflect the high cysteine requirements of hair and nails [68], since hair and nail keratins and keratin-associated proteins contain many cysteine residues that participate in disulfide bonding. In fact, we performed a computational analysis of the human proteome to identify proteins with cysteine content above the proteome average (3%) and found that 25 of the top 30 proteins with the highest percentages of cysteine (35-41%) are keratin-associated proteins (unpublished data, Table 1). Additionally, gene ontology analysis of genes encoding proteins with higher than average cysteine content showed an enrichment for gene products associated with ‘keratinization’ (GO:0031424; p-value = 4.35e-28) (Table S2). Since disease-associated *CARS1* variants cause a loss-of-function effect, tRNA^{Cys} charging is likely impaired, which may lead to defects in cysteine-rich protein translation, including keratins and keratin-associated proteins; such an effect may explain the brittle hair and nail phenotype. For *TARS1*, it has been suggested that pathogenic variants cause brittle hair due to reduced protein translation [69]; however, this does not explain why this phenotype is only observed in patients with *TARS1* and *CARS1* variants. Though keratin-associated proteins are not the most threonine-rich proteins in the proteome, many cysteine-rich, keratin-associated proteins also have threonine content higher than the proteome average (5%). For example, keratin-associated protein 9-2 (KRTAP9-2), which is 33% cysteine, is also 16% threonine. Gene ontology analysis of genes encoding proteins with higher than average threonine content showed an enrichment for gene products associated with adhesion including ‘cell-cell adhesion’ (GO:0098609; p-value = 1.83e-10) (Table S2), which is an important process for keratinocytes [70]. Further work is needed to determine if cysteine- and threonine-rich proteins are affected by *CARS1* and *TARS1* variants. Our analyses revealed another group of proteins that have high cysteine content: metallothioneins (MTs), which are metal-binding proteins that function in detoxification of heavy metals such as cadmium and copper [71]. If MTs are affected by disease-associated *CARS1* variants, patients may be more susceptible to heavy metal toxicity. Experiments in relevant models in the environment of depleted *CARS1* function are needed to evaluate the effect on metallothionein protein levels and to test for differential sensitivity to heavy metal exposure.

Sensorineural hearing loss: lysine and the inner ear

The inner ear has a complex cellular organization and, therefore, variants in a large panel of genes can cause hearing loss [72, 73]. Lysyl-tRNA synthetase 1 (*KARS1*; MIM 601421) encodes the enzyme that charges tRNA^{Lys} with lysine in both the cytoplasm and the mitochondria [74]. Interestingly, bi-allelic *KARS1* variants have been associated with recessive, non-syndromic hearing impairment [73], suggesting that inner ear cells may be particularly sensitive to impairments in tRNA charging with lysine. In addition to non-syndromic hearing impairment, *KARS1* variants have been implicated in recessive, syndromic hearing loss. In these latter patients, hearing loss is comorbid with other symptoms commonly associated with *ARS* variants including developmental delay, neurocognitive decline, seizures, and leukoencephalopathy [75-77].

It is not currently understood how the inner ear is particularly sensitive to mutations in *KARS1*. It has been suggested that aminoacylation impairments in the cochlea might affect

many cellular processes and lead to hearing impairment [73]. A general sensitivity to impaired translation may explain why variants in other ARS loci (*LARS2* [55], *HARS2* [54], *HARS1* [78], *AARS1* [79], *RARS2* [80]) have also been associated with broader syndromes that include hearing impairment, but it does not explain the non-syndromic hearing loss associated with *KARS1* variants. We wondered if inner ear cells have high lysine requirements that may explain this tissue-specific phenotype. We analyzed the human proteome to identify proteins with percentages of lysine higher than the proteome average (6%) and then considered if those proteins had roles in the inner ear (unpublished data, Table 1). One protein of particular interest with high lysine content is growth arrest-specific 8 (GAS8 [MIM 605178]), which contains 12% lysine and is a component of the dynein regulatory complex that is important for ciliary motility and for ear development [81]. Similarly, the transmembrane inner ear expressed gene (*TMIE* [MIM 607237]) has been associated with autosomal recessive non-syndromic hearing loss [82] and encodes a protein with 15% lysine. Gene ontology analysis of genes encoding proteins with higher than average lysine content showed the greatest enrichment for gene products associated with protein targeting including ‘SRP-dependent cotranslational protein targeting to membrane’ (GO:0006614; p-value = 3.83e-8) and ‘protein targeting to ER’ (GO:0045047; p-value = 1.61e-8) (Table S2); however, it is unclear how perturbations in these processes would specifically affect the process of hearing. Future work is needed to investigate the roles of lysine-rich proteins in the inner ear and if the translation of lysine-rich proteins are specifically affected by *KARS1* variants.

