A Novel Homozygous *YARS2* Mutation in Two Italian Siblings and a Review of Literature

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Abstract *YARS2* encodes the mitochondrial tyrosyl-tRNA synthetase that catalyzes the covalent binding of tyrosine to its cognate mt-tRNA. Mutations in *YARS2* have been identified in patients with myopathy, lactic acidosis, and sideroblastic anemia type 2 (MLASA2). We report here on two siblings with a novel mutation and a review of literature. The older patient presented at 2 months with marked anemia and lactic acidemia. He required periodic blood transfusions until 14 months of age. Cognitive and motor development was normal. His younger sister was diagnosed at birth, presenting with anemia and lactic acidosis at 1 month of age requiring periodical transfusions. She is now 14 months old and doing well. For both our patients, there was no clinical evidence of muscle involvement. We found a new homozygous mutation in *YARS2*, located in the α -anticodon-binding (α ACB) domain, involved in the interaction with the anticodon of the cognate mt-tRNA^{Tyr}.

Our study confirms that MLASA must be considered in patients with congenital sideroblastic anemia and underlines the importance of early diagnosis and supportive therapy in order to prevent severe complications. Clinical severity is variable among *YARS2*-reported patients: our review of the literature suggests a possible phenotype-genotype correlation, although this should be confirmed in a larger population.

Introduction

Mitochondrial protein synthesis requires the RNA apparatus encoded by mtDNA genes (2 rRNAs and 22 tRNAs) and a remarkable number of protein factors encoded by nuclear genes, including ribosomal, structural, and assembly proteins; tRNA-modifying enzymes; rRNA-methylating enzymes: initiation, elongation, and termination translation factors; and aminoacyl-tRNA synthetases (aaRSs) that charge the 20 amino acids to the cognate mt-tRNA molecule (Rötig 2011). With the exception of *GARS* and *KARS*, mitochondrial and cytoplasmic aaRSs are encoded by two distinct nuclear genes (Diodato et al. 2014). Mutations in sixteen mt-aaRSs genes have been found to cause heterogeneous mitochondrial disorders, often characterized by a remarkable tissue or organ specificity (Konovalova and Tyynismaa 2013; Diodato et al. 2014; Hallmann et al. 2014; Schwartzentruber et al. 2014).

YARS2 encodes the mitochondrial tyrosyl-tRNA synthetase. Mutations in *YARS2* have been identified in patients with myopathy, lactic acidosis, and sideroblastic anemia (MLASA) phenotype (Riley et al. 2010, 2013; Sasarman et al. 2012; Shahni et al. 2013; Nakajima et al. 2014). MLASA is a rare autosomal recessive condition associated

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with two distinct genetic forms: MLASA1 (MIM #600462) results from mutations in *PUS1* gene (Bykhovskaya et al. 2004; Fernandez-Vizarra et al. 2007) and MLASA2 (MIM #613561) caused by *YARS2* mutations. *PUS1* encodes pseudouridine synthase, a tRNA-modifying enzyme that stabilizes the tRNA structure and facilitates the peptidyl transfer reaction of rRNAs (Fernandez-Vizarra et al. 2007). Both *PUS1* and *YARS2* mutations lead to decreased mitochondrial protein synthesis resulting in mitochondrial respiratory chain dysfunction. *PUS1* mutations have been associated with combined defects of complex I (CI) and IV (CIV) and *YARS2* with CI, complex III (CIII), and CIV deficiency (Riley et al. 2010, 2013; Sasarman et al. 2012).

Congenital sideroblastic anemia may be caused by mutations in several nuclear DNA genes involved in mitochondrial pathways: (1) heme biosynthesis, *ALAS2* (erythroid-specific delta-aminolevulinate synthase) and *SLC25A38* (a mitochondrial carrier protein of still unknown function); (2) iron-sulfur cluster biosynthesis, *ABCB7* (adenosine triphosphate binding cassette B7) and *GLRX5* (glutaredoxin 5); and (3) mitochondrial protein synthesis, *PUS1* and *YARS2*. Congenital sideroblastic anemia has also been described in association with sporadic single mtDNA deletions in Pearson's syndrome (Fujiwara and Harigae 2013) and more recently in a patient with an mtDNA point mutation in *ATP6* gene (Burrage et al. 2014).

