The phenotypic spectrum of germline YARS2 variants: from isolated sideroblastic anemia to mitochondrial myopathy, lactic acidosis and sideroblastic anemia 2

Lisa G. Riley, 1,2,3 Matthew M. Heeney, 4,5 Joëlle Rudinger-Thirion, 6 Magali Frugier, 6 Dean R. Campagna, 6 Ronghao Zhou, 7 Gregory A. Hale, 7 Lee M. Hilliard, 8 Joel A. Kaplan, 9 Janet L. Kwiatkowski, 10,11 Colin A. Sieff, 1,3 David P. Steensma, 12,13 Alexander J. Rennings, 14 Annet Simons, 15 Nicolaas Schaap, 16 Richard J. Roedenburg, 17 Tjitske Kleefstra, 15 Leonor Arenillas, 18 Josep Fita-Torró, 19 Rasha Ahmed, 20 Miguel Abboud, 20 Elie Bechara, 21 Roula Farah, 21 Rienk Y. J. Tamminga, 22 Sylvia S. Bottomley, 23 Mayka Sanchez, 10,24,25 Gerwin Huls, 26 Dorine W. Swinkels, 27 John Christodoulou 2,28,29,30 and Mark D. Fleming 3,6,13,29

* LGR and MMH contributed equally to this work. #JC and MDF contributed equally to this work as co-senior authors.

1 Genetic Metabolic Disorders Research Unit, Kids Research Institute, Children’s Hospital at Westmead, Sydney, Australia; 2 Discipline of Child & Adolescent Health, Sydney Medical School, University of Sydney, Australia; 3 Dana Farber-Boston Children’s Center for Cancer and Blood Disorders, Boston, MA, USA; 4 Department of Pediatrics, Harvard Medical School, Boston, MA, USA; 5 Architecture et Réactivité de l’ARN, Université de Strasbourg, CNRS, IBMC, Strasbourg, France; 6 Department of Pathology, Boston Children’s Hospital, Boston, MA, USA; 7 Johns Hopkins All Children’s Hospital, St. Petersburg, FL, USA; 8 Division of Pediatric Hematology Oncology, University of Alabama at Birmingham, AL, USA; 9 Levine Children’s Hospital, Charlotte, NC, USA; 10 The Children’s Hospital of Philadelphia, Division of Hematology, Philadelphia, PA, USA; 11 University of Pennsylvania School of Medicine, Philadelphia, PA, USA; 12 Adult Leukemia Program, Dana-Farber Cancer Institute, Boston, MA, USA; 13 Harvard Medical School, Boston, MA USA; 14 Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, the Netherlands; 15 Department of Human Genetics, Radboud University Medical Centre, Nijmegen, the Netherlands; 16 Department of Hematology, Radboud University Medical Centre, Nijmegen, the Netherlands; 17 Rambou Center for Mitochondrial Medicine, Translational Metabolic Laboratory, Department of Pediatrics, Radboud University Medical Centre, Nijmegen, the Netherlands; 18 Laboratory CitoLògia Hèmatòlogica, Servicio Patología, GRETNHE, IMIM Hospital del Mar Research Institute, Hospital del Mar, Barcelona, Spain; 19 Iron metabolism: regulation and disease group, Josep Carreras Leukaemia Research Institute (JCI), Campus ICO-Germans Trias i Pujol, Campus Can Ruti, Carretera de Can Ruti, Camí de les Escoles, Badalona, Spain; 20 Department of Pediatrics and Adolescents, American University of Beirut Medical Center, Beirut, Lebanon; 21 Department of Pediatrics, Saint George Hospital University Medical Center, Beirut, Lebanon; 22 Beatrix Children’s Hospital, Department of Pediatric Hematology, University Medical Center Groningen, University of Groningen, the Netherlands; 23 Department of Medicine, University of Oklahoma College of Medicine, Oklahoma City, OK, USA; 24 Program of Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona, Spain; 25 BloodGenetics, S.L., Esplugues de Llobregat, Barcelona, Spain; 26 Department of Hematology, University Medical Center Groningen, the Netherlands; 27 Department of Laboratory Medicine, Translational Metabolic Laboratory, Radboud University Medical Centre, Nijmegen, the Netherlands; 28 Neurodevelopmental Genomics Research Group, Murdoch Childrens Research Institute, Melbourne, Australia and 29 Department of Paediatrics, Melbourne Medical School, University of Melbourne, Australia

