BRAIN COMMUNICATIONS

Dominant *NARS1* mutations causing axonal Charcot–Marie–Tooth disease expand *NARS1*-associated diseases

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Pathogenic variants in six aminoacyl-tRNA synthetase (ARS) genes are implicated in neurological disorders, most notably inherited peripheral neuropathies. ARSs are enzymes that charge tRNA molecules with cognate amino acids. Pathogenic variants in asparaginyl-tRNA synthetase (*NARS1*) cause a neurological phenotype combining developmental delay, ataxia and demyelinating peripheral neuropathy. *NARS1* has not yet been linked to axonal Charcot–Marie–Tooth disease. Exome sequencing of patients with inherited peripheral neuropathies revealed three previously unreported heterozygous *NARS1* variants in three families. Clinical and electrophysiological details were assessed. We further characterized all three variants in a yeast complementation model and used a knock-in mouse model to study variant p.Ser461Phe. All three variants (p.Met236del, p.Cys342Tyr and p.Ser461Phe) co-segregate with the sensorimotor axonal neuropathy phenotype. Yeast complementation assays show that none of the three NARS1 variants support wild-type yeast growth when tested in isolation (i.e. in the absence of a wild-type copy of NARS1), consistent with a loss-of-function effect. Similarly, the homozygous knock-in mouse model (p.Ser461Phe/Ser472Phe in mouse) also demonstrated loss-of-function characteristics. We present three previously unreported *NARS1* variants segregating with a sensorimotor neuropathy phenotype in three families. Functional studies in yeast and mouse support variant pathogenicity. Thus, *NARS1* is the seventh ARS implicated in dominant axonal Charcot–Marie–Tooth disease, further stressing that all dimeric ARSs should be evaluated for Charcot–Marie–Tooth disease.

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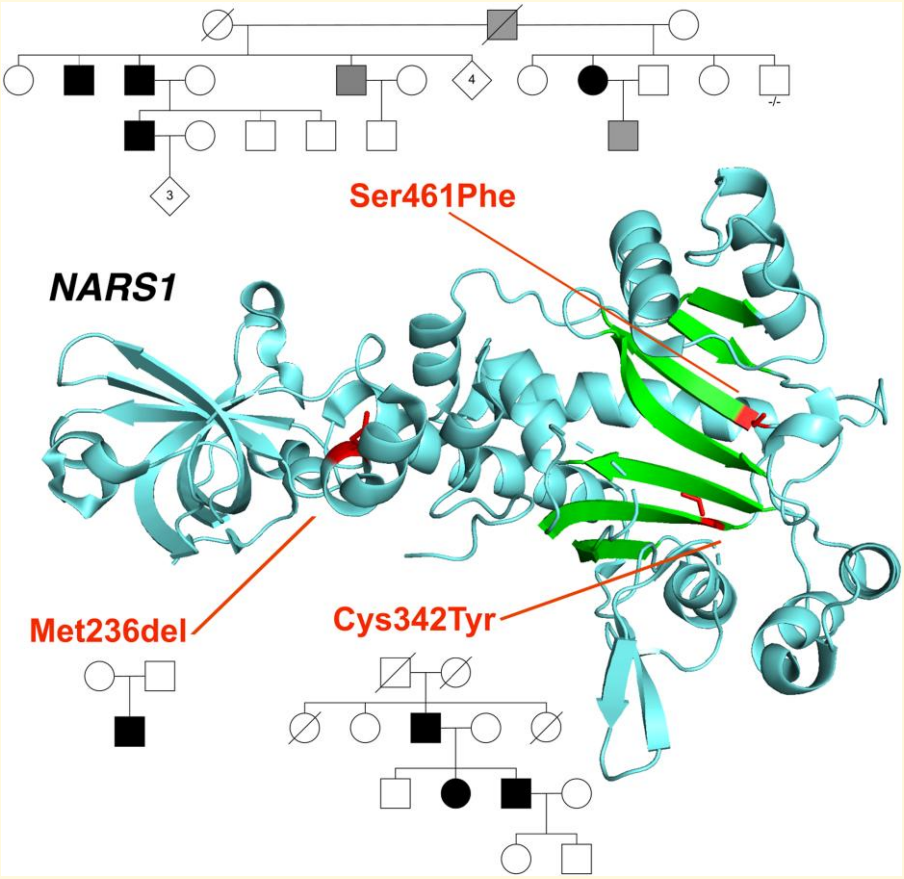
Graphical Abstract



