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Clinical Features, Molecular Heterogeneity, and Prognostic Implications in *YARS2*-Related Mitochondrial Myopathy

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IMPORTANCE *YARS2* mutations have been associated with a clinical triad of myopathy, lactic acidosis, and sideroblastic anemia in predominantly Middle Eastern populations. However, the identification of new patients expands the clinical and molecular spectrum of mitochondrial disorders.

OBJECTIVES To review the clinical, molecular, and genetic features of *YARS2*-related mitochondrial disease and to demonstrate a new Scottish founder variant.

DESIGN, SETTING, AND PARTICIPANTS An observational case series study was conducted at a national diagnostic center for mitochondrial disease in Newcastle upon Tyne, England, and review of cases published in the literature. Six adults in a well-defined mitochondrial disease cohort and 11 additional cases described in the literature were identified with *YARS2* variants between January 1, 2000, and January 31, 2015.

MAIN OUTCOME AND MEASURES The spectrum of clinical features and disease progression in unreported and reported patients with pathogenic *YARS2* variants.

RESULTS Seventeen patients (median [interquartile range] age at onset, 1.5 [9.8] years) with *YARS2*-related mitochondrial myopathy were identified. Fifteen individuals (88%) exhibited an elevated blood lactate level accompanied by generalized myopathy; only 12 patients (71%) manifested with sideroblastic anemia. Hypertrophic cardiomyopathy (9 [53%]) and respiratory insufficiency (8 [47%]) were also prominent clinical features. Central nervous system involvement was rare. Muscle studies showed global cytochrome-*c* oxidase deficiency in all patients tested and severe, combined respiratory chain complex activity deficiencies. Microsatellite genotyping demonstrated a common founder effect shared between 3 Scottish patients with a p.Leu392Ser variant. Immunoblotting from fibroblasts and myoblasts of an affected Scottish patient showed normal YARS2 protein levels and mild respiratory chain complex defects. Yeast modeling of novel missense *YARS2* variants closely correlated with the severity of clinical phenotypes.

CONCLUSIONS AND RELEVANCE The p.Leu392Ser variant is likely a newly identified founder *YARS2* mutation. Testing for pathogenic *YARS2* variants should be considered in patients presenting with mitochondrial myopathy, characterized by exercise intolerance and muscle weakness even in the absence of sideroblastic anemia irrespective of ethnicity. Regular surveillance and early treatment for cardiomyopathy and respiratory muscle weakness is advocated because early treatment may mitigate the significant morbidity and mortality associated with this genetic disorder.

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Supplemental content

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Childhood-onset mitochondrial disease is among the most common inherited neurologic disorders, with an estimated minimum point prevalence of 5 (95% CI, 4.0-6.2) in 100 000 population.[1] Moreover, recent studies have estimated the prevalence of mitochondrial disease in affected adults at 9.6 per 100 000 caused by mutations in mitochondrial DNA (mtDNA) and 2.9 per 100 000, caused by nuclear mutations.[2] Hence, overall disease burden is potentially extensive. Patients exhibit marked phenotypic and genotypic heterogeneity, with mitochondrial respiratory chain complex deficiency a hallmark feature in tissues.

The amino-acyl transfer RNA (tRNA) synthetases are a group of enzymes critical for protein synthesis that are required for the recognition and conjugation of specific amino acids. Two sets of synthetases are encoded by separate genes in human cells, distinguished by cytoplasmic (referred to as *ARS*) or mitochondrial (referred to as *ARS2*) localization. *ARS2* mutations have emerged as an important cause of mitochondrial translation disorders that first appeared to manifest in tissue and cell-type specific phenotypes, including central nervous system involvement (*CARS2*, *DARS2*, *EARS2*, *FARS2*, *GARS*, *IARS2*, *MARS2*, *NARS2*, *PARS2*, *RARS2*, *TARS2*, and *VARS2*); myopathy, lactic acidosis, and sideroblastic anemia (MLASA) (*YARS2*); hypertrophic cardiomyopathy (*AARS2*); sensorineural hearing loss and ovarian dysgenesis (Perrault syndrome) (*LARS2* and *HARS2*); and hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (*SARS2*).[3-18]

Mutations of *YARS2* encoding mitochondrial tyrosyl-tRNA synthetase have been described predominantly in patients with infantile- to childhood-onset autosomal recessive MLASA syndrome with mitochondrial respiratory chain complex deficiencies.[4,19-23] The MLASA syndrome was first described in patients with *PUS1* mutations,[24] with *YARS2* mutations later identified in patients with a clinically similar phenotype,[4] and most recently in 2 patients with a novel de novo heteroplasmic m.8969G>A (*MT-ATP6*) and recessive *LARS2* variants.[25,26] Although rare, sideroblastic anemia is a prominent feature in Pearson syndrome caused by single, large-scale mtDNA deletions.[27,28]

We present 6 adult patients with *YARS2* variants, including 4 previously unreported patients of Scottish descent, and demonstrate a new *YARS2* founder effect within this population, with immunoblotting and yeast modeling of novel missense variants to confirm pathogenicity. Together with previous cases from the literature, we review the spectrum of clinical features and progression of *YARS2*-associated mitochondrial disease to fully delineate the clinical phenotype and genotype correlates.