ABSTRACT

YARS2 variants have previously been described in patients with myopathy, lactic acidosis and sideroblastic anemia 2 (MLASA2). YARS2 encodes the mitochondrial tyrosyl-tRNA synthetase, which is responsible for conjugating tyrosine to its cognate mt-tRNA for mitochondrial protein synthesis. Here we describe 14 individuals from 11 families presenting with sideroblastic anemia and YARS2 variants that we identified using a sideroblastic anemia gene panel or exome sequencing. The phenotype of these patients ranged from MLASA to isolated congenital sideroblastic anemia. As in previous cases, inter- and intra-familial phenotypic variability was observed, however, this report
Importantly, some patients carry a common divergent, affected and unaffected, clinical phenotypes. We describe two pairs of genotypically identical siblings with mild myopathy and lactic acidosis. In addition, we show that some of which were associated with milder effects on mitochondrial tyrosyl-tRNA synthetase, YARS2-related myopathy in the absence of sideroblastic anemia 2 (MLASA2; OMIM #613561), and recently cases of YARS2-related myopathy in the absence of sideroblastic anemia have been reported. YARS2 encodes the mitochondrial tyrosyl-tRNA synthetase, YARS2, which is responsible for the ATP-dependent conjugation of tyrosine to its cognate tRNA, required to support mitochondrial protein synthesis. YARS2 catalyses this reaction in a two-step process. In the first step, tyrosine and ATP bind to the catalytic domain to form the tyrosyl-adenylate intermediate. In the second step, cognate tRNA binds the synthetase and the tyrosyl moiety is transferred to the tRNA CCA-end. The resulting tyrosyl-tRNA will be delivered to the ribosome.

The most commonly reported YARS2 variant, p.(Phe52Leu), prevalent in patients of Lebanese Christian descent, has been shown to reduce YARS2 amino-acylation catalytic efficiency by approximately 9-fold, and leads to a reduction in mitochondrial protein synthesis in patients with MLASA2. Here we report YARS2 variants, some of which were associated with milder effects on amino-acylation, in patients with isolated CSA, or CSA with mild myopathy and lactic acidosis. In addition, we describe two pairs of genotypically identical siblings with divergent, affected and unaffected, clinical phenotypes. Importantly, some patients carry a common YARS2 c.572 G>T variant, p.(Gly191Val), that we and others have previously shown has a mild effect on amino-acylation activity, and suggest that these milder alleles may be the basis of the reduced penetrance and expressivity.

Methods

Clinical data

The patients and their immediate family members were referred to MMH, MDF, NS or LA for clinical consultation. Written informed consent was obtained from participants in the study, as approved by the Institutional Review Boards of Boston Children's Hospital, USA, the Radboud University Medical Center, the Netherlands, and the Hospital Germans Trias i Pujol, Badalona, Spain. In each case, CSA was ascertained by complete blood counts (CBCs), and peripheral blood or bone marrow morphology. Clinical data are provided in the Online Supplementary Appendix.

Variant detection

Targeted sequencing of nuclear encoded CSA genes, and the mitochondrial genome as well as mitochondrial DNA deletion analysis was performed on the probands of families 1-3 and 5-9. Genomic DNA was isolated from peripheral blood or skin fibroblasts, using the Puregene DNA Purification Kit (Qiagen, Valencia, CA, USA). DNA templates for sequencing were amplified from genomic DNA by PCR, enzymatically cleaned, bidirectionally sequenced using fluorescent dye termination sequencing chemistry, and analyzed with the Sequencher 5.3 DNA sequence assembly software (Gene Codes, Ann Arbor, MI, USA), as previously described.