Introduction

Aminoacyl-tRNA synthetases (ARSs) are a group of ubiquitously expressed enzymes catalysing the ligation of tRNA to cognate amino acids, an essential step in the process of protein translation.[1] ARSs can be subdivided into cytoplasmic and mitochondrial proteins and although mutated aminoacyl-tRNA synthetase (ARS) enzymes are implicated in various recessive and dominant disorders most of them are neurological diseases.[2] Most notably, missense or small, in-frame deletions in six dimeric, cytoplasmic ARSs—alanyl-(*AARS1*), glycyl-(*GARS1*), histidyl-(*HARS1*), seryl-(*SARS1*), tryptophanyl-(*WARS1*) and tyrosyl-tRNA synthetase (*YARS1*)—are implicated in inherited peripheral neuropathies (IPN).[2-12]

Pathogenic variants in *NARS1*, encoding the asparaginyl-tRNA synthetase, have been found in patients with dominant and more frequently recessive disease phenotypes encompassing central and peripheral nervous system (CNS and PNS) involvement including developmental delay, ataxia, seizures and demyelinating peripheral neuropathy;[13,14] however, *NARS1* variants have not yet been associated with a PNS-only phenotype.

Here, we describe three previously unreported variants in *NARS1* associated with an axonal sensorimotor neuropathy in three families, with variable severity and—in one case—additional CNS features including ataxia. We provide genetic and functional evidence in yeast complementation assays and a *NARS1* knock-in mouse supporting pathogenicity of these variants.

Materials and methods

Participant consent

Informed consent was obtained based on the local and legal guidelines. Our study complies with the Declaration of Helsinki and was approved by the ethical committee of the University of Antwerp, University of Sao Paolo, and Austin Health centre in Melbourne.

Genetic studies

Exome sequencing (ES) was performed on DNA extracted from whole blood samples as previously described.[15] ES data analysis was performed using the GENESIS platform.[16] Filtering was performed based on allele frequency, variant type and conservation and identified a heterozygous variant in *NARS1* in all three families. Sanger sequencing of the identified *NARS1* variants was performed in available family members (Fig. 1).

Clinical information

An international collaboration of three specialized neuromuscular centres gathered the clinical data from three families with segregating *NARS1* variants. All patients were examined by experienced neurologists, and phenotype information was collected using a standardized clinical record form.

Structural protein modelling

Missense3D prediction and Pymol modelling were performed for the wild-type and missense variants p.Cys342Tyr and p.Ser461Phe based on 5xix model of human asparaginyl-tRNA synthetase though lacking the N-terminal domain-only modelling (98–548), which encompasses all variants discussed in the manuscript. In addition, Phyre2 was used to model the in-frame p.Met236del variant, which was not able to be modelled by Missense3D.

Generation of a haploid ΔDED81 yeast strain

A haploid Δ*DED81* strain that contains a *DED81*-bearing pRS316 maintenance vector (to support cell viability) was generated. The yeast *DED81* locus (the yeast ortholog of human *NARS1*) was amplified from yeast genomic DNA with Gateway (Invitrogen) compatible primers. Gel-purified PCR products were Gateway cloned into a *URA3*-bearing pRS316 vector according to the manufacturer's specifications. The *URA3*-bearing pRS316 vector containing wild-type *DED81* was transformed into a commercially available diploid heterozygous Δ*DED81* yeast strain (Thermo Scientific) and grown on

