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BRIEF COMMUNICATION

FARS2 mutations presenting with pure spastic paraplegia and lesions of the dentate nucleiSupreet K. Sahai^{1,*}, Rebecca E. Steiner^{2,3,4,*}, Margaret G. Au⁵, John M. Graham⁵, Noriko Salamon⁶, Michael Ibba^{2,3,4} & Tyler M. Pierson^{1,5,7}¹Department of Neurology, Cedars-Sinai Medical Center, Los Angeles, California²Department of Microbiology, Ohio State University, Columbus, Ohio 43210³Center for RNA Biology, Ohio State University, Columbus, Ohio 43210⁴Ohio State Biochemistry Program, Ohio State University, Columbus, Ohio 43210⁵Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, California⁶Department of Radiological Sciences, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California⁷Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, California**Correspondence**

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Introduction

Mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) play an integral role in mitochondrial protein synthesis and translation by charging transfer RNAs (tRNA) with their associated amino acids. To date, most mt-aaRS mutations involve a wide spectrum of central nervous system disorders with a broad range of phenotypes.¹ For example, mutations in *GARS* cause peripheral neuropathy, *MARS2* mutations have been associated with spastic ataxia with leukoencephalopathy, and *DARS2* variants

Abstract

Mutations in *FARS2*, the gene encoding the mitochondrial phenylalanine-tRNA synthetase (mtPheRS), have been linked to a range of phenotypes including epileptic encephalopathy, developmental delay, and motor dysfunction. We report a 9-year-old boy with novel compound heterozygous variants of *FARS2*, presenting with a pure spastic paraplegia syndrome associated with bilateral signal abnormalities in the dentate nuclei. Exome sequencing identified a paternal nonsense variant (Q216X) lacking the catalytic core and anticodon-binding regions, and a maternal missense variant (P136H) possessing partial enzymatic activity. This case confirms and expands the phenotype related to *FARS2* mutations with regards to clinical presentation and neuroimaging findings.

have been implicated in leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation.^{1–4}

FARS2 is a nuclear gene encoding the mitochondrial phenylalanyl-tRNA synthetase (mtPheRS; MIM#611592), which transfers phenylalanine to its specific mitochondrial tRNA^{Phe}. mtPheRS contains four major domains: a mitochondrial localization signal (residues 1–37) in the N-terminal region (residues 1–83), a catalytic domain (residues 84–325), a linker region (residues 326–358), and an anticodon-binding domain (359–451).⁵ Similar to many

mt-aaRSs, *FARS2* has been linked with a severe mitochondrial syndrome associated with an infantile-onset epileptic encephalopathy and/or fatal Alpers-like syndrome.^{5–9} More moderate phenotypes consisting of cognitive disability, motor delays, and epilepsy have also been reported.^{10,11} In its mildest form, *FARS2* presented with a pure spastic paraplegia syndrome in a consanguineous Chinese family with homozygous mutations (spastic paraplegia type 77; SPG77 MIM#617046).¹²

We report a 9-year-old boy with progressive lower extremity spasticity and normal cognition associated with bilateral signal abnormalities in the dentate nuclei. Exome sequencing revealed novel compound heterozygous *FARS2* mutations: a paternal nonsense variant upstream of the catalytic core and anticodon-binding region (c.646 C>T; p.Q216X) and a maternal missense variant (c.407 C>A; p.P136H) associated with decreased enzyme activity. This combination of alleles with null and partial activity resulted in this unique case of SPG77.

Material and Methods

A detailed description of materials and methods used can be found in the Data S1.

Results

Subject

The subject was a 9-year-old boy, born to healthy non-consanguineous parents. His parents were of northern European and Ashkenazi Jewish ancestry and were without neurological issues. Family history was negative. His early development was normal. He started walking at 14 months and was speaking in sentences by age 2. At approximately 2.5 years of age, the patient was noted to develop toe-walking with a tendency to trip and skip. His ambulation has progressively worsened, but his development has otherwise been normal.