Exome sequencing for Patient 4 was performed on genomic DNA isolated from whole blood. The experimental workflow was performed at BGI Europe (Beijing Genome Institute Europe, Copenhagen, Denmark) using an Illumina Hiseq (Illumina, CA, USA) platform. Variants in genes previously associated with Mendelian diseases (OMIM), including CSAs, were analyzed bioinformatically.

Patient 10 DNA was analyzed using a targeted gene panel for congenital and acquired sideroblastic anemias, including ABCB7, ALAS2, GLRX5, PUS1, SF3B1, SLC19A2, SLC25A38, STEAP3, TRNT1 and YARS2. The library was constructed using the Custom HaloPlex™ Target Enrichment System (Agilent, Santa Clara, CA, USA) and sequenced on a MiSeq platform (Illumina, San Diego, CA, USA). Data were analyzed with SureCall software (Agilent, Santa Clara, CA, USA).

Patient 11 DNA was analyzed using a targeted gene panel for sideroblastic anemia (ABCB7, ALAS2, GLRX5, HSCB, HSPA9, PUS1, SLC25A38, STEAP3, YARS2) and ion semiconductor sequencing as developed by Ion Torrent systems.

In silico predictions of variant pathogenicity were performed using the Alamut Visual suite of genetic analysis software (Interactive Biosoftware, Rouen, France), and linking externally to the PolyPhen2 and SIFT analytical tools. Minor allele frequencies are reported as in gnomAD (gnomad.broadinstitute.org) current as of September 2017.

Amino-acylation assays

Recombinant wild-type and the p.(Leu61Val), p.(Met195Ile), p.(Ser203Ile), p.(Tyr236Cys) and p.(Gly244Ala) YARS2 variants were expressed in E. coli, purified to homogeneity and assayed for
tyrosylation activity as previously described. Apparent kinetic parameters were determined from Lineweaver-Burk plots in the presence of 4.8 to 6.5 nM YARS2 and 0.28 to 1.12 μM native E. coli tRNA^Tyr (Sigma, St. Louis, MO, USA). Experimental errors on $k_{cat}$ and $K_m$ varied at most by 20%. Numerical values are averages of at least two independent experiments.

**Results**

**Phenotypic spectrum**

Eleven probands with CSA were identified with potentially pathogenic YARS2 variants by targeted gene sequencing panels or exome sequencing (Table 1A and 1B). The majority of these families were derived from a group of more than 200 probands with CSA referred to SSB, MDF and MMH, in which approximately 4% of cases were attributed to YARS2 variants. YARS2 variants have previously been identified in patients with myopathy, lactic acidosis and sideroblastic anemia 2 (MLASA2); however, some patients in this study did not have overt clinical features of MLASA2 other than CSA, and several individuals with biallelic variants had no phenotype whatsoever. In two families, the proband had moderate sideroblastic anemia (P8a and P9a), while a sibling with the same YARS2 genotype was not anemic and was otherwise...
asymptomatic (P8b and P9b) (Table 1B). In a third family (P2a and P2b) (Table 1A), the proband was identified with a severe, new onset anemia at six years of age, and, subsequent to her brother’s diagnosis, the younger sibling was found to be anemic. Four of the probands presented within the first two years of life (P5, P6, P7, P9a), and 4 presented in adolescence (P1, P4, P8a, P11). Two patients have died (P1, P5), both from multi-organ failure, one of these following two unsuccessful hematopoietic stem cell transplantations (HSCTs). One patient (P4) has undergone successful HSCT.