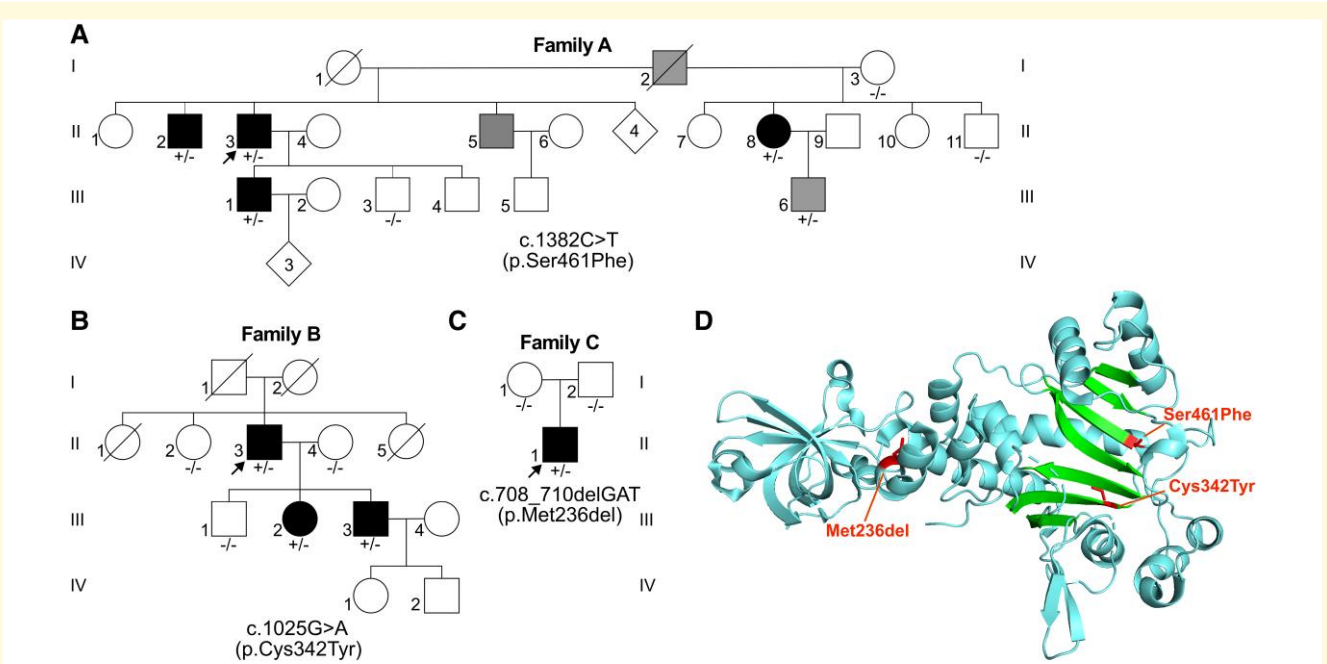


Figure 1 Families carrying NARS1 variants and variant protein modelling. (A–C) pedigrees of families with *NARS1* (NM_004539.4) variants showing co-segregation of the respective variant with the disease phenotype, with affected individuals (filled), unaffected individuals (unfilled) and hearsay affected individuals without clinical investigation (intermediate). (**A**) Family 1: c.1382C>T: (p.Ser461Phe); (**B**) family 2: c.1025G>A: (p.Cys342Tyr); (**C**) family 3: c.708_710delGAT: (p.Met236del). (**D**) 3D protein model of NARS1 (PDB 5xix) with the identified variants indicated (red) and the beta-sheet structure indicated in green.