Examination showed spasticity in his lower extremities with preserved bulk and mild weakness. Deep tendon reflexes were brisk in both legs. He had bilateral extensor plantar responses and unsustained ankle clonus. He had a spastic gait with toe-walking. Sensation and coordination were normal. Diagnostic studies included neuroimaging at 9 years with nonspecific signal abnormalities in the dentate nuclei consistent with edema (hypointense abnormalities on T1-weighted images and hyperintense abnormalities on T2-weighted images; Fig. 1); otherwise, serum

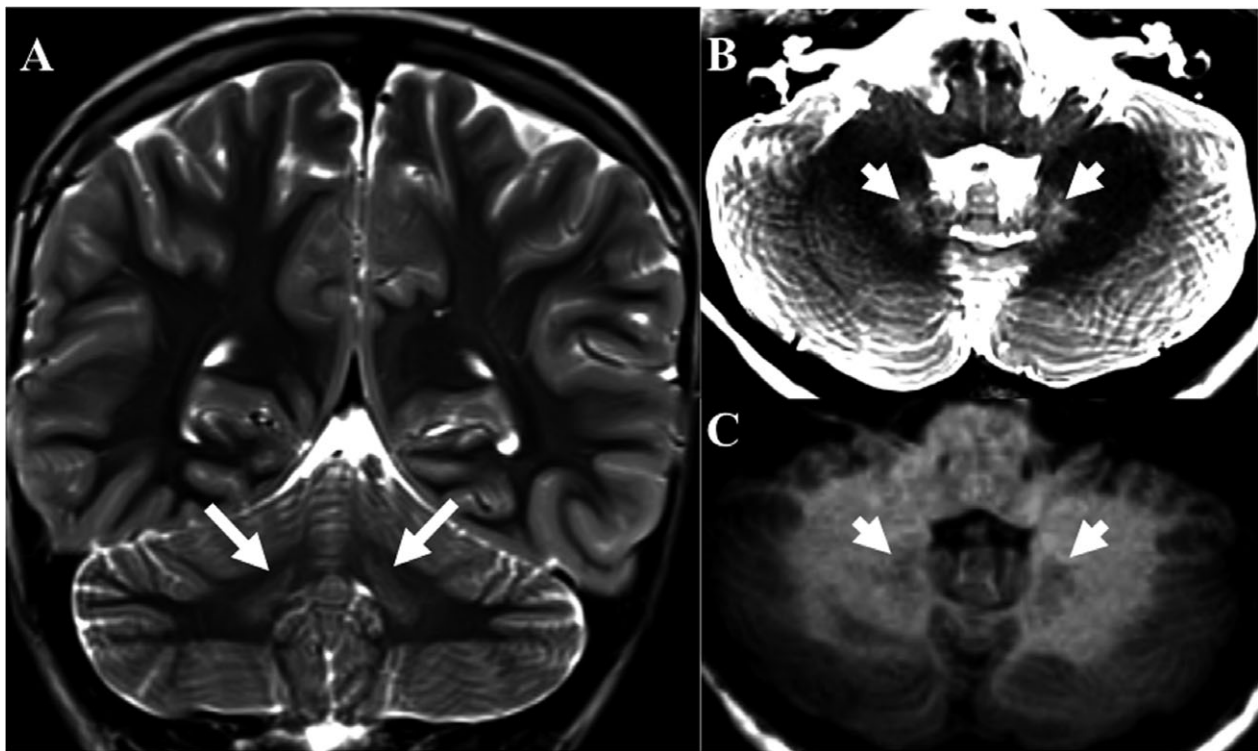


Figure 1. Magnetic resonance imaging of the brain (A) T2-weighted coronal and axial (B) images demonstrates abnormal signal hyperintensities in the bilateral dentate nuclei (arrows and arrowheads, respectively) (C) T1-weighted axial images demonstrated abnormal signal hypointensities. Together these findings are consistent with localized edema of the dentate nuclei.

lactate, plasma amino acids (including alanine), urine organic acids, and acylcarnitine profile were normal.

Genetic findings

Exome sequencing identified two novel variants. One paternal nonsense variant, (c.646 C>T; p.Q216X) and a maternal missense variant, (c.407 C>A; p.P136H) (Fig. 2). Neither of these were observed in ~6500 individuals in the NHLBI Exome Sequencing Project. The P136H variant is a nonconservative amino acid substitution at a residue that is conserved among vertebrates (Fig. 2). The new residue differs in size, polarity, and charge. The Q216X variant truncates mtPheRS near its midpoint, omitting the enzymatic catalytic core and anticodon-binding domain (Fig. 2).