Key Points

Question What is the genotypic and clinical phenotypic spectrum of *YARS2*-related mitochondrial disease?

Findings In this case series study, the triad of myopathy, lactic acidosis, and sideroblastic anemia was not identified in all cases. The p.Leu392Ser variant was identified as a possible new founder *YARS2* mutation in the Scottish ancestry.

Meaning Testing for pathogenic *YARS2* variants should be considered in patients presenting with mitochondrial myopathy and lactic acidosis even in the absence of sideroblastic anemia, irrespective of ethnicity.

Methods

Patients

Six patients (1.1, 1.2, 2, 3, 4.1, and 4.2) were referred to a national diagnostic and clinical center in Newcastle upon Tyne, England. Patients 1.1 and 1.2 were initially described in 1974.[29] Total genomic DNA was extracted and muscle biopsies were taken with written informed consent by standard procedures. We performed a literature review to identify all previously published cases with *YARS2* variants.[4,19-23] This study was approved and performed under the ethical guidelines issued by North East-Newcastle and North Tyneside institutional review board and complied with the Declaration of Helsinki.[30] Patients provided written informed consent.

Muscle Histochemistry and Respiratory Chain Enzyme Analysis

Muscle biopsy samples were subjected to cytochrome-*c* oxidase (COX), succinate dehydrogenase (SDH), and sequential COX-SDH histochemical reaction.[31] Measurement of mitochondrial respiratory chain complex activities in skeletal muscle homogenates was performed as previously described.[32]

Mitochondrial DNA Studies

Whole mitochondrial genome sequencing was performed using skeletal muscle homogenate to exclude pathogenic point mutations. Mitochondrial DNA rearrangements and mtDNA depletion were excluded using established, diagnostic, long-range polymerase chain reaction, and quantitative real-time polymerase chain reaction assays, respectively. Haplogroups were classified using HaploGrep.[33]

Identification of Pathogenic *YARS2* Variants

YARS2-targeted gene screening or variants identified by whole exome sequencing were confirmed by Sanger sequencing using custom-designed primers for all 5 exons and intronic regions of *YARS2* (GenBank NM_001040436.2). Patient 4.1 was the first to have the diagnosis identified by whole exome sequencing,[34] followed by patients 2 and 3 with a separate exome filtering pipeline.[35] Targeted *YARS2* gene screening was performed on patient 1.1.

Identification of Founder *YARS2* Mutation

Short tandem repeat tracts flanking the *YARS2* gene were identified using the Repeat Masker track of the University of California, Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu),[36] and primers were designed using Primer3 (http://primer3.ut.ee/).[37] Patient DNA was amplified across the various short tandem repeat loci by using polymerase chain reaction. Use of a fluorescently tagged forward primer facilitated electrophoresis on an ABI3130xl genetic analyzer. Sizing and genotyping were performed (Peak

Scanner, version 1.0; Applied Biosystems) with standard analysis parameters; ROX500 (Applied Biosystems) was used as a size standard.

Immunoblotting

Total protein aliquots from fibroblasts and myoblasts of patient 3 plus 2 controls for each cell line were separated through the use of sodium dodecyl sulfate polyacrylamide gel electrophoresis. Membranes were probed with antibodies specific to YARS2 (AP7838; Abgent), SDHA (ab14715; Abcam), ATP5B (ab14730; Abcam), UQCRC2 (ab14745; Abcam), MT-COI (ab14705; Abcam), MT-COII (ab110258; Abcam), and NDUFB8 (ab110242; Abcam). β-Actin (A5316; Sigma-Aldrich) and VDAC1/Porin (ab14734; Abcam) were used as loading controls.