We report the clinical, biochemical, and molecular findings of two Italian siblings with a new mutation in *YARS2* and a review of literature.

Case Report

The two siblings are children of unrelated healthy parents. Family history is unremarkable.

Patient 1 is a boy born after uncomplicated pregnancy and delivery. At 2 months of age, hematological examination performed because of extreme pallor with no other symptom revealed marked anemia (Hb 5.2 g/dl, Hct 14.8 %, RBC $1.52 \times 10^6/\mu\text{l}$) with mild neutropenia ($1.36 \times 10^3/\mu\text{l}$) but normal total leukocyte count and platelets. Serial controls confirmed anemia and occasionally neutropenia and thrombocytopenia. Parvovirus 19 infection, Fanconi anemia and Diamond-Blackfan anemia were ruled out. Liver and renal function and cardiac and abdominal ultrasound were normal. Bone marrow analysis performed at 3 months of age revealed mild erythroblastopenia and marked vacuolization of erythroid precursors. He was found to have lactic acidemia 4,416 $\mu\text{mol/l}$ (normal range 580–2,100 $\mu\text{mol/l}$). At this age he received the first transfusion, and since then he required transfusions every 3–4 weeks until 14 months when he spontaneously improved with normalization of blood parameters. The patient was examined in our unit at 13

months of age: he presented good general conditions, normal physical development, and mild psychomotor delay without achievement of autonomous gait. Laboratory data showed recovery of anemia (Hb 10.4 g/dl, Hct 31.7 %) and a normal blood lactate (2077 $\mu\text{mol/l}$). Analysis of urinary organic acids showed mild elevation of lactic, 3-OH butyric, and 3-OH isovaleric acids. EEG and ophthalmological examination were normal. T2-weighted MRI showed mild hyperintensity in the pallida and two tiny areas of abnormal signal in the pontine tegmentum. HMR spectroscopy was normal. Muscle biopsy performed at 16 months showed a few hypo-atrophic fibers, normal content of lipids, and glycogen with minimal increase of SDH activity in subsarcolemmal fibers. Activities of CI, CIII, and CIV were markedly reduced in muscle homogenate (CI = 13%, CIII = 18%, and CIV = 15% of the mean control values) but normal in fibroblasts; however, seahorse-based microscale oxygraphy revealed a consistent reduction of maximal respiratory rate in fibroblasts (28% of controls). As these results suggested a mitochondrial respiratory chain disorder, treatment with coenzyme Q₁₀ and riboflavin was started. He had a good clinical evolution with normal psychomotor development and achieved autonomous gait at the age of 18 months. At the last examination, at 6 years of age, neurological examination was normal, body weight and height were at the 50th centile, and no other signs of organ dysfunction appeared.

Patient 2, the younger sister, was born after uncomplicated pregnancy and delivery. The parents refused prenatal diagnosis. At 1 month of age, hematological examination revealed high lactate 9,900 $\mu\text{mol/l}$ (normal 580–2,100) and mild anemia: Hb 10.5 g/dl, Hct 27.7 %, RBC $3 \times 10^6/\mu\text{l}$, normal leukocytes, and platelets. Serial controls confirmed anemia, and she was started on periodical transfusions, the last one at 9 months of age. She is now 14 months old with a normal psychomotor development without any other major problems.

After receiving written informed consent from the parents, in agreement with the Declaration of Helsinki and approved by the Ethical Committees of the Foundation 'Carlo Besta' Neurological Institute, Milan, Italy, we performed Southern blot analysis of mtDNA extracted both from blood and muscle of patient 1 that did not show alteration, as well as the sequence analysis of mtDNA. On the basis of the similarity between the clinical features of our patient and of previously reported MLASA patients, we sequenced *PUS1* and *YARS2*. While the *PUS1* gene was normal, we identified a homozygous missense mutation c.933C>G/p.Asp311Glu in *YARS2*, which affects amino acid residue in the α -anticodon-binding (α ACB) domain that is invariant from humans to yeast. The mutation was heterozygous in the healthy parents and absent in the public single nucleotide polymorphism databases, including dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>) and