Biochemical evaluation

Both the wild-type- (WT) and P136H-mtPheRS recombinant proteins were overexpressed and purified; however, the Q216X variant was unable to be isolated despite numerous attempts with nickel affinity and anion exchange columns. We were unable to isolate the Q216X protein in the context of the c.646 C>T point mutation, nor in our attempt to specifically only express the first 216 amino acids of mtPheRS. This was presumably due

to the instability or protease degradation of the truncated peptide and indicates that the truncated protein has no residual activity as a result of this instability.

Aminoacylation activity of the WT and P136H mtPheRS was evaluated (Fig. 3). The P136H mutant had a 50% reduction in activity compared to the WT enzyme (Fig. 3). This was further shown to equate to a 10-fold decrease in the k_{cat}/K_M for P136H compared to the WT mtPheRS (Fig. 3). A large decrease in k_{cat}/K_M indicates a potentially physiologically significant reduction in the efficiency with which tRNA^{Phe} can be aminoacylated by phenylalanine. Thermostability assays were completed with active site titrations after 1-h incubation at room temperature and 37°C. These studies revealed that mtPheRS-P136H's thermal stability was similar to WT (Fig. S1).

Discussion

Pure spastic paraplegia consists of lower extremity spasticity and dysfunction, without other neurological or cognitive issues. Our subject possessed novel compound heterozygous mutations in *FARS2* (Q216X and P136H) resulting in a pure spastic paraplegia syndrome that was also associated with bilateral signal abnormalities of the dentate nuclei. His phenotype was similar to a previously reported consanguineous Chinese family with pure spastic

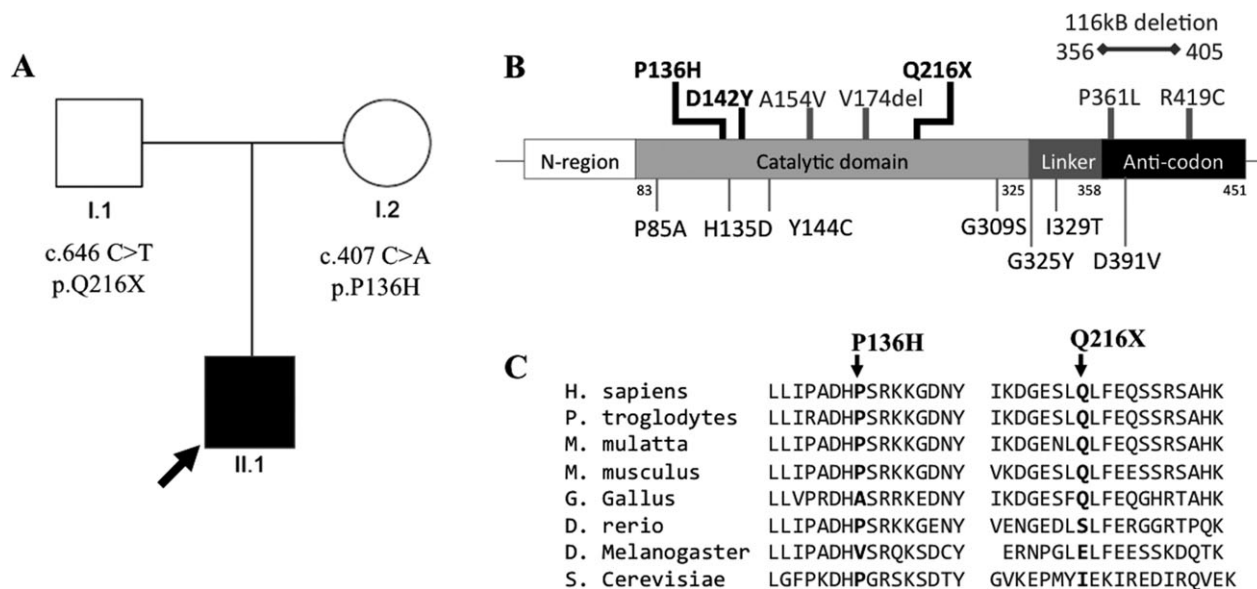


Figure 2. Pedigree and *FARS2* mutations. (A) Family Pedigree. The proband is indicated by an arrow and the affected individual is in black. (B) *FARS2* gene schematic and location of mutations. Our subject's mutations (P136H and Q216X) and previously reported cases with pure spastic paraplegia are in black. Previously reported moderate cases are in gray and above the schematic, previously reported severe cases are in gray and below the schematic. (C) Protein homology at mutated residues across species. The P136 and Q216 residues are conserved across most vertebrate species.