The 11 probands all had moderate to severe normocytic to macrocytic anemia. In nine probands, the presence of ringed sideroblasts, ranging from 10% to over 50% of bone marrow erythroblasts, was documented on bone marrow aspiration; marrows were not examined in 3 other patients and 2 clinically unaffected siblings (Table 1A and B). Eight patients required transfusion; however, one patient spontaneously became transfusion independent at 16 months of age (P7), and 5 patients had periods of hematologic remission (P4, P5, P9a), transiently becoming RBC transfusion independent. In addition to anemia, 3 probands had variable neutropenia and/or thrombocytopenia (P1, P6, P8a). Four patients were treated with pyridoxine with no improvement in their anemia (P4, P5, P6, P11).

Two patients had severe lactic acidosis (P1, P5), but the remaining cases in which it was studied had mild or no lactic acidosis (Table 1A and B). Two patients had elevated blood lactate upon light exercise (P4, P8a); those with mild lactic acidosis also tended to have mild myopathy, although one patient with no reported lactic acidosis had

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>P7</th>
<th>P8a</th>
<th>P8b</th>
<th>P9a</th>
<th>P9b</th>
<th>P10</th>
<th>P11</th>
</tr>
</thead>
<tbody>
<tr>
<td>YARS2 variant 1</td>
<td>c.<a href="NM_001040436.2">572G&gt;T;731G&gt;C</a></td>
<td>c.572G&gt;T</td>
<td>c.572G&gt;T</td>
<td>c.98C&gt;A</td>
<td>c.98C&gt;A</td>
<td>c.608G&gt;T</td>
<td>c.933C&gt;G</td>
</tr>
<tr>
<td>YARS2 variant 2</td>
<td>c.933C&gt;G</td>
<td>p.(Asp311Glu)</td>
<td>c.1360_1361insG</td>
<td>p.(Ile454Serfs*10)</td>
<td>p.(Tyr236Cys)</td>
<td>p.(Tyr236Cys)</td>
<td>p.(Asp311Glu)</td>
</tr>
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<td>Gender</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
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<td>Ethnicity</td>
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<td>Caucasian/</td>
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<td>Dutch</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Age at presentation</td>
<td>3 months</td>
<td>18 years</td>
<td>49 years</td>
<td>3 months</td>
<td>3 months</td>
<td>23 years</td>
<td>13 years</td>
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<tr>
<td>Sideroblastic anemia</td>
<td>Severe, transfusion dependent until 16 months</td>
<td>Moderate</td>
<td>None</td>
<td>Severe, transfusion intermittently from 3 months</td>
<td>None</td>
<td>Moderate</td>
<td>Severe transfusion dependent from 13 years</td>
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<tr>
<td>Hemoglobin, g/dL</td>
<td>5.8</td>
<td>9.9</td>
<td>13.9</td>
<td>2.4</td>
<td>12.8</td>
<td>9.6</td>
<td>6.6</td>
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<tr>
<td>MCV, fL</td>
<td>94.6</td>
<td>111.9</td>
<td>82</td>
<td>113.8</td>
<td>94.1</td>
<td>86</td>
<td>95</td>
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<tr>
<td>Abs Retic, M/μL</td>
<td>0.057</td>
<td>0.059</td>
<td>0.106</td>
<td>0.015</td>
<td>0.053</td>
<td>0.088</td>
<td>0.018</td>
</tr>
<tr>
<td>Retic, %</td>
<td>1.8</td>
<td>2.3</td>
<td>2.1</td>
<td>2.4</td>
<td>1.3</td>
<td>2.38</td>
<td>0.8</td>
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<tr>
<td>WBC x10^9/L</td>
<td>8.01</td>
<td>6</td>
<td>6.8</td>
<td>10.1</td>
<td>9.8</td>
<td>7.65</td>
<td>4.9</td>
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<tr>
<td>ANC x10^9/L</td>
<td>1201</td>
<td>3600</td>
<td>4340</td>
<td>1919</td>
<td>3180</td>
<td>4280</td>
<td>1700</td>
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<tr>
<td>Platelets x10^9/L</td>
<td>337</td>
<td>149</td>
<td>182</td>
<td>537</td>
<td>414</td>
<td>243</td>
<td>257</td>
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<tr>
<td>RS, % of BM erythroblasts</td>
<td>47</td>
<td>40</td>
<td>ND</td>
<td>&gt;50</td>
<td>ND</td>
<td>32</td>
<td>81</td>
</tr>
<tr>
<td>Chelation (Year started)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes (2017)</td>
<td></td>
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<tr>
<td>Lactic acidosis</td>
<td>None</td>
<td>Exercise induced only</td>
<td>None</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Mild</td>
</tr>
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<td>Myopathy</td>
<td>Moderate</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Mild</td>
</tr>
<tr>
<td>Other clinical features</td>
<td>Intermittent diarrhea and abdominal pain</td>
<td>Dependent edema, leukopenia, thrombocytopenia, atypical pulmonary carcinoid tumor (age 53)</td>
<td>None</td>
<td>Facial dysmorphism</td>
<td>Facial dysmorphism</td>
<td>Asthenia</td>
<td>None</td>
</tr>
</tbody>
</table>