Infantile hepatopathy: leucine and liver function

Infantile liver disease is a life-threatening condition that develops in early infancy and can be difficult to diagnose [83]. Patients present with failure to thrive, jaundice, and distended abdomen, and may have elevated liver transaminases and hyperbilirubinemia [83]. Leucyl-tRNA synthetase 1 (*LARS1*) encodes the enzyme that charges tRNA^{Leu} with leucine in the cytoplasm [7]. Bi-allelic variants in *LARS1* cause infantile liver failure syndrome type 1 (ILFS1; MIM 615438), a multi-system disorder involving infantile liver failure, developmental delay, anemia, seizures, and recurrent liver dysfunction triggered by febrile illness [83-86]. Notably, bi-allelic variants in many ARS genes result in phenotypes that affect the liver [21, 87], which may indicate that this organ is generally sensitive to impaired protein translation. However, *LARS1* variants seem to predominantly affect the liver and cause earlier onset and a more severe liver phenotype; perhaps this liver-predominant effect may be due to impaired translation of proteins with high leucine content. It has been proposed that proteins with high levels of leucine were most likely to be affected in patients with *LARS1* variants [83]. Previous analysis of the human proteome determined that immune-related proteins contain very high percentages of leucine, which correlates with the patient phenotype of hepatic dysfunction triggered with illness [83]. Additionally, leucine-rich proteins are involved in the phospholipid biosynthetic process and, in support of this observation, large fat deposits were seen in affected livers [83]. Our gene ontology analysis of genes encoding proteins with higher than average leucine content showed an enrichment for similar terms including ‘lipid biosynthetic process’ (GO:0008610; p-value = 1.79e-4) (Table S2). Furthermore, when analyzing the human proteome for leucine-rich proteins, we

found that the gene associated with infantile liver failure syndrome 2 (MIM 616483) [88] encodes a protein with a higher percentage of leucine than the proteome average of 10%; NBAS (MIM 608025) is 13% leucine.

As mentioned above, loss of a secondary function could explain the tissue-specific effects of ARS variants and there are data suggestive of this for *LARS1*-associated disease. It has been reported that LARS1 is a leucine sensor and an activator of mTORC1 (an autophagy inhibitor), and that LARS1 deficiency results in reduced activation of mTORC1 and increased autophagy [89]. Reduced hepatic mTORC1 activity has been shown to cause liver cell damage; therefore, variants in *LARS1* may contribute to disease through reduced mTORC1 activity and abnormal autophagy [84]. Aberrant mTORC1 signaling may also contribute to the seizure phenotype observed in these patients since mTOR signaling has been associated with neurological disorders including epilepsy [90]. Future research is needed to determine if leucine-rich protein expression is specifically affected by *LARS1* variants, and if the disease-associated alleles affect mTORC1 activity.

Future directions toward defining the mechanisms of tissue-specific ARS phenotypes

Variants in ARSs have been implicated in a variety of human diseases with dominant and recessive inheritance patterns, and many questions on the mechanisms of disease pathogenesis remain unanswered (see Outstanding Questions). Variants in five ARSs have been implicated in dominant peripheral neuropathy, and both dominant-negative and gain-of-function mechanisms have been proposed to explain the peripheral nerve phenotype (Figure 2). Further work is needed to address critical questions about ARS-associated neuropathy including: Is there is a common, unifying feature among the five implicated enzymes that explains the dominant neuropathy? and Can mutations in any ARS enzyme cause dominant neuropathy or are there unique features about the five enzymes implicated to date?

As outlined above, variants in cytoplasmic ARSs cause recessive phenotypes often affecting a wide array of tissues; however, these variants have also been linked with tissue-specific or tissue-predominant effects and the mechanisms of the tissue specificity is not defined. Given that genetic and functional data point to a loss of enzyme function as the molecular mechanism of ARS-mediated recessive disease, impaired ARS activity likely leads to impaired protein translation. Since many ARS-associated recessive phenotypes include overlapping features, a global effect on protein translation may occur when any ARS enzyme is mutated; however, tissue-predominant effects may not be explained by a global reduction in protein translation.

One potential explanation for the observed tissue specificity of ARS-associated recessive disease is that tissues may have different demands for different amino acids (Figure 3A). For example, as discussed above, patients with *CARS1* variants present with brittle hair and nails, which both express cysteine-rich keratins and keratin-associated proteins. Therefore, if tRNA^{Cys} charging with cysteine is impaired, hair and nails may be more susceptible to aminoacylation defects because of the high cysteine demand for keratin translation. Two

lines of research would test this hypothesis. First, ribosome profiling studies [91] will be useful to determine if ribosomes are stalling at cysteine codons, in particular along transcripts with many cysteine codons. Second, quantitative proteomics studies using metabolic isotopic labeling [92] or label-free [93] quantitative mass spectrometry on relevant tissue samples would determine if the expression of cysteine-rich proteins is significantly reduced in the environment of pathogenic *CARS1* variants.