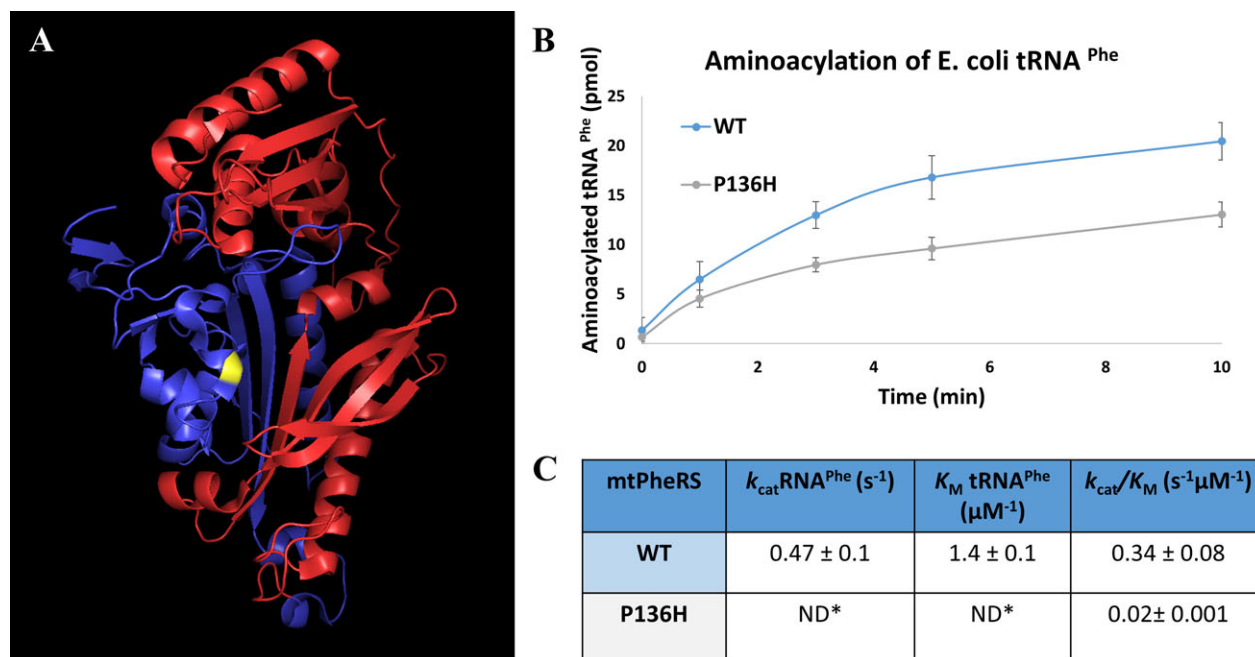


Figure 3. Biochemical analysis of variants. (A) Crystal structure of mtPheRS. Yellow residue denotes the P136H residue. Red region denotes the portion of mtPheRS removed in Q216X truncation. Blue region is the portion of mtPheRS present in Q216X variant¹⁵ (B) Aminoacylation curve of generation of Phe-tRNA^{Phe}. Blue line is WT mtPheRS and gray line is P136H mtPheRS. (C) Steady-state kinetic parameters tRNA^{Phe}. *Individual kinetic parameters could not be obtained due to the high K_M compared to practical tRNA concentrations ($[S] \ll [E]$). Therefore, the k_{cat}/K_M was directly estimated from $V = k_{cat}/K_M([E][S])$.

paraplegia due to homozygous mutations within the catalytic domain of mtPheRS (D142Y), although their neuroimaging was normal.¹² To date, these subjects represent the mildest cases of *FARS2*-related disease. Overall, approximately 21 subjects with *FARS2*-related disease have been reported, with most cases (12 of 21; see Table S1) presenting with a severe phenotype consisting of epileptic encephalopathy and diffuse cortical dysfunction (lower extremity spasticity, motor delays, and cognitive disability). Many of these severe cases were also associated with lactic acidosis or liver disease.^{6–8} Several moderate phenotypes have also been reported with diffuse cortical dysfunction, but had more functional capabilities.^{10–12} Examples included two siblings with compound heterozygous mutations (R419C & microdeletion) presenting with intellectual disability, truncal hypotonia, appendicular hypertonicity, and neonatal epilepsy.¹¹ Additional moderate subjects included two unrelated children with diffuse cortical dysfunction and lactic acidosis.¹⁰ One child (P361L & V174del) had additional findings of bradykinesia and dystonia, whereas the other (P361L & A154V) also developed epilepsy. Both had neuroimaging abnormalities in the frontopontine pyramidal tracts, thalamus, and cerebellar atrophy.¹⁰ In contrast to the subjects with pure spastic paraplegia, these individuals