MCV: mean corpuscular volume; retic: reticulocytes; WBC: white blood cell count; ANC: absolute neutrophil count; RS: ringed sideroblasts; BM: bone marrow; ND: not determined.
moderate myopathy (P7). Patient 1 (P1) with severe lactic acidosis and myopathy had combined respiratory chain deficiency in skeletal muscle, and the muscle biopsy showed histopathological features typical of a mitochondrial myopathy, including ragged red fibers on trichrome stain and "parking lot" inclusions and whorled arrays of mitochondrial cristae by transmission electron microscopy (data not shown). In one family, the proband (P9a) and his clinically unaffected, but genotypically identical sibling (P9b), had distinctive "triangular" faces, unlike their parents or genotypically normal sibling, which has not previously been reported in association with YARS2 variants, but has been described in mitochondrial myopathy with lactic acidosis and sideroblastic anemia 1 (MLASA1; OMIM #600462) due to pseudouridine synthase 1 (PUS1) variants.17

Table 2. In silico predictions of pathogenicity for YARS2 missense variants.

<table>
<thead>
<tr>
<th>YARS2 variant</th>
<th>SIFT prediction</th>
<th>SIFT score</th>
<th>PolyPhen2 prediction</th>
<th>PolyPhen2 score</th>
<th>gnomAD frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.(Leu61Val)</td>
<td>Deleterious</td>
<td>0.03</td>
<td>Benign</td>
<td>0.001</td>
<td>0.0016*</td>
</tr>
<tr>
<td>p.(Met195Ile)</td>
<td>Tolerated</td>
<td>0.17</td>
<td>Possibly damaging</td>
<td>0.827</td>
<td>0</td>
</tr>
<tr>
<td>p.(Ser203Ile)</td>
<td>Deleterious</td>
<td>0.02</td>
<td>Probably damaging</td>
<td>0.989</td>
<td>0</td>
</tr>
<tr>
<td>p.(Tyr236Cys)</td>
<td>Tolerated</td>
<td>0.09</td>
<td>Probably damaging</td>
<td>1.000</td>
<td>0.0008*</td>
</tr>
<tr>
<td>p.(Gly244Ala)</td>
<td>Deleterious</td>
<td>0.00</td>
<td>Probably damaging</td>
<td>0.995</td>
<td>0.0047*</td>
</tr>
</tbody>
</table>

*No homozygotes reported.