Yeast Modeling

Yeast strains were generated and cultured as described in the eMethods in the Supplement. Human YARS2 is highly conserved in phylogenesis including yeast. The human residue Leu392 is conserved from human to yeast (Leu411) (eFigure, C in the Supplement), and the mutant allele corresponding to the novel missense variant identified in the patient of our cohort was generated (msy^{L411S}). The human residue Cys369 is, however, not conserved in yeast (Leu391) (eFigure, C in the Supplement). For this nonconserved residue, we mutagenized the yeast sequence to replace the amino acid leucine of the wild-type Msy1 with the amino acid cysteine of the wild-type human YARS2, thus creating the so-called "humanized" version (hL391C). If the humanized wild-type variant is able to complement the oxidative growth defect of the *msy1Δ* strain, it is possible to evaluate the effects of the novel missense variant identified in our cohort (Cys369Tyr) by creating the corresponding yeast mutant allele $msy1^{L391Y}$.

Oligonucleotides are provided in eTable 1 in the Supplement. Oxidative growth and mitochondrial respiration were measured as described previously.[19] The full methodology is provided in the eMethods in the Supplement.

Statistical Analysis

A paired, 2-tailed *t* test was performed to examine the yeast mitochondrial respiratory rate, and the level of statistical significance was set at $P \leq .05$. Microsoft Excel 2016 (Microsoft Corp) was used for statistical analysis.

Results

Clinical Features

We present 6 patients referred to our national diagnostic center with confirmed *YARS2* variants (**Table 1**), including 4 individuals of Scottish ancestry. A literature review identified an additional 11 patients with *YARS2* variants.[4,19-23] Hence, there is a total of 17 patients with *YARS2* variants (eTable 2 in the Supplement). A summary of the clinical features of all 17 patients (13 pedigrees) is given in **Table 2**. The median (interquartile range [IQR]) age at onset was 1.5 [9.8] years (range, 1 week to 31 years); 14 patients (82%) presented within the first decade of life. One patient presented in adolescence and 2 patients presented in adulthood. Six patients (35%) died (median [IQR] age at death, 25.5 [46.5] years; range, 3 months to 52 years). Two patients who died at age 3 months both presented with symptoms within 8 weeks of birth. A third patient presenting at age 10 weeks survived into adolescence, and the longest-surviving patients presented in childhood and survived into adulthood. Death in all 6 of these patients was preceded by progressive respiratory muscle weakness, which often required noninvasive ventilation, and cardiac failure (**Figure 1**).

Muscle Histochemistry and Muscle Respiratory Chain Enzyme Activity Analysis

Skeletal muscle biopsy specimens were available for 8 of the 17 patients (47%) (eTable 2 in the Supplement). Global COX deficiency was evident in 4 patients (**Figure 2**); ragged red fibers were observed in 2 patients. Patient 7 had a marked increase in SDH activity, but patient 13.1 exhibited only a minimal increase. Other abnormalities included lipid vacuoles (patient 9) and a few myopathic hypoatrophic fibers (patient 13.1).

Muscle respiratory chain enzyme activity was measured in 10 patients. Combined complex I, III, and IV deficiencies were found in 6 patients; complex I and IV deficiencies were found in the remaining 4 individuals.

Identification of Pathogenic *YARS2* Variants

In our cohort, we identified 3 novel pathogenic *YARS2* variants (c.1106G>A p.Cys369Tyr, c.1147_1164dup p.Val383_Glu388dup, and c.1175T>C p.Leu392Ser) and a previously reported variant (c.137G>A p.Gly46Asp).[22] The p.Cys369Tyr substitution is absent from external databases (ExAC, ESP6500, and 1000 Genomes Project) and affects a highly conserved residue; the impact of the missense substitution is predicted to be deleterious according to SIFT and MutationTaster; PolyPhen2 and AlignGVGD are more conservative with their predictions. Because of the proximity of the c.1106G>A nucleotide to the intron-exon boundary, the c.1106G>A substitution may cause aberrant messenger RNA splicing and, in addition, it is predicted to abolish a SC35 exonic splicing enhancer according to the in silico splicing prediction tool ESEfinder. The p.Val383_Glu388dup variant was found in 2 of 121 360 (1.648×10^{-5}) alleles in ExAC from 2 non-Finnish European individuals. The p.Leu392Ser missense change was found in 1 of 121 354 alleles in ExAC in a single non-Finnish European individual and is predicted to be deleterious according to SIFT and MutationTaster; however, PolyPhen2 and AlignGVGD predict a milder effect of the p.Leu392Ser substitution (ExAC, ESP6500, and 1000 Genomes Project frequency data, accessed June 22, 2016). To date, there is a total of 9 pathogenic variants described in the *YARS2* gene (**Figure 3**A). All variants lead to missense changes except for a 6-amino acid duplication within the S4-like domain and a nonsense variant that is likely to lead to degradation of messenger RNA by nonsense-mediated decay.[21] All patients harbored homozygous missense changes except for patients 3 and 12, who had compound heterozygous changes. The Lebanese founder p.Phe52Leu variant was the most frequent pathogenic *YARS2* variant, occurring in 5 patients.