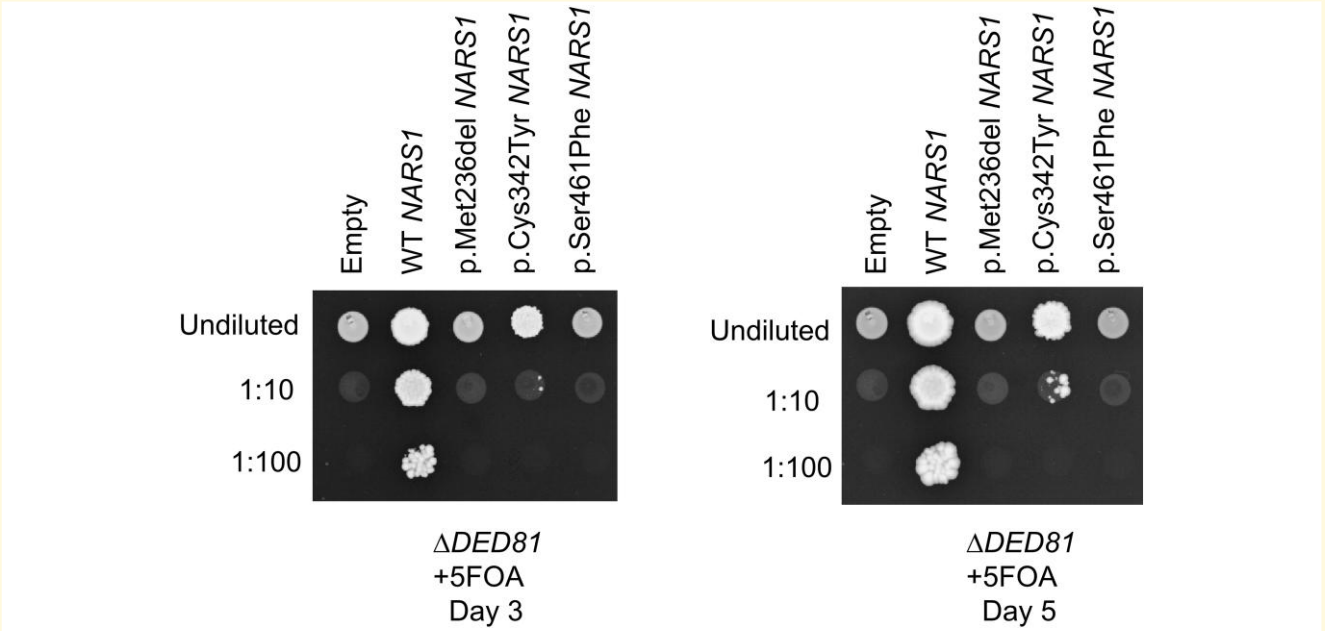


Figure 2 Yeast complementation of NARS1 variants. Yeast cells lacking endogenous *DED81* (the yeast ortholog of *NARS1*) were transformed with vectors containing wild-type (WT) or mutant (p.Met236del, p.Cys342Tyr or p.Ser461Phe) *NARS1*, or with a vector with no *NARS1* insert ('Empty'). Resulting cultures were diluted, plated on 5-FOA (0.1% 5-fluoroorotic acid) solid growth medium and grown at 30°C for 3 days (left panel) or 5 days (right panel). Two independent transformations were performed, and at least two colonies per transformation were test.

medium lacking uracil. Strains were sporulated as previously described.[17] Sporulated strains were dissected using a MSM 400 dissecting microscope (Singer Instruments) and plated on YPD plates. Resulting spores were patched onto solid growth media containing Geneticin (G418) or 0.1% 5-fluoroorotic acid (5-FOA), or standard growth media lacking uracil (Teknova). Two spores that grew on G418 and on yeast media lacking uracil, but did not grow on 5-FOA media, were selected for use in the yeast complementation assays.

Yeast complementation assays

NARS1 expression constructs were generated using Gateway cloning technology (Invitrogen). Wild-type human *NARS1* open reading frame was amplified from human cDNA and confirmed via Sanger sequencing. Mutagenesis corresponding to the identified variants (p.Met236del, p.Cys342Tyr and p.Ser461Phe) was performed using the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene). Sanger validated entry clones were purified and LR-cloned into a pYY1 vector,[18] harbouring a yeast *ADH* promotor directing *NARS1* expression.

Constructs harbouring wild-type or mutant *NARS1*, or a construct with no *NARS1* insert ('Empty' in Fig. 2), were transformed into the haploid *Saccharomyces cerevisiae* strain with a deleted endogenous *DED81* locus, which also harbours wild-type *DED81* on a *URA3*-bearing construct. Subsequent selection was performed as previously described.[17,19] Yeast were imaged after 3 and 5 days of incubation at 30°C. Two independent transformations were performed, and at least two colonies per transformation were tested.

Mouse model

The *Nars1* S472F mice were generated by CRISPR/Cas9 genome editing. The TCT S472 codon (final codon of exon 13 in mouse *Nars1*) was changed to TTT encoding phenylalanine. A sgRNA guide introduced a double strand break at a PAM site 13 base pair upstream target nucleotide.

Donor template oligo, sgRNA (50 ng/µl) and Cas9 mRNA (100 ng/µl) were microinjected into C57BL6/J embryos, and potential founders were screened by Sanger sequencing. Two founders of interest were identified: one with the S472F allele (official designation C57BL/6J-*Nars*^{em1Rwb}/Rwb, MGI:7310304) and a second with an upstream 8 base pair frame shift (C57BL/6J-*Nars*^{em2Rwb}/Rwb, MGI:7310307). Prior to producing study cohorts, the alleles were passed two generations through wild-type C57BL6/J mice to eliminate mosaicism and minimizing possible off-target effects.