An additional possibility to explain tissue-specific phenotypes associated with ARS variants relates to the availability of tRNA [59, 60] and amino acids [61, 62], which may vary among tissues—due to differences in pathways including biosynthesis and salvage—and influence the cellular response to impaired ARS activity (Figure 3A). For example, a mutant *CARS1* enzyme with reduced activity would likely charge more tRNA^{Cys} molecules in a tissue with abundant tRNA^{Cys} and cysteine compared to a tissue with low availability of these molecules. Studies investigating tissue-specific tRNA expression and amino acid abundance are needed to address this hypothesis. First, to quantify tRNA abundance, tRNA sequencing techniques [94, 95] or tRNA microarrays [59] could be applied to different tissues, which would indicate if patient phenotypes occur in tissues with low levels of tRNA^{Cys}. Second, amino acid content across different tissues could be determined using chromatography [69, 96] to determine if patient phenotypes occur in tissues with low levels of cysteine.

A final consideration on the mechanisms behind tissue-predominant ARS-associated phenotypes is that secondary functions have been reported for some ARSs (Figure 3B). More basic science research is needed to uncover secondary functions of ARSs and to determine how these functions may relate to ARS-associated disease. Extending our *CARS1* example reveals two interesting possibilities: (i) *CARS1* functions in cysteine polysulfidation [97] and ferroptosis [98]; and (ii) alternative splicing results in two widely expressed *CARS1* protein isoforms that contain different C-terminal amino-acid sequences [99]. Studies measuring cysteine hydropersulfide [100] in relevant patient tissues would indicate if cysteine polysulfidation is affected by impaired *CARS1*. Additionally, knockdown of *CARS1* has been reported to inhibit ferroptosis induced by cysteine deprivation and to induce the transsulfuration pathway [98]; studies investigating components of these pathways in the environment of pathogenic *CARS1* variants would be informative. To further investigate the two widely expressed *CARS1* isoforms and their potential functions, subcellular fractionation [101] and fluorescence imaging [102] experiments would indicate if the isoforms have different subcellular localizations. Co-immunoprecipitation and mass spectrometry experiments [103] would also indicate if the isoforms have differential binding partners. By defining secondary functions of ARS enzymes, the community may then determine if—and how—pathogenic variants affect these activities.

Concluding Remarks

Variants in nearly all 37 human ARS loci have been implicated in genetic disease. Five ARS genes have been linked with dominant peripheral neuropathies, which may reflect sensitivity of the peripheral nervous system to impairments in protein translation and/or to aberrant protein interactions that affect signaling pathways. Variants in the majority of ARSs have

been linked with recessive phenotypes and certain ARSs are associated with tissue-specific phenotypes despite the ubiquitous expression of these essential genes. The explanation for the observed tissue specificity is not understood and may reflect: *(i)* differing requirements across tissues to translate proteins enriched for specific amino acids; *(ii)* differing availability of tRNA and amino acids across tissues; and/or *(iii)* impairment of secondary functions of ARS enzymes that are important for specific tissues. Experiments designed to test these hypotheses will provide insights into the biological function of ARS enzymes, advance our understanding of the mechanisms of ARS-associated diseases, and more broadly enable us to characterize other clinical phenotypes caused by mutations in the protein translation machinery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Ramskold D, et al. (2009) An abundance of ubiquitously expressed genes revealed by tissue transcriptome sequence data. *PLoS Comput Biol* 5, e1000598 [PubMed: 20011106]
2. Da Costa L, et al. (2018) An update on the pathogenesis and diagnosis of Diamond-Blackfan anemia. *F1000Res* 7 [PubMed: 29527296]
3. Da Costa L, et al. (2018) Molecular approaches to diagnose Diamond-Blackfan anemia: The EuroDBA experience. *Eur J Med Genet* 61, 664–673 [PubMed: 29081386]
4. Cudny H, et al. (1986) Cloning, sequencing, and species relatedness of the *Escherichia coli* cca gene encoding the enzyme tRNA nucleotidyltransferase. *J Biol Chem* 261, 6444–6449 [PubMed: 3009457]
5. Chakraborty PK, et al. (2014) Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD). *Blood* 124, 2867–2871 [PubMed: 25193871]
6. DeLuca AP, et al. (2016) Hypomorphic mutations in TRNT1 cause retinitis pigmentosa with erythrocytic microcytosis. *Hum Mol Genet* 25, 44–56 [PubMed: 26494905]
7. Antonellis A and Green ED (2008) The role of aminoacyl-tRNA synthetases in genetic diseases. *Annu Rev Genomics Hum Genet* 9, 87–107 [PubMed: 18767960]
8. Ribas de Pouplana L and Schimmel P (2001) Two classes of tRNA synthetases suggested by sterically compatible dockings on tRNA acceptor stem. *Cell* 104, 191–193 [PubMed: 11269237]
9. Pak D, et al. (2018) Aminoacyl-tRNA synthetase evolution and sectoring of the genetic code. *Transcription* 9, 205–224 [PubMed: 29727262]
10. Giege R, et al. (1998) Universal rules and idiosyncratic features in tRNA identity. *Nucleic Acids Res* 26, 5017–5035 [PubMed: 9801296]
11. Guo M and Schimmel P (2013) Essential nontranslational functions of tRNA synthetases. *Nat Chem Biol* 9, 145–153 [PubMed: 23416400]
12. Kim S, et al. (2011) Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping. *Nat Rev Cancer* 11, 708–718 [PubMed: 21941282]