Table 1. Clinical, Molecular, and Genetic Details of Patients With *YARS2*

	Patient					
Characteristic	1.1	1.2	2	3	4.1	4.2
Sex	Male	Male	Female	Male	Male	Male
Age at onset	Childhood	Childhood	Childhood	Childhood	Childhood	Childhood
Current age decade, y	50s[a]	40s[a]	70s	20s	30s[a]	30s
Ethnicity	Scottish	Scottish	Scottish	Irish/Scottish	Jordanian	Jordanian
Consanguinity	No	No	Yes	No	No	No
Clinical features						
Myopathy[b]	4/5	Yes	4−/5	4/5	4/5	3/5
Respiratory insufficiency	NA	NA	NIV	Severe restrictive pattern	Yes	NIV
Cardiomyopathy	High-output cardiac failure	High-output cardiac failure	No	Yes	End-stage cardiac failure	No
Sideroblastic anemia	Yes	Yes	No	No	Yes	No
Transfusion	No	No	No	No	Yes	No
Lactic acidosis	Yes	Yes	No	Yes	Yes	Yes
Other features	No	No	Facial weakness	No	No	Scapular winging
Muscle biopsy						
Histochemistry	Global COX deficiency	NA	Global COX deficiency	Global COX deficiency, RRFs	Global COX deficiency	RRFs
RC enzyme deficiency	CI, CIII, CIV	NA	CI, CIV	CI, CIII, CIV	CI, CIV	ND
YARS2 variant						
cDNA change	Homozygous c.1175T>C	ND	Homozygous c.1175T>C	c.1106G>A/ c.1147_1164dup	Homozygous c.137G>A	ND
Amino acid change	Homozygous p.Leu392Ser	ND	Homozygous p.Leu392Ser	p.Cys369Tyr/ p.Val383_Glu388dup	Homozygous p.Gly46Asp	ND
mtDNA haplogroup	H1m1	ND	G2a3a	T2e1a	HV13	ND

Abbreviations: cDNA, complementary DNA; CI, complex I; CIII, complex III; CIV, complex IV; COX, cytochrome-*c* oxidase; mtDNA, mitochondrial DNA; NA, not available; ND, not done; NIV, noninvasive ventilation; RC, mitochondrial respiratory chain; RRFs, ragged red fibers.

[a] Age at death.

[b] Scored according to the Medical Research Council scale for muscle strength (https://www.mrc.ac.uk/research/facilities-and-resources-for-researchers/mrc-scales/mrc-muscle-scale/). The muscle scale grades muscle power on a scale of 0 to 5 in relation to the maximum expected for that muscle: 0, no contraction; 1, flicker or trace of contraction; 2, active movement, with gravity eliminated; 3, active movement against gravity; 4, active movement against gravity and resistance; and 5, normal power. Grades 4−, 4, and 4+ may be used to indicate movement against slight, moderate, and strong resistance, respectively.

We identified 2 patients (1.1 and 2) from 2 apparently unrelated families of Scottish ancestry with a homozygous p.Leu392Ser variant in the S4-like domain. Patient 2 was born to consanguineous parents. Formal genetic testing of patient 1.2, a clinically affected sibling of patient 1.1, was not feasible; however, their clinically unaffected sister was tested and did not harbor the familial variant. We also identified a third patient of Scottish ancestry (patient 3) with unreported (p.Cys369Tyr and p.Val383_Glu388dup) compound heterozygous variants in which familial segregation studies confirmed recessive inheritance.

Identification of Founder *YARS2* Mutation

Microsatellite genotyping of dinucleotide repeat regions flanking the *YARS2* gene supported a common founder across a region of at least 183 kb shared between unrelated patients 1.1 and 2 with a T-1-2-3 haplotype consisting of the *YARS2* p.Leu392Ser mutation plus markers ms3, ms2, and ms1. Analysis of DNA from the unaffected sister of patient 1.1, whose genetic status was homozygous wild type at the p.Leu392Ser locus, was as anticipated: consistent with inheritance of the normal paternal and maternal alleles (Figure 3B).

Assessment of Mitochondrial DNA

Pathogenic or likely pathogenic mtDNA point mutations, rearrangements, and depletion were excluded. All of the patients from our center belonged to different haplogroups (Table 1 and eTable 3 in the Supplement).

Immunoblotting Analysis

Immunoblotting of total protein aliquots from cultured skin fibroblasts and myoblasts of patient 3 showed no change in immunoreactive YARS2 levels (eFigure, A and B in the Supplement). A minimal reduction of MT-COI was observed in both fibroblasts and myoblasts, whereas a marked decrease in NDUFB8 was observed only in myoblasts.