The numbers of animals included in examination of neuropathy-relevant phenotypes, histopathology, neurophysiology and molecular analyses were based on previous experience with related mouse models of CMT and power analyses of nerve conduction velocities and histopathology results (axon number) in wild-type mice with sensitivity to detect a 20% change from wild-type values. No animals (data points) were excluded from the analysis; final number of animals per group is presented in Supplementary Table 1. Animal grouping was based on genotype, when a subset of samples was analysed

[histopathology or quantitative real-time PCR (qRT-PCR), for example], samples were selected at random within a genotype. Experimenters were not blind to genotype.

All mouse studies were performed in compliance with the Guide to the Care and Use of Laboratory Animals, and all protocols were reviewed and approved by the Animal Care and Use Committee of The Jackson Laboratory. Mice were maintained under standard house conditions with food and water provided *ad libitum* and a 12:12 light/dark cycle.

Peripheral neuropathy phenotyping

Mice were evaluated for peripheral neuropathy using established methods.[20-22] In brief, grip strength and endurance are tested using an inverted wire grid and timing the latency to falling (max 60 s), with three repetitions per session. Nerve conduction velocity (NCV) is measured at the sciatic nerve determining the latency and distance, from which the nerve conduction velocity is derived.

Molecular analysis of mouse spinal cord

Markers of the integrated stress response were examined by qRT-PCR and RNAScope *in situ* hybridization as previously described.[23] Spinal cord mRNA was also used for quantitative RT-PCR analysis of *Nars1* mRNA levels.

Statistical analyses

All mouse phenotype characteristics were tested by one-way ANOVA unless otherwise noted. NCV was tested using Student's *t*-test, significance was considered at a $P < 0.05$, and all data are presented as mean ± standard deviation.

Results

The three families reported are part of ongoing research applying next-generation sequencing (NGS) to unsolved inherited peripheral neuropathies patients. ES analysis in three probands identified two missense and one in-frame deletion variant in *NARS1* (NM_004539.4) in patients with Charcot–Marie–Tooth disease (CMT) from three separate families (Fig. 1). None of these variants were associated with disease previously. We identified missense variants: c.1382C>T (p.Ser461Phe) in family A and c.1025G>A (p.Cys342Tyr) in family B, and identified the in-frame deletion c.708_710delGAT (p.Met236del) in family C. Subsequent Sanger sequencing revealed a dominant or *de novo* co-segregation of the *NARS1* variant and the neuropathy phenotype (Fig. 1). All three *NARS1* variants identified, are absent from GnomADv3, affect highly conserved residues, and prediction scores support pathogenicity (Supplementary Table 2).[24] ES data analysis did not reveal other relevant and/or segregating variants in genes associated with peripheral neuropathy, ataxia or other neuromuscular diseases.

Family A is of Belgian descent and consists of three affected half-siblings (A:II:2, A:II:3 and A:II:8), one of whom has an affected son (A:III:1). The deceased father (A:I:2) of the half-siblings was affected by history, but was never clinically assessed. Affected individuals in family A demonstrated a variable severity of the phenotype, their overall presentation was of a typical late adult-onset slowly progressive length dependent axonal CMT neuropathy affecting both upper and lower limbs, though overall starting in the lower limbs, as well as affecting both sensory and motor functions. Family B is a family from Australia consisting of an affected father (B:II:3) and two affected children (B:III:2 and B:III:3). Like family A, affected individuals in family B display noticeable variability in the severity of the phenotype, but the overall phenotype falls within the classification of an adult-onset slowly progressive length dependent axonal CMT neuropathy, affecting the lower limbs more than upper limbs, and affecting sensory functions before motor functions. Family C is a family from Brazil consisting of an affected male proband (C:II:1) and his unaffected parents. In contrast to families A and B, the proband in family C presented with a more complex neurological phenotype, which consists of an axonal sensorimotor neuropathy, but also signs of pyramidal tract degeneration (Babinski signs and spasticity), cerebellar ataxia and a neurodevelopmental component with delayed motor milestones and intellectual disability. Cerebellar atrophy was observed on brain MRI. Comparatively, none of the individuals in families A and B displayed signs of psychomotor delay or intellectual disability, and motor milestones were obtained normally and there is no evidence of pyramidal features, ataxia or contractures.