13. Guo M, et al. (2010) Functional expansion of human tRNA synthetases achieved by structural inventions. *FEBS Lett* 584, 434–442 [PubMed: 19932696]
14. Wakasugi K, et al. (2002) A human aminoacyl-tRNA synthetase as a regulator of angiogenesis. *Proc Natl Acad Sci U S A* 99, 173–177 [PubMed: 11773626]
15. Lo WS, et al. (2014) Human tRNA synthetase catalytic nulls with diverse functions. *Science* 345, 328–332 [PubMed: 25035493]
16. Antonellis A, 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 [PubMed: 12690580]
17. Jordanova A, 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 [PubMed: 16429158]
18. Latour P, 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 [PubMed: 20045102]
19. Tsai PC, et al. (2017) A recurrent WARS mutation is a novel cause of autosomal dominant distal hereditary motor neuropathy. *Brain* 140, 1252–1266 [PubMed: 28369220]
20. Vester A, 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 [PubMed: 22930593]
21. 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 [PubMed: 28633377]
22. Rips J, et al. (2018) MARS variant associated with both recessive interstitial lung and liver disease and dominant Charcot-Marie-Tooth disease. *Eur J Med Genet* 61, 616–620 [PubMed: 29655802]
23. Sagi-Dain L, et al. (2018) Whole-exome sequencing reveals a novel missense mutation in the MARS gene related to a rare Charcot-Marie-Tooth neuropathy type 2U. *J Peripher Nerv Syst* 23, 138–142 [PubMed: 29582526]
24. Gonzalez M, 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 [PubMed: 23729695]
25. Hyun YS, et al. (2014) Rare variants in methionyl- and tyrosyl-tRNA synthetase genes in late-onset autosomal dominant Charcot-Marie-Tooth neuropathy. *Clin Genet* 86, 592–594 [PubMed: 24354524]
26. Nam SH, et al. (2016) Identification of Genetic Causes of Inherited Peripheral Neuropathies by Targeted Gene Panel Sequencing. *Mol Cells* 39, 382–388 [PubMed: 27025386]
27. Oprescu SN, et al. (2017) Predicting the pathogenicity of aminoacyl-tRNA synthetase mutations. *Methods* 113, 139–151 [PubMed: 27876679]
28. Seburn KL, et al. (2006) An active dominant mutation of glycyl-tRNA synthetase causes neuropathy in a Charcot-Marie-Tooth 2D mouse model. *Neuron* 51, 715–726 [PubMed: 16982418]
29. Motley WW, et al. (2011) Charcot-Marie-Tooth-linked mutant GARS is toxic to peripheral neurons independent of wild-type GARS levels. *PLoS Genet* 7, e1002399 [PubMed: 22144914]
30. Lek M, et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536, 285–291 [PubMed: 27535533]
31. Wei N, et al. (2019) Neurodegenerative Charcot-Marie-Tooth disease as a case study to decipher novel functions of aminoacyl-tRNA synthetases. *J Biol Chem* 294, 5321–5339 [PubMed: 30643024]
32. He W, et al. (2011) Dispersed disease-causing neomorphic mutations on a single protein promote the same localized conformational opening. *Proc Natl Acad Sci U S A* 108, 12307–12312 [PubMed: 21737751]
33. Blocquel D, et al. (2017) Alternative stable conformation capable of protein misinteraction links tRNA synthetase to peripheral neuropathy. *Nucleic Acids Res* 45, 8091–8104 [PubMed: 28531329]
34. He W, et al. (2015) CMT2D neuropathy is linked to the neomorphic binding activity of glycyl-tRNA synthetase. *Nature* 526, 710–714 [PubMed: 26503042]