Yeast Modeling

Oxidative growth of the strains expressing msy^{L411S} and the humanized version $msy1^{hL391C}$ was similar to the wild type at 28°C and 36°C, whereas growth of the strain expressing $msy1^{L391Y}$ was reduced, particularly at 36°C (eFigure, D in the Supplement). Oxygen consumption of cells grown at 28°C showed a reduction for both of the mutants: mutant $msy1^{L391Y}$ showed a 27% reduction of the respiratory rate with respect to its

Table 2. Summary of the Clinical Features of All 17 Patients

Clinical Features	No. (%)
Myopathy/exercise intolerance	15 (88)
Lactic acidosis	15 (88)
Sideroblastic anemia	13 (76)
Transfusion dependent	7 (41)
Spontaneous recovery	3 (18)
MLASA triad	12 (71)
Hypertrophic cardiomyopathy	9 (53)
Respiratory insufficiency	8 (47)
Ventilatory support	5 (29)
Facial muscle weakness	2 (12)
Ptosis	2 (12)
Ophthalmoparesis	1 (6)
Strabismus	1 (6)
Nystagmus	1 (6)
Delayed motor milestones	1 (6)
Scoliosis	1 (6)
Seizure	1 (6)
Gastrointestinal symptoms	1 (6)
Hepatomegaly	1 (6)
Proximal renal tubulopathy	1 (6)

Abbreviation: MLASA, myopathy, lactic acidosis, and sideroblastic anemia.

humanized version msy^{hL391C} and mutant $msy1^{L411S}$ showed a 21% reduction with respect to the wild-type strain (eFigure, E in the Supplement).

Discussion

We describe the variable clinical phenotype of 17 patients with *YARS2* variants, including 6 new adult cases identified by targeted *YARS2* gene sequencing or whole exome sequencing. Our study suggests that the classic clinical triad of MLASA is not essential for identifying *YARS2*-related mitochondrial disease. Demonstrable proximal muscle weakness may be subtle during the early stage of *YARS2*-related mitochondrial disease onset. Episodic vomiting coupled with fatigue, headache, and cardiac palpitations following physical exertion in children and adults should raise a clinical suspicion of exercise intolerance with associated lactic acidemia. We show that almost all affected patients (n = 15) exhibited lactic acidemia together with marked exercise intolerance and progressive myopathy.

Sideroblastic anemia has been considered to be an important clinical feature of Pearson syndrome-,[26] *PUS1*-,[24] or *YARS2*-related[4] mitochondrial disorders. Pearson syndrome, characterized by infantile-onset exocrine pancreatic failure and sideroblastic anemia,[27] can be differentiated from *PUS1*- and *YARS2*-related mitochondrial disease by its clinical phenotype and presence of a single, large-scale mtDNA deletion in blood and other tissues. Both *YARS2* and *PUS1* mutations have been reported in MLASA, but there may be additional clinical pointers to help discern between them. Mutations in *PUS1* have been associated with microcephaly,[38-41] variable degrees of learning disability and cognitive impairment,[38-40,42] dysmorphic features,[38-40] and failure to thrive or to experience growth restriction[39,41,43]; these features are conspicuously absent in YARS2-related mitochondrial disease. In contrast, hypertrophic cardiomyopathy is a clinical feature that has been identified independently in 8 family pedigrees with *YARS2* mutations (eTable 2 in the Supplement) compared with only a single case of *PUS1* mutations.[41] Although there are emerging phenotypic differences observed between these 2 genetic mutations, caution should be applied on drawing firm conclusions given the relatively small number of patients in these 2 cohorts to date.