YARS2 variants in patients with congenital sideroblastic anemia

We identified three previously described YARS2 variants and ten novel variants in patients with CSA: the Lebanese Christian founder variant, p.(Phe52Leu),4 was in the homozygous state in 4 patients; the p.(Asp311Glu) variant5 homozygous in one patient; and a novel variant, p.(Leu61Val) homozygous in one patient. The remaining six families had compound heterozygous variants including four novel missense variants: p.(Met195Ile), p.(Ser203Ile), p.(Tyr236Cys), p.(Gly244Ala); a novel nonsense variant p.(Ser33*); three novel indels, p.(Thr197-Leu208del), p.(Leu389Gysfs*6), p.(Ile454Serfs*10); one novel splicing variant, c.1104-1G>A; and two previously reported missense variants, p.(Gly191Val) and p.(Asp311Glu).5,5 Three patient had two indel or splicing variants.
The five novel missense variants all lie in the catalytic domain of YARS2 (Figure 1A) and are rare in the gnomAD database (gnomad.broadinstitute.org) (Table 2). In silico predictions of pathogenicity for p.(Leu61Val), p.(Met195Ile) and p.(Tyr236Cys) vary between the SIFT and PolyPhen2 prediction programs while p.(Ser203Ile) and p.(Gly244 Ala) are predicted to be damaging to the YARS2 protein by both algorithms (Table 2 and Figure 1B). Conservation among species for each missense variant is shown in Online Supplementary Figure S1.

The nonsense variant, the splicing variant and three novel indels are likely to be deleterious. The splicing variant c.1104-1G>A alters a canonical position in the 3’ splice acceptor site of intron 3 and it is predicted to result in skipping of exon 4. The YARS2 c.98C>A, p.(Ser33*) nonsense variant and the c.1165_1166insG, p.(Leu389Cysfs*6) frameshift variant both lie greater than 55 nucleotides upstream of the last exon-exon junction and are most likely targeted for nonsense mediated decay.13 The p.(Thr197_Leu208) in frame deletion results in loss of 12 residues in α-helical regions of the catalytic domain, and more precisely of cluster 1, which is important for tRNA acceptor end recognition19 (Figure 1B). The c.1360_1361insG, p.(Ile454Serfs*10) variant lies in the last exon of YARS2 and is not predicted to be targeted for nonsense mediated decay.18 This variant would cause a frameshift at position 454 in the S4-like domain, which is found in all prokaryotic and organellar tyrosyl-tRNA synthetases, and is thought to stabilize the interaction between the tRNA and YARS2.19,20

Amino-acylation activity of YARS2 missense variants

Amino-acylation assays are commonly used to evaluate the effect of variants on aminoacyl-tRNA synthetase activity, with reduced activity being a strong predictor of pathogenicity.21 Consequently, the effect of the five missense variants, p.(Leu61Val), p.(Met195Ile), p.(Ser203Ile), p.(Tyr236Cys) and p.(Gly244 Ala) on amino-acylation activity was measured by the incorporation of [35S]-tyrosine into an E. coli tRNA40 substrate and compared to wild-type YARS2 activity. In vitro studies of the YARS2 variants revealed that amino-acylation efficiency was mildly reduced for p.(Leu61Val) and, p.(Met195Ile), while p.(Tyr236Cys) was not affected as compared to the wild-type enzyme (Table 3). YARS2 p.(Ser203Ile) and p.(Gly244 Ala) demonstrated a 17-fold loss in catalytic efficiency. The reduced activity of YARS2 p.(Ser203Ile) is a consequence of an increased $K_{cat}$, indicating that its affinity for tRNA was reduced. On the other hand, the YARS2 p.(Gly244 Ala) is characterized by a 13-fold lower $k_{cat}$ suggesting that the variant hinders efficient transfer of the tyrosyl moiety from the active site to the tRNA.