35. Sleight JN, 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 [PubMed: 28351971]
36. Mo Z, et al. (2018) Aberrant GlyRS-HDAC6 interaction linked to axonal transport deficits in Charcot-Marie-Tooth neuropathy. *Nat Commun* 9, 1007 [PubMed: 29520015]
37. Benoy V, et al. (2018) HDAC6 is a therapeutic target in mutant GARS-induced Charcot-Marie-Tooth disease. *Brain* 141, 673–687 [PubMed: 29415205]
38. Morelli KH, et al. (2019) Allele-specific RNA interference prevents neuropathy in Charcot-Marie-Tooth disease type 2D mouse models. *J Clin Invest*
39. Coughlin CR 2nd, 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 [PubMed: 25787132]
40. Cho JS, et al. (2017) FARS2 mutation and epilepsy: Possible link with early-onset epileptic encephalopathy. *Epilepsy Res* 129, 118–124 [PubMed: 28043061]
41. Sofou K, et al. (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 [PubMed: 25629079]
42. Mizuguchi T, et al. (2017) PARS2 and NARS2 mutations in infantile-onset neurodegenerative disorder. *J Hum Genet* 62, 525–529 [PubMed: 28077841]
43. Zhang J, et al. (2018) Distinct magnetic resonance imaging features in a patient with novel RARS2 mutations: A case report and review of the literature. *Exp Ther Med* 15, 1099–1104 [PubMed: 29434700]
44. Diodato D, et al. (2014) VARS2 and TARS2 mutations in patients with mitochondrial encephalomyopathies. *Hum Mutat* 35, 983–989 [PubMed: 24827421]
45. Scheper GC, et al. (2007) Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nat Genet* 39, 534–539 [PubMed: 17384640]
46. Steenweg ME, et al. (2012) Leukoencephalopathy with thalamus and brainstem involvement and high lactate 'LTBL' caused by EARS2 mutations. *Brain* 135, 1387–1394 [PubMed: 22492562]
47. Talim B, et al. (2013) Multisystem fatal infantile disease caused by a novel homozygous EARS2 mutation. *Brain* 136, e228 [PubMed: 23008233]
48. Sahin S, et al. (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 [PubMed: 27206875]
49. Oliveira R, et al. (2017) Lethal Neonatal LTBL Associated with Biallelic EARS2 Variants: Case Report and Review of the Reported Neuroradiological Features. *JIMD Rep* 33, 61–68 [PubMed: 27571996]
50. Bayat V, 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 [PubMed: 22448145]
51. Webb BD, et al. (2015) Novel, compound heterozygous, single-nucleotide variants in MARS2 associated with developmental delay, poor growth, and sensorineural hearing loss. *Hum Mutat* 36, 587–592 [PubMed: 25754315]
52. Theisen BE, et al. (2017) Deficiency of WARS2, encoding mitochondrial tryptophanyl tRNA synthetase, causes severe infantile onset leukoencephalopathy. *Am J Med Genet A* 173, 2505–2510 [PubMed: 28650581]
53. Sommerville EW, et al. (2017) Clinical Features, Molecular Heterogeneity, and Prognostic Implications in YARS2-Related Mitochondrial Myopathy. *JAMA Neurol* 74, 686–694 [PubMed: 28395030]
54. Pierce SB, et al. (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 [PubMed: 21464306]

55. Pierce SB, et al. (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 [PubMed: 23541342]
56. Dallabona C, et al. (2014) Novel (ovario) leukodystrophy related to AARS2 mutations. *Neurology* 82, 2063–2071 [PubMed: 24808023]
57. Moosa S, 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 [PubMed: 28328135]
58. Gonzalez-Serrano LE, et al. (2019) When a common biological role does not imply common disease outcomes: Disparate pathology linked to human mitochondrial aminoacyl-tRNA synthetases. *J Biol Chem* 294, 5309–5320 [PubMed: 30647134]
59. Dittmar KA, et al. (2006) Tissue-specific differences in human transfer RNA expression. *PLoS Genet* 2, e221 [PubMed: 17194224]
60. Sagi D, et al. (2016) Tissue- and Time-Specific Expression of Otherwise Identical tRNA Genes. *PLoS Genet* 12, e1006264 [PubMed: 27560950]
61. Barle H, et al. (1996) The concentrations of free amino acids in human liver tissue obtained during laparoscopic surgery. *Clin Physiol* 16, 217–227 [PubMed: 8736710]
62. Bergstrom J, et al. (1974) Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol* 36, 693–697 [PubMed: 4829908]
63. Shimomura Y and Ito M (2005) Human hair keratin-associated proteins. *J Invest Dermatol Symp Proc* 10, 230–233
64. Rice RH, et al. (2010) Proteomic analysis of human nail plate. *J Proteome Res* 9, 6752–6758 [PubMed: 20939611]
65. Rice RH (2011) Proteomic analysis of hair shaft and nail plate. *J Cosmet Sci* 62, 229–236 [PubMed: 21635850]
66. Chamcheu JC, et al. (2011) Keratin gene mutations in disorders of human skin and its appendages. *Arch Biochem Biophys* 508, 123–137 [PubMed: 21176769]
67. Schweizer J, et al. (2007) Hair follicle-specific keratins and their diseases. *Exp Cell Res* 313, 2010–2020 [PubMed: 17428470]
68. Kuo ME, et al. (2019) CysteinyI-tRNA Synthetase Mutations Cause a Multi-System, Recessive Disease That Includes Microcephaly, Developmental Delay, and Brittle Hair and Nails. *Am J Hum Genet* 104, 520–529 [PubMed: 30824121]
69. Theil AF, et al. (2019) Bi-allelic TARS Mutations Are Associated with Brittle Hair Phenotype. *Am J Hum Genet* 105, 434–440 [PubMed: 31374204]
70. Cabrijan L and Lipozencic J (2011) Adhesion molecules in keratinocytes. *Clin Dermatol* 29, 427–431 [PubMed: 21679870]
71. Thirumoorthy N, et al. (2011) A review of metallothionein isoforms and their role in pathophysiology. *World J Surg Oncol* 9, 54 [PubMed: 21599891]
72. Yaeger D, et al. (2006) Outcomes of clinical examination and genetic testing of 500 individuals with hearing loss evaluated through a genetics of hearing loss clinic. *Am J Med Genet A* 140, 827–836 [PubMed: 16532460]
73. Santos-Cortez RL, et al. (2013) Mutations in KARS, encoding lysyl-tRNA synthetase, cause autosomal-recessive nonsyndromic hearing impairment DFNB89. *Am J Hum Genet* 93, 132–140 [PubMed: 23768514]
74. Tolkunova E, et al. (2000) The human lysyl-tRNA synthetase gene encodes both the cytoplasmic and mitochondrial enzymes by means of an unusual alternative splicing of the primary transcript. *J Biol Chem* 275, 35063–35069 [PubMed: 10952987]
75. Sun C, et al. (2019) Loss-of-function mutations in Lysyl-tRNA synthetase cause various leukoencephalopathy phenotypes. *Neurol Genet* 5, e565 [PubMed: 31192300]
76. Zhou XL, et al. (2017) Mutations in KARS cause early-onset hearing loss and leukoencephalopathy: Potential pathogenic mechanism. *Hum Mutat* 38, 1740–1750 [PubMed: 28887846]