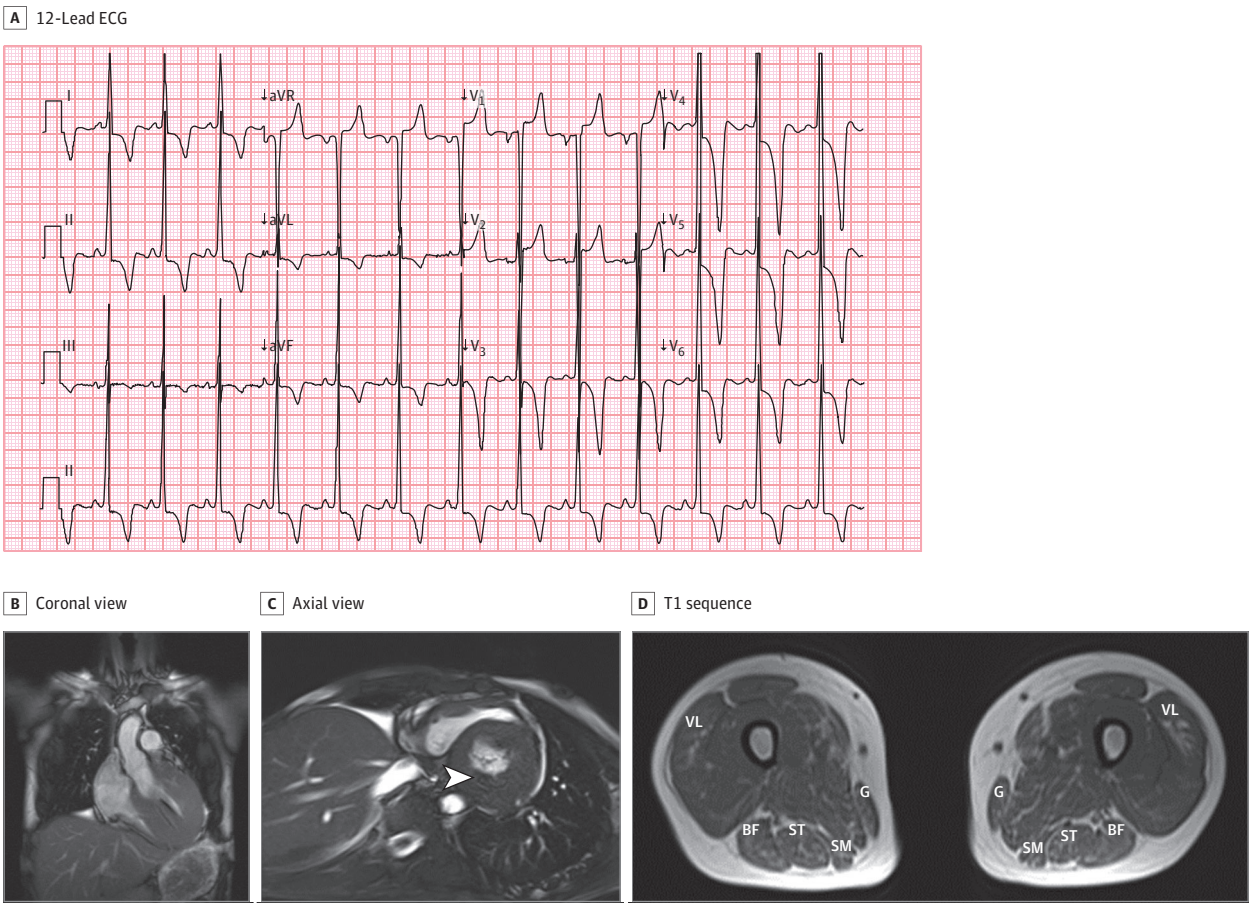
We have observed full resolution of sideroblastic anemia in 2 of the patients from our center and other previously reported patients with *YARS2* mutations. The exact mechanism of such a phenomenon in *YARS2* mutations is unknown given the other salient clinical features, including myopathy and cardiomyopathy, frequently progress over time. In another form of mitochondrial disease, the spontaneous resolution of sideroblastic anemia in Pearson syndrome has been speculated to be the result of negative selection of mutant mtDNA in the bone marrow.[44] This is a feature that warrants further work but is beyond the scope of this article.

Severe respiratory muscle weakness leading to ventilation failure, especially in adult patients, is a rare clinical feature in mitochondrial disease[45-47] compared with other genetic muscle diseases.[48] Analysis of all cases would suggest this is a key, terminal feature combined with hypertrophic cardiomyopathy and/or refractory transfusion-dependent sideroblastic anemia in the natural progression of *YARS2*-associated mitochondrial disease. These findings highlight the importance of regular surveillance of respiratory and cardiac function. Furthermore, early instigation of supportive treatment, such as noninvasive ventilation and pharmacologic cardiac remodeling agents,[49] may slow disease progression.

The muscle biopsy findings of severe, global COX deficiency in muscle fibers were consistent with a diagnosis of mitochondrial myopathy and prompted further investigation. Immunoblotting of fibroblasts and myoblasts from patient 3 demonstrated no change in YARS2 protein levels; immunoblotting of fibroblasts, myoblasts, or MyoD-forced myotubes from 2 patients (8.2 and 12) also showed no decrease.[4,21] In contrast, YARS2 levels were undetectable in myoblasts and myotubes from patient 7.[22] This may reflect the tissue-specific nature of *YARS2* and other aminoacyl tRNA synthetase mutations and variation in the threshold for expression between different individuals and tissues. One factor contributing to this variation may be the rate of protein turnover in an individual tissue. Our data illustrating only a minor decrease in MT-COI and MT-COII levels in patient fibroblasts and myoblasts despite marked COX deficiency in muscle lend support to this argument.