Although all three families share the core phenotype of an axonal sensorimotor neuropathy with variable severity, the CNS features in proband C are reminiscent of previously reported patients with dominant *NARS1*-associated disease.[14] For a detailed overview of the clinical features, see Table 1 and Supplementary Table 3.

We next examined the 3D location of the neuropathy-associated variants in a structural protein model of NARS1, using human asparaginyl-tRNA synthetase model AsnRS1 (5XIX), previous predictions on domain functions and the programs Missense3D and Pymol.[13,25] While the 5XIX model lacks the N-terminal domain-only modelling (residues 98–548), it encompasses all variants described here. In this model, all three variants reported (p.Met236del, p.Cys342Tyr and p.Ser461Phe) are located in the catalytic domain.[13,26] We also observed that the two missense variants associated with the pure neuropathy phenotype (p.Cys342Tyr and p.Ser461Phe) are in close proximity to the catalytic site; in particular, p.Cys342Tyr is located near the amino acid and ATP binding sites (Fig. 1D).[26] These two variants are predicted to break hydrogen bonds and cause a change from a buried residue to an exposed residue according to Missense3D predictions.[27] Missense3D is not able to model the in-frame deletion p.Met236del that was found in family C. Overall, the location to the catalytic site (particularly near the amino acid and ATP binding pocket)

Table 1 Summarized clinical phenotype per patient

Proband (gender)	A:II:2 (M)	A:II:3 (M)	A:II:8 (F)	A:III:1 (M)	A:III:6 (M)	B:II:4 (M)	B:III:6 (F)	B:III:7 (M)	C:II:1 (M)
Variant (protein level)	p.Ser461Phe	p.Ser461Phe	p.Ser461Phe	p.Ser461Phe	p.Ser461Phe	p.Cys342Tyr	p.Cys342Tyr	p.Cys342Tyr	p.Met236del (*de novo*)
Age at onset—age at (last) examination	59	56	55	33	26	69	39	33	30
Current age	60	57	56	34	27	72	41	36	30
Ethnicity	Belgian	Belgian	Belgian	Belgian	Belgian	Australian	Australian	Australian	Brazilian
CNS involvement	-	-	-	-	-	-	-	-	impaired psychomotor development, intellectual disability, impaired speech, cerebellar ataxia, seizures age 7y
Foot deformities	Pedes cavi, hammer toes	Pedes cavi	Pedes cavi	Pedes cavi, hammer toes	Pedes cavi	None	Pedes cavi	Pedes cavi	Pedes cavi, hammer toes
Walking impairment	+ (AFO)	+	–	+	+	–	+ (AFO, cane)	+ (AFO)	+ (AFO)
Distal sensory loss	PP + VS (LL)	VS (LL)	VS (LL)	VS (LL)	VS (LL)	PP (LL)	VS (UL + LL), PC (LL), PP (LL)	VS (UL + LL), PC (LL), PP (LL)	VS (LL)
Muscle weakness	Distal LL	Distal UL + LL	-	Distal LL	-	-	Distal (UL + LL), proximal (LL)	Distal (UL + LL), proximal (LL)	Distal (UL + LL), proximal (LL)
Additional features	-	-	-	-	-	-	-	-	Babinski signs (spasticity?), cerebellar ataxia
NCS + EMG	CMT2	CMT2	N/A	CMT2	CMT2	CMT2?	CMT2	CMT-INT	CMT2+
Additional exams	-	-	-	-	-	-	-	-	Brain MRI: mild cerebellar atrophy (21y), spinal MRI normal

AFO, ankle foot orthotics; CMT2, axonal Charcot–Marie–Tooth disease; CMT2+, complex axonal Charcot–Marie–Tooth disease; CMT-INT, intermediate Charcot–Marie–Tooth disease; CNS, central nervous system; F, female; LL, lower limb(s); M, male; N/A, not available; PC, proprioception; PP, pinprick sensation; UL, upper limb(s); VS, vibration sense.

deems these variants similar to other neuropathy-associated ARS variants (e.g. DETAQ GARS1) and supports the notion that they reduce gene function.[4,6,28]