Discussion

Here we expand the clinical spectrum associated with YARS2 variants and describe patients with milder phenotypes who do not display all the features of MLASA2. Rather, most of the patients we describe presented principally with a normo- or macro-cytic CSA; they are mostly non-syndromic and unlike the most common forms of non-syndromic sideroblastic anemia (e.g. ALAS2 or SLC25A38 deficiency), the anemia is not microcytic. Nevertheless, in addition to ringed sideroblasts, some of these patients had vacuolization of marrow precursors and/or other cytopenias that are often seen in the syndromic sideroblastic anemias (e.g. Pearson syndrome), which may be a diagnostic clue.

Patients 1 and 3 had all the typical features of MLASA2, whereas Patients 2a, and 2b, who share homozygosity for the YARS2 Lebanese founder allele, p.(Phe52Leu), had only anemia. Patient 1 also had other features not typically associated with MLASA2, including neutropenia, thrombocytopenia, pericardial effusion, and premature ovarian failure. Neutropenia and pericardial effusion have each been reported in one other patient homozygous for the p.(Phe52Leu) variant.5,22 Two other patients in the current series with other genotypes also had mild or intermittent neutropenia. Premature ovarian failure is associated with variants in several mitochondrial aminoacyl-tRNA synthetase-encoding genes including HARS2, LARS2 and AARS2,23-25 and thus may be a feature common to mitochondrial protein synthesis defects. There are now 10 reported individuals homozygous for the YARS2 p.(Phe52Leu) variant22 and all have been symptomatic, supporting complete penetrance of this allele. However, the great range of phenotypic severity strongly suggests the presence of other genetic and environmental influences that can modify the effects of YARS2 deficiency.

Patient 4 presented in late adolescence with sideroblastic anemia without myopathy and has a homozgyous p.(Leu61Val) variant that diminished the amino-acylation catalytic efficiency 4-fold. Leu61 is located in a region of the catalytic domain specific to mitochondrial YARSs that was proposed to contact the tRNA75 acceptor helix (Figure 1B).17 In this case, HSCT appeared to be an effective treatment, restoring the patient’s hemoglobin levels to normal.

Patient 5 presented in infancy with CSA and was transfusion dependent other than a remission occurring between three and six years of age; she had no myopathy until her post-HSCT terminal illness. This patient had a YARS2 c.1165_1166insG variant predicted to result in a null allele, and a novel p.(Met195Ile) variant which lies within cluster 1, in a region involved in recognition of the tyrosine accepting arm of tRNA75 (Figure 1B).17 Some YARS proteins (e.g. yeast) have an isoleucine (Ile) at this position, suggesting that it might be a milder allele. Indeed, in vitro this mutant had little effect on YARS2 catalytic efficiency.

Patient 10 is a compound heterozygote for a splicing mutation (c.1104-1G>A) predicted to cause skipping of exon 4, and a missense variant p.(Ser203Ile), also located
in cluster 1. YARS2 (p.Ser203Ile) led to a reduced affinity for tRNA\(^{\text{tyr}}\), resulting in a 17-fold loss in catalytic efficiency (Figure 1C). Patient 10 has no lactic acidosis or myopathy, and presented with isolated normocytic anemia and asthenia, and has not required transfusion.

Patient 7 presented with anemia in infancy requiring two transfusions within the first 16 months of life and then became transfusion independent. She has moderate myopathy and no lactic acidosis and a compound heterozygous genotype: a missense variant, p.(Gly191Val), occurring in cis with p.(Gly191Val) and in trans with the p.(Asp311Glu) variant. Gly244 is a critical residue for tyrosyl-adenylate binding.\(^{19}\) YARS2 p.(Gly244Ala) only affects the p.(Asp311Glu) variant. Gly244 is a critical residue for tyrosyl-adenylate binding in the active site (Figure 1C). YARS2 Asp311 is involved in the recognition of anticodon residue G34 of tRNATyr.\(^{19}\) The p.(Asp311Glu) variant was observed in Patient 11 who was homozygous for p.(Asp311Glu), with transfusion-dependent MLASA2.