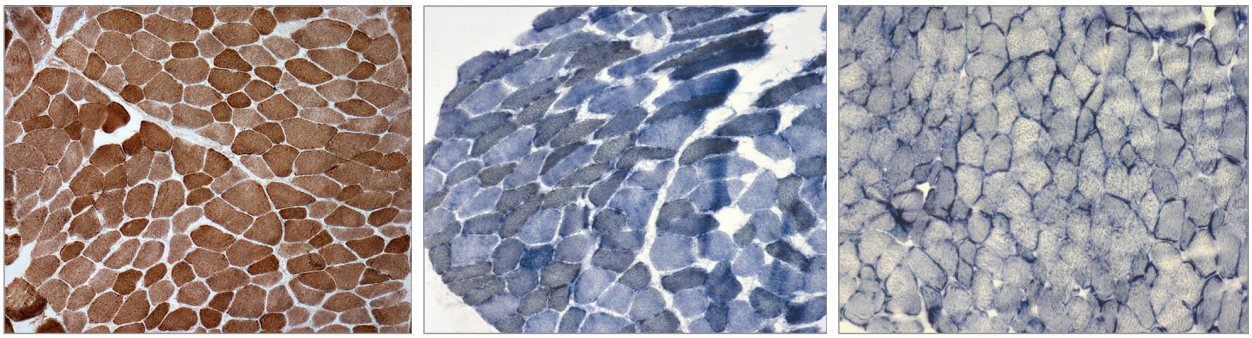
Yeast modeling to confirm pathogenicity of the reported p.Asp311Glu variant, corresponding to msy^{D333E}, had been performed previously demonstrating an OXPHOS defect by failure to grow on media containing ethanol.[19] We replicated this for our unreported missense changes, p.Leu392Ser and p.Cys369Tyr, corresponding to msy^{L411S} and msy^{L391Y}. Both

Figure 1. Findings of Cardiac Investigations and Magnetic Resonance Imaging (MRI) of the Muscle in Patient 3



A, Twelve-lead electrocardiogram (ECG) showed voltage criteria consistent with left ventricular hypertrophy (S wave in V_1 + R wave in V_6 = 48 mm + 53 mm = 101 mm; >35 mm or 7 large squares are suggestive of left ventricular hypertrophy) with strain pattern. B and C, Coronal and axial views of cardiac MRI. Concentric hypertrophic cardiomyopathy was evident (arrowhead). D, T1-sequence MRI of the muscle showed mild atrophy affecting the vastus lateralis (VL), bicep femoris (BF), semitendinosus (ST), semibranosus (SM), and gracilles (G).

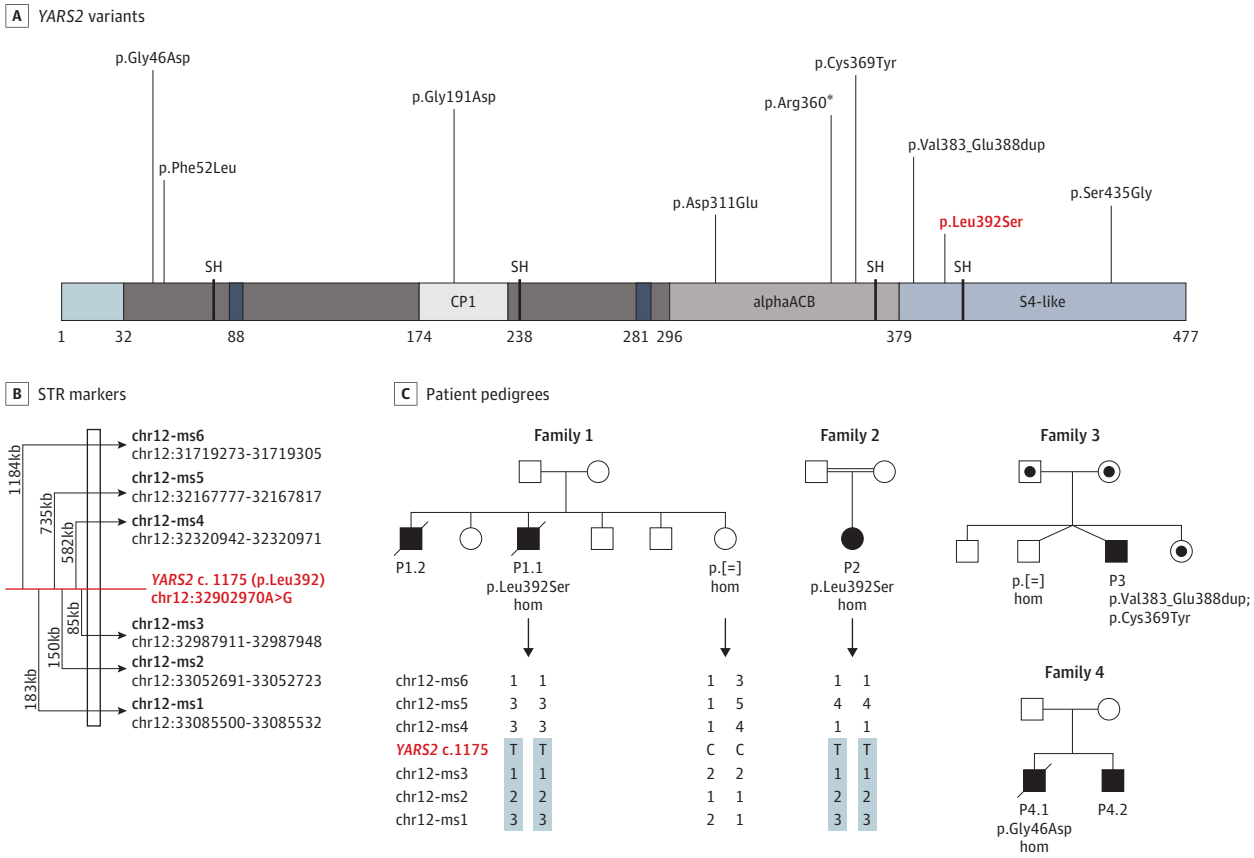
Figure 2. Skeletal Muscle Histochemistry Analyses