To further assess variant pathogenicity, we employed a yeast complementation assay testing the impact of each variant on gene function. First, we determined if human *NARS1* rescues deletion of the yeast ortholog. Wild-type human *NARS1* rescued yeast viability, whereas a vector with no *NARS1* insert ('Empty') did not allow any yeast growth (Fig. 2). This confirms that *DED81* is an essential gene and that human *NARS1* complements the loss of endogenous *DED81*. Transforming each of the *NARS1* variants (p.Met236del, p.Cys342Tyr and p.Ser461Phe) into the yeast strain showed that the p.Met236del and p.Ser461Phe variants did not support any yeast cell growth consistent with these variants ablating *NARS1* function in this assay (Fig. 2). In contrast, the p.Cys342Tyr variant exhibited severely reduced yeast growth compared to wild-type *NARS1*, but did not ablate yeast cell growth, consistent with a hypomorphic allele (Fig. 2); interestingly, this hypomorphic effect has been observed for other neuropathy-associated ARS alleles and is consistent with the reduced computational pathogenicity prediction score of this variant (Supplementary Table 2).[29] Based on loss-of-function effects observed for other neuropathy-associated ARS variants,[30] these yeast functional data support the pathogenicity of the *NARS1* variants reported in this study.

To evaluate if the p.Ser461Phe allele of NARS1 is deleterious in mice, the equivalent variant was introduced into the mouse genome (p.Ser472Phe in the mouse NARS1 protein). The p.Ser461Phe allele was chosen because of the strong association with disease in family A. In addition to the anticipated p.Ser472Phe amino acid substitution, the second allele recovered (see Supplementary Fig. 1) included the p.Ser472Phe variant but was preceded by an 8 base pair deletion resulting in a frame shift and premature truncation (referred to here as 'del8'). In qRT-PCR experiments, *Nars1* mRNA was not reduced in heterozygous p.Ser472Phe/+ mice, but was down ~50% in heterozygous del8/+ mice, consistent with nonsense mediated decay of the frame shifted transcript (see Supplementary Fig. 2). Both variants were non-viable in homozygous state, with no homozygous animals recovered from heterozygous breedings (0 for 31 for p.Ser427Phe, $P = 0.008$ by Chi-square test; 0 for 50 for the del8 frameshift, $P = 9 \times 10^{-5}$ by Chi-square test). The alleles were also non-viable as compound heterozygotes, with no homozygotes recovered in 24 offspring ($P = 0.016$ by Chi-square test), consistent with the p.Ser472Phe allele negatively impacting gene function comparably to the frameshift and with the results in yeast.

Heterozygous mice were evaluated for signs of peripheral neuropathy, including body weight, grip strength and endurance, sciatic motor nerve conduction velocity and peripheral nerve histopathology (see Supplementary Fig. 2). No differences from wild-type control animals were found at multiple time points examined out to 18 months of age. The motor and sensory branches of the femoral nerve had normal axon numbers and normal axon sizes at 18 months of age. Mice were also evaluated for gene expression signatures identified in other mouse models of tRNA synthetase-associated IPN; specifically, activation of the integrated stress response detected through expression of ATF4-target genes in motor neurons.[23] No increased expression of markers such as *Fgf21* or *Gdf15* was detected by qRT-PCR from spinal cord in mice heterozygous for either allele at 18 months of age (see Supplementary Fig. 2). To confirm that signal from a small number of compromised cells was not lost in analysis of bulk spinal cord mRNA, we also performed RNAScope *in situ* hybridization, probing for *Fgf21* and *Gdf15* mRNA and co-labelling with *Chat* mRNA probes to identify motor neurons. Consistent with the qRT-PCR results, there was no detectable difference from controls in expression of these genes in the spinal cord in either *Nars1* genotype, although positive control samples from *Gars*/CMT2D mice showed the anticipated upregulation of ATF4-target genes.[23] Thus, our results in mice confirm that the p.Ser461Phe allele is deleterious in mice (p.Ser472Phe), but we did not detect signs of peripheral neuropathy in mice heterozygous for this variant.