77. Scheidecker S, et al. (2019) Mutations in KARS cause a severe neurological and neurosensory disease with optic neuropathy. *Hum Mutat*
78. Puffenberger EG, et al. (2012) Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One* 7, e28936 [PubMed: 22279524]
79. McLaughlin HM, 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 [PubMed: 22009580]
80. Glamuzina E, et al. (2012) Further delineation of pontocerebellar hypoplasia type 6 due to mutations in the gene encoding mitochondrial arginyl-tRNA synthetase, RARS2. *J Inherit Metab Dis* 35, 459–467 [PubMed: 22086604]
81. Colantonio JR, et al. (2009) The dynein regulatory complex is required for ciliary motility and otolith biogenesis in the inner ear. *Nature* 457, 205–209 [PubMed: 19043402]
82. Naz S, et al. (2002) Mutations in a novel gene, TMIE, are associated with hearing loss linked to the DFNB6 locus. *Am J Hum Genet* 71, 632–636 [PubMed: 12145746]
83. Casey JP, et al. (2012) Identification of a mutation in LARS as a novel cause of infantile hepatopathy. *Mol Genet Metab* 106, 351–358 [PubMed: 22607940]
84. Casey JP, et al. (2015) Clinical and genetic characterisation of infantile liver failure syndrome type 1, due to recessive mutations in LARS. *J Inherit Metab Dis* 38, 1085–1092 [PubMed: 25917789]
85. Lin WX, et al. (2017) [Clinical feature and molecular diagnostic analysis of the first non-caucasian child with infantile liver failure syndrome type 1]. *Zhongguo Dang Dai Er Ke Za Zhi* 19, 913–920 [PubMed: 28774368]
86. Peroutka C, et al. (2019) Severe Neonatal Manifestations of Infantile Liver Failure Syndrome Type 1 Caused by Cytosolic Leucine-tRNA Synthetase Deficiency. *JIMD Rep* 45, 71–76 [PubMed: 30349989]
87. Antonellis A, et al. (2018) Compound heterozygosity for loss-of-function FARSB variants in a patient with classic features of recessive aminoacyl-tRNA synthetase-related disease. *Hum Mutat* 39, 834–840 [PubMed: 29573043]
88. Haack TB, et al. (2015) Biallelic Mutations in NBAS Cause Recurrent Acute Liver Failure with Onset in Infancy. *Am J Hum Genet* 97, 163–169 [PubMed: 26073778]
89. Han JM, et al. (2012) Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* 149, 410–424 [PubMed: 22424946]
90. Bockaert J and Marin P (2015) mTOR in Brain Physiology and Pathologies. *Physiol Rev* 95, 1157–1187 [PubMed: 26269525]
91. Ingolia NT, et al. (2012) The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. *Nat Protoc* 7, 1534–1550 [PubMed: 22836135]
92. Ong SE, et al. (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics* 1, 376–386 [PubMed: 12118079]
93. Weisser H, et al. (2013) An automated pipeline for high-throughput label-free quantitative proteomics. *J Proteome Res* 12, 1628–1644 [PubMed: 23391308]
94. Gogakos T, et al. (2017) Characterizing Expression and Processing of Precursor and Mature Human tRNAs by Hydro-tRNAseq and PAR-CLIP. *Cell Rep* 20, 1463–1475 [PubMed: 28793268]
95. Goodarzi H, et al. (2016) Modulated Expression of Specific tRNAs Drives Gene Expression and Cancer Progression. *Cell* 165, 1416–1427 [PubMed: 27259150]
96. Cheng S, et al. (2009) Trichothiodystrophy and fragile hair: the distinction between diagnostic signs and diagnostic labels in childhood hair disease. *Br J Dermatol* 161, 1379–1383 [PubMed: 19754869]
97. Akaike T, et al. (2017) Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat Commun* 8, 1177 [PubMed: 29079736]
98. Hayano M, et al. (2016) Loss of cysteinyl-tRNA synthetase (CARS) induces the transsulfuration pathway and inhibits ferroptosis induced by cystine deprivation. *Cell Death Differ* 23, 270–278 [PubMed: 26184909]