Normal cytochrome-*c* oxidase (COX)–succinate dehydrogenase histochemical analysis from a healthy control sample (A) compared with a global COX deficiency observed in the muscle fibers of patient 2 (B) and patient 4.1 (C) (scale bar, 100 µm).

mutated alleles showed mild OXPHOS defects, although *msy*^{L391Y} had a slightly more severe OXPHOS phenotype. However, both mutants were phenotypically milder than the previously characterized *msy*^{D333E} mutant. More important, when compared, all modeled missense changes reflected the human clinical phenotypes.

Figure 3. Genetic Characterization of Patients



A, All reported pathogenic *YARS2* variants. Novel p.Leu392Ser founder mutation from this study is shown in red. B, Location of short tandem repeat (STR) markers used for analysis and their genomic distance relative to *YARS2*. C, Pedigrees of patients (P), including 1.1, 1.2, and 2 with STR haplotype results (phase assumed when no parental samples are available) and a shared haplotype (highlighted in blue) involving the *YARS2* locus (red type), ms1, ms2, and ms3 STR markers. Pedigrees 3 and 4 are also shown.

Limitations

Predicting the severity and speed of disease progression is challenging given the genetic heterogeneity of this disorder. Homozygous mutations in p.Gly46Asp appear to manifest later in childhood compared with the p.Phe52Leu and p.Ser435Gly variants. We now describe 3 unrelated patients of Scottish ancestry with homozygous p.Leu392Ser mutations who presented with slower disease progression and survival into late adulthood. It has been hypothesized that mtDNA haplotype background may influence phenotypic expression.[21,50,51] Within our patient cohort, haplotype H was associated with slower disease progression and a less severe phenotype as previously observed.[21] Patient 2, who presented with the mildest phenotype of all *YARS2* patients, belonged to haplogroup G (Table 1 and eTable 3 in the Supplement), which, to our knowledge, has not been previously reported. However, as with previous phylogenetic analysis and characterization of mtDNA haplogroups in patients with *YARS2*,[21] there are likely additional, indeterminate genetic factors that modify phenotypic expression of disease.

The advent of next-generation sequencing has permitted the identification of patients with *YARS2* mutations who do not manifest with the full MLASA syndromic features, thus further expanding the phenotypic and genotypic heterogeneity of these mitochondrial disorders. This is exemplified by the identification of a 73-year-old patient with characteristic global COX deficiency in muscle who harbored recessive *YARS2* variants and manifests remarkably indolent, progressive, mitochondrial myopathy.

Conclusions

YARS2-related mitochondrial disease is phenotypically heterogeneous and has a variable prognosis ranging from infantile-onset—and often fatal—MLASA syndrome to later adolescent-onset, slowly progressive myopathy. Progressive respiratory muscle weakness and cardiomyopathy are the major causes of death in these patients. We suggest that *YARS2*-associated mitochondrial disease may be an underdiagnosed clinical entity, particularly in populations outside the Middle East and in adult patients who do not exhibit the full clinical spectrum of MLASA, as we demonstrate a new Scottish *YARS2* founder effect within our patient population.

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