Discussion

Here, we describe three previously unreported *NARS1* variants in three families with a dominant axonal peripheral neuropathy phenotype. The two missense variants (p.Cys342Tyr and p.Ser461Phe) are associated with a pure axonal neuropathy, whereas the in-frame deletion p.Met236del is associated with an axonal neuropathy with mild spasticity and cerebellar ataxia. Previous studies associated *NARS1* variants with dominant and recessive neurodevelopmental phenotypes including developmental delay, microcephaly, ataxia and seizures.[13,14] While eight of the patients did present with a peripheral neuropathy as part of their broader phenotype, this was a demyelinating neuropathy, rather than the axonal neuropathy that we have observed in the presented three families.[14] It is unclear why different *NARS1* variants would cause different types of peripheral neuropathies as this might indicate that neurons and glial cells are differentially susceptible to aberrant *NARS1* function. Of note is that a substantial number of previously published *NARS1* variants were found in complex recessive syndromes, and only a smaller number of variants were seen in dominant cases.

In contrast, the *de novo* variant identified in family C was associated with a more complex phenotype of axonal neuropathy with CNS involvement, reminiscent of the previous studies. As *NARS1* variants have been associated with neurodevelopmental features, careful evaluation of possible CNS symptoms was performed, which failed to detect any CNS abnormalities in families A and B and moderate CNS involvement in family C.

Modelling of the three variants described in this study predicts a significant impact for each on the structure of the protein. The residues affected by p.Cys342Tyr and p.Ser461Phe are both located in the beta-barrel part of the

protein in the catalytic site (particularly near the amino acid and ATP binding pocket), and both are predicted to break hydrogen bonds. Based on the localization, and compared to other previously reported mutations such as p.Arg545Cys and p.Arg534*, we do not expect these variants so significantly alter the linker region or the homodimerization. We rather presume changes in the catalytic capabilities of AsnRS1 due to disruption of amino acid and ATP binding for the p.Cys342Tyr and p.Ser461Phe variants affecting the overall function of the protein. This is supported by our findings in the yeast model, which demonstrated a loss-of-function effect for all three variants and by the homozygous lethality of mice carrying the p.Ser461Phe variant. Despite these findings, it is unclear if a loss-of-function or gain-of-function effect is the mechanism of action in these neuropathy patients; we were unable to obtain patient cells to assess protein expression and enzymatic activity. A gain-of-function effect of these variants cannot be ruled out, as several ARS genes IPN-associated heterozygous variants have been shown to work via a toxic gain-of-function mechanism (e.g. via inappropriate interactions with NRP1 or TrkB).[21,31,32]

The lack of a neuropathy phenotype in the p.Ser472Phe mice is likely reflective of the mild phenotype in patients carrying this variant, who were still ambulatory even in the seventh decade of life. When other pathogenic variants, heterozygous in patients, in tRNA synthetase genes have been introduced into mice, neuropathy phenotypes were observed.[22,23,33] However, in the case of *Gars1*, the pathogenic variant introduced caused an early-onset, severe motor neuropathy in the patient,[22] and in the case of *Yars1*, modest gene expression changes were observed in heterozygous mice,[23] but neuropathy-associated phenotypes were only present in homozygous animals.[33]

Therefore, mice may only manifest corresponding phenotypes with the most severe human disease alleles, perhaps making the p.Met236del allele a better candidate than p.Ser472Phe for future studies. Alternatively, the lack of phenotype in heterozygous mice may indicate that pathogenic variants in *NARS1* lead to neuropathy in humans through a mechanism that is not conserved in mice. Regardless, the known role of *NARS1* in neurological disease, the association of *NARS1* with neuropathy across multiple families described here (and also in previously published families with broader PNS/CNS syndromes), the segregation of the disease alleles within these families, and our functional studies in yeast and homozygous mice strongly support the pathogenicity of the *NARS1* variants reported in this study.

In summary, our work extends the clinical heterogeneity associated with pathogenic *NARS1* variants, which now includes a primarily axonal sensorimotor neuropathy. Furthermore, we implicate the seventh dimeric, cytoplasmic ARS in dominant neuropathy, indicating that: (i) the function of these enzymes plays a role in disease aetiology; and (ii) all genes encoding dimeric, cytoplasmic ARSs (e.g. *TARS1* and *KARS1*) should be carefully evaluated in patients with dominant axonal neuropathy.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

The authors report no competing interests.

Data availability

The NGS datasets supporting the current study have not been deposited in a public repository due to limitations of patient consent for public data sharing, but like other data in this study are available from the corresponding author on reasonable request and appropriate handling.

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