99. Davidson E, et al. (2001) Isolation of two cDNAs encoding functional human cytoplasmic cysteinyl-tRNA synthetase. *Biol Chem* 382, 399–406 [PubMed: 11347887]
100. Doka E, et al. (2016) A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems. *Sci Adv* 2, e1500968 [PubMed: 26844296]
101. La Bella V, et al. (2000) Expression and subcellular localization of two isoforms of the survival motor neuron protein in different cell types. *J Neurosci Res* 62, 346–356 [PubMed: 11054803]
102. Kohnhorst CL, et al. (2016) Subcellular functions of proteins under fluorescence single-cell microscopy. *Biochim Biophys Acta* 1864, 77–84 [PubMed: 26025769]
103. Maccarrone G, et al. (2017) Characterization of a Protein Interactome by Co-Immunoprecipitation and Shotgun Mass Spectrometry. *Methods Mol Biol* 1546, 223–234 [PubMed: 27896772]

HIGHLIGHTS

- Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed, essential enzymes that ligate amino acids onto cognate tRNA molecules.
- Variants in loci encoding ARS enzymes have been implicated in myriad dominant and recessive human inherited disease phenotypes.
- Five of the 37 human ARS loci are associated with dominant peripheral neuropathy with a primarily axonal pathology; the vast majority of the alleles cause a loss-of-function effect.
- Nearly all 37 human ARS loci are associated with recessive multi-system disorders, and every genotype result in severely impaired ARS function.
- Interestingly, for both dominant and recessive phenotypes, certain tissues are exclusively or more severely affected; the mechanism(s) by which specific tissues are affected is not defined.
- Defining the molecular mechanism(s) of ARS-related disease and determining the cell-type specificity to impaired ARS function will provide insight into the biology of these essential enzymes and will reveal potential therapeutic targets.

OUTSTANDING QUESTIONS

- What are the downstream consequences of impaired ARS function on protein translation? Does this result in a global defect in translation or does this cause a more dramatic impairment in the translation of a specific subset of proteins?
- Do disease-associated ARS alleles impact non-canonical functions and does this process play a role in human disease pathogenesis?
- For recessive diseases caused by ARS variants, are some tissues more affected than others due to a requirement for proteins enriched for the associated amino acid? Similarly, are some tissues more affected due to lower levels of the associated amino acid or tRNA?
- For ARS variants that cause dominant axonal neuropathy, does impaired enzyme function (possibly exacerbated by a dominant-negative effect) lead to the axonal dysfunction? Alternatively, is a toxic gain-of-function effect common to all neuropathy-associated ARS alleles? Finally, can variants in any ARS enzyme cause dominant neuropathy, or is there something unique about the five that have been implicated to date?

Figure 1.

The two-step aminoacylation reaction. **(A)** First, the aminoacyl-tRNA synthetase (ARS) binds the amino acid (AA) and an ATP molecule to form the aminoacyl adenylate intermediate; this process results in the release of a pyrophosphate molecule. **(B)** Second, a cognate tRNA molecule binds the ARS and the amino acid is transferred to the tRNA; an AMP molecule and the charged tRNA are then released. The image represents a dimeric enzyme where each subunit is capable of charging tRNA.

Figure 2.

Potential mechanisms of ARS-associated dominant peripheral neuropathy. **(A)** Non-functional mutant ARS subunits may dimerize with wild-type subunits in a heterozygous patient cell and drastically reduce tRNA charging—and subsequently protein translation—via a dominant-negative effect. **(B)** Mutant ARS enzymes may aberrantly bind NRP1, TRK, or HDAC6 and perturb neuronal signaling pathways. A key is provided to define each component; see text for details.