In two families in this study (Families 6 and 8), affected patients have the common p.(Gly191Val) allele (MAF = 0.1259) in trans of a predicted null allele. Importantly, all of the unaffected carriers of predicted null alleles in these and other families, where probands had the ancestral p.Gly191 variant in trans, were asymptomatic (data not shown). Patient 6 presented in infancy with CSA requiring transfusions every three weeks. She has mild lactic acidosis, no myopathy and intermittent neutropenia. She has a c.590_645del variant resulting in a 12 amino acid deletion in the catalytic domain (Figure 1A), which would almost certainly lead to a completely dysfunctional protein, in trans with p.(Gly191Val). Individuals 6a and 8b also carry p.(Gly191Val) in trans with a predicted null or severe loss-of-function allele, c.1360_1360insG, p.(Ile454Serfs*10). This variant truncates the S4-like domain which is thought to stabilize the interaction with tRNA\(^{\text{tyr}}\), and the deletion of the YARS2 S4-like domain leads to a 100-fold reduced amino-acylation activity in vitro.\(^{20}\) Patient 8a had sideroblastic anemia and edema. Lactate was elevated only on exertion and the patient did not have myopathy. Her sister (P8b) is asymptomatic. Patient 8a also had a somatic mutation in SF3B1 p.(Lys700Glu) that is strongly associated with myelodysplastic syndromes with ringed sideroblasts.\(^{26}\) Based on the childhood presentation of her anemia and exercise intolerance that was exacerbated significantly decades later, and the fact that a mutation in SF3B1 would be exceptional in a patient under 30 years of age, we infer the YARS2 mutations to be the primary cause of her anemia with the SF3B1 mutation occurring as a secondary somatic event, which exacerbated her anemia, bringing her to clinical attention. In addition to the reduced activity in vitro,\(^{20}\) support of the notion that YARS2 p.(Gly191Val) contributes to the disease phenotype in these patients comes from the observation that this variant is a disease modifier in Leber Hereditary Optic Neuropathy (LHON); the three common LHON mitochondrial DNA mutations have incomplete penetrance. However, all patients who carry both the LHON m.11778G>A mtDNA disease-associated variant in combination with a homozygous YARS2 p.(Gly191Val) genotype were symptomatic.\(^{21}\)

Patients 9a and 9b carried the YARS2 c.98C>A, p.(Ser33*) nonsense variant, which would result in a null allele, and the p.(Tyr236Cys) variant (Figure 1A and B) that did not alter amino-acylation activity in vitro. In addition, in silico analysis using Alamut did not predict that this variant would lead to alteration of an exonic splicing enhancer site. Patient 9a presented in infancy with sideroblastic anemia that has come and gone throughout his life. He has no lactic acidosis or myopathy. He and his unaffected brother have some dysmorphic features, which have not previously been reported in association with YARS2 variants, but are typical of MLASA1 patients with pseudouridine synthase 1 (PUS1) mutations.\(^{22,23}\) His genotypically concordant fraternal twin (P9b) has only mild anemia and similar facial dysmorphism, once again highlighting the potential for decreased penetrance and/or expressivity of the disorder.

Interestingly, some YARS2 patients with myopathy, but no sideroblastic anemia, have recently been reported by Sommerville et al.\(^{24}\) They report siblings with a homozygous YARS2 p.(Leu392Ser) variant who had MLASA2, while another individual homozygous for the same variant had myopathy without sideroblastic anemia or lactic acidosis.

To summarize, the inter- and intra-familial phenotypic variability, intermittent transfusion dependence of some YARS2 cases, and the association of a common variant with disease, suggest that all MLASA2 phenotypes may be susceptible to subtle changes in YARS2 function, which may in turn be influenced by genetic and/or environmental modifiers. This study shows that YARS2 variants can result in CSA in the absence of clinically significant myopathy or lactic acidosis. Thus, we recommend that YARS2 variants be considered as a cause of isolated sideroblastic anemia as well as MLASA2 or mitochondrial myopathy.

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