could be classified as complex spastic paraplegia due to their combination of spasticity and other significant neurological issues.

The variable severity of the *FARS2* phenotypes may be related to the amount of residual mtPheRS activity each individual possesses. This residual activity may play a role in both neuro-development, as well as the long-term maintenance of the nervous system. Previous work revealed mitochondrial dysfunction can disrupt migration of cortical interneurons and lead to epileptic encephalopathy and/or intellectual disability.¹³ One could hypothesize that individuals with reduced mtPheRS activity leading to severe *FARS2* phenotypes (associated with larger energy deficits) could possess these migrational anomalies, as well as being vulnerable to developing later-onset injury in cells with higher energy requirements (e.g., cerebellar Purkinje or cortical pyramidal cells), leading to their complex spastic paraplegia. Alternatively, individuals with higher residual mtPheRS activity may generate enough energy for normal interneuron migration, resulting in their phenotype solely being a function of later-onset injury to cells with higher energy requirements. Our subject had residual mtPheRS activity as a result of a partially active substitution mutation (P136H) combined with an unstable null truncation variant

(Q216X). This may be why his phenotype is currently limited to a pure spastic paraplegia syndrome.

Although our subject presented with the same phenotype as the consanguineous Chinese family, his neuroimaging revealed bilateral signal abnormalities of the dentate nuclei. Of note, our subject was not the only *FARS2*-related patient with changes in this location. Similar abnormalities were also seen in a child with a more severe clinical phenotype and additional neuroimaging features that included a thin corpus callosum, white matter changes, and diffuse cerebral cortical atrophy.⁹ Interestingly, another case with a similar presentation to our subject involved *SARS2*, a gene encoding a mt-aARS for serine.¹⁴ In that report, homozygous splice site mutations led to partial activity of the mitochondrial seryl-tRNA synthetase (mtSerRS) resulting in spastic paraplegia and mild intellectual disability. Interestingly, this child also presented with increased T2 hyperintensities in the dentate nuclei with later development of cerebellar atrophy. Both our subject and this child were without clinical cerebellar findings. It may be that in both subjects' dentate changes are not contributing to their spasticity; nevertheless, cerebellar dysfunction is frequently associated with spastic paraplegia and in this context, may be playing a role. It could also be that with time, both subjects develop cerebellar symptoms. The identification and long-term follow-up of more *FARS2* or other mt-aARS patients with pure spastic paraplegia will be required to better understand these associations.

Aminoacyl-tRNA synthetases have been associated with a range of symptoms from early-onset mitochondrial phenotypes to milder forms with later-onset neurological findings. The more common phenotype of *FARS2* disorders is a severe disorder with epileptic encephalopathy and severe neurological abnormalities affecting the function of deep gray nuclei and the cerebral cortex. We report a subject presenting with pure spastic paraplegia without familial consanguinity. This case further underscores the idea that the phenotype related to *FARS2* and other mt-aARSs may have a wide spectrum of disease severity. The subject reported here will require continued monitoring to determine if his course has plateaued or may progress; however, in the context of reported *FARS2*-related disease, it may be that pure spastic paraplegia is the mildest presentation.

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Conflict of Interest

None declared.

Author Contributions

S.K.S., J.M.G., M.A., N.S., and T. M. P. evaluated subject and collected clinical data. R.S., M.I., and T. M. P. conceived and designed the experiments. R.S., M.I., and T. M. P. performed the experiments. S.K.S., R.S., M.I., J.M.G., M.A., N.S., and T. M. P. analyzed the data. S.K.S., R.S., M.I., and T. M. P. wrote the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Data S1.

Table S1. Currently Identified *FARS2* Subjects and Data.

Figure S1. Thermostability of mtPheRS mutant.