Figure 3. Potential mechanisms of ARS-associated recessive disease. **(A)** Wild-type CARS1 (left) charges tRNA^{Cys} with cysteine, and ribosomes are then able to translate transcripts with many cysteine codons. In contrast, mutant CARS1 (right) has reduced charging activity, which could result in uncharged tRNA^{Cys} and the stalling of ribosomes along transcripts with many cysteine codons (indicated by small curved lines around ribosome). The impaired charging activity may be exacerbated by reduced levels of tRNA^{Cys} (grayed out tRNA) and/or cysteine (grayed out circles) in specific tissues. **(B)** Wild-type LARS1 (left) is an activator of mTORC1, which inhibits autophagy. Activation of mTORC1 may be impaired by mutant LARS1 (right), which could lead to abnormally increased autophagy. A key is provided to define each component; see text for details.

Figure 4.

Variants in ARS genes have been associated with tissue-specific or tissue-predominant recessive phenotypes. Variants in *CARS1* and *TARS1* have been implicated in brittle hair (blue), and *CARS1* variants have also been implicated in brittle nails (blue). Variants in *KARS1* have been associated with sensorineural hearing impairment (red). Variants in *MARS1* have been implicated in interstitial lung disease and pulmonary alveolar proteinosis (yellow). Variants in *LARS1* have been implicated in infantile hepatopathy (green).

Table 1.

Top 30 human proteins with the highest percentages of each indicated amino acid.

Cysteine		Threonine		Lysine		Leucine	
Protein	% Cys	Protein	% Thr	Protein	% Lys	Protein	% Leu
SCYGR5	41%	IGHD1-1	60%	TMA7	31%	PPY2P	38%
SCYGR1	40%	MUC2	35%	H1F0	29%	MYMX	30%
SCYGR3	39%	MUC22	34%	HIST1H1B	29%	SFTA2	26%
SCYGR7	39%	MUC3A	29%	HIST1H1E	28%	MFSD3	25%
SCYGR6	38%	TRBJ2-3	25%	RPL41	28%	OCLM	25%
SCYGR4	37%	TRBD1	25%	HIST1H1C	28%	MT-ND3	24%
KRTAP4-6	37%	MUC17	24%	HIST1H1D	28%	TTY13	24%
SCYGR8	37%	MUC12	22%	H1-1	27%	SCGB3A2	24%
KRTAP4-12	37%	MUC3B	21%	SREK1IP1	26%	MT-ND4L	23%
KRTAP4-4	37%	MUC5B	21%	CYLC2	26%	TMEM216	23%
SCYGR2	37%	MUC5AC	20%	FAM133B	25%	GLYCAM1	23%
KRTAP4-5	36%	PRG4	20%	FAM133A	25%	TSPO2	23%
KRTAP4-3	36%	TRBJ2-7	20%	HMG2	24%	GPIBB	23%
KRTAP4-7	36%	TRBJ1-2	20%	HMG4	23%	TMEM203	23%
KRTAP17-1	36%	TRBJ1-1	20%	LLPH	23%	SMIM40	23%
KRTAP5-7	36%	MUC19	20%	RPL38	23%	SLC39A5	23%
KRTAP9-1	36%	MUC21	19%	CYLC1	23%	TMEM82	23%
KRTAP5-9	36%	TRGJ1	19%	FAU	22%	BPIFA1	23%
KRTAP4-11	35%	TRDJ1	19%	TRDN	22%	IL11	23%
KRTAP5-8	35%	HCG22	19%	RPS25	22%	SLC35A4	23%
KRTAP5-11	35%	HAVCR1	18%	RPL39P5	22%	NRM	23%
KRTAP4-9	35%	KLF18	18%	FAM32A	21%	SFT2D3	22%
KRTAP4-8	35%	MUCL1	18%	C19orf53	21%	IGFALS	22%
KRTAP5-6	35%	MUC16	18%	RPL35	21%	TMEM35B	22%
KRTAP4-2	35%	TRBJ1-6	18%	HMG1	21%	CYB561D2	22%
MT1B	34%	PLAC4	17%	RPL36A	21%	BIK	22%
KRTAP4-1	34%	MUC6	17%	RPL13AP3	21%	IL27	22%
KRTAP5-3	33%	TMEM123	17%	TMSB4X	20%	LRRC32	22%
MT1F	33%	MTRNR2L9	17%	SERF2	20%	TMEM140	22%
MT1E	33%	MTRNR2L6	17%	RPL29	20%	MYMX	30%

The human proteome was obtained from UniProt (Proteome ID UP000005640; accessed November 3, 2019). Proteins from the Swiss-Prot UniProt database are shown and uncharacterized proteins were removed. Biostrings in R was used to determine the number of each amino acid in each protein. For each protein, percent for a given amino acid was calculated by dividing the number of that specific amino acid by the total number of amino acids. Here we show the top 30 proteins for each of the listed amino acids. See also online supplemental information Table S2.