Fig. 1 (a) Chromatogram of the *YARS2* region comprising the identified mutation in patient 1; (b) molecular organization of human mtTyrRS (in green, the α -anticodon-binding – α ACB – domain); (c)

multiple alignment of *YARS2* protein domain contained the amino acid Asp311 (conserved and semi-conserved residues are highlighted in red – background and script, respectively)

EVS (<http://evs.gs.washington.edu/EVS>), which altogether contain approximately 12,000 alleles. In addition, the p. Asp311Glu change scored very high for the likelihood to be deleterious according to several softwares for pathogenicity prediction (damaging for Polyphen2, $p = 0.99$; Panther, 0.92; MutPred, 0.93; SIFT; and MutationTaster) (Fig. 1). The younger sister underwent genetic analysis immediately after birth, and she also resulted homozygous for the c.933C>G mutation.

The orthologous gene in *Saccharomyces cerevisiae* is *MSY1*, where the human p.Asp311Glu corresponds to p.Asp333Glu. To validate the pathogenic role of the human mutation, we introduced the corresponding mutant allele (*msyl*^{D333E}) in the W303-1B yeast strain disrupted in *MSY1* (*msyl* Δ).

We tested the possible deleterious impact of the allegedly pathogenic mutation *msyl*^{D333E} on oxidative

phosphorylation. The *MSY1*-defective yeast strain failed to grow in medium containing 2% ethanol (Fig. 2) as expected since the absence of the gene leads to complete loss of mtDNA (Edwards and Schimmel 1987). The OXPHOS-negative phenotype was rescued by reexpressing the wild-type *MSY1* gene, whereas very limited correction was obtained by expressing *msyl*^{D333E} (Fig. 2).

Measurement of the mitochondrial cytochrome content is an index of the structural integrity of the respiratory chain complexes. The null mutant *msyl* Δ transformed with the empty vector displayed a complete loss of cytochromes b and aa3; the strain transformed with the *msyl*^{D333E} allele displayed a dramatic reduction of cytochromes b and aa3; in both cases the cytochrome c was unaffected. As expected, the mitochondrial respiratory activities were concordant with the cytochrome profiles. These results clearly indicate that the human *YARS2* mutation is deleterious in yeast.

Fig. 2 Study in yeast strain W303-1B *msy1D*. (a) Oxidative growth phenotype; (b) cytochromes absorption spectra; (c) respiratory rate

Discussion and Conclusion

As disease mutations in mt-aaRS hit housekeeping nuclear DNA genes, they should in principle cause impaired mitochondrial translation in all organs. Nevertheless, a tissue-organ specificity of mt-aaRS disease mutations has been observed for each of the fourteen aaRS2 genes associated with disease mutations in humans (Diodato et al. 2014; Hallmann et al. 2014; Schwartzenruber et al. 2014).

To date nine patients with *YARS2* mutations have been described (Table 1). Two different homozygous missense mutations affecting the catalytic domain of mtTyrRS have been reported in seven different subjects of Lebanese origin: c.156C>G/p.Phe52Leu and c.137G>A/p.Gly46Asp. Later, Riley et al. described a French compound heterozygous patient carrying two novel *YARS2* mutations: c.572G>A/p.Gly191Asp and c.1078C>T/p.Arg360*. The p.Gly191Asp mutation results in a 38-fold loss in catalytic efficiency; the p.Arg360* mutation produces an unstable protein (Riley et al. 2013). The most recent *YARS2*-mutated patients reported in the literature (Nakajima et al. 2014) carried a homozygous missense mutation c.1303A>G/p.Ser435Gly. The p.Ser435Gly mutation is located in the S4-like domain of the C-terminal portion of the protein, which is essential to strengthen the interaction between mt-tRNA^{Tyr} and the mtTyrRS (Bonnefond et al. 2007). The mutation found in our patients is located in the α ACB domain. Asp311 is at the

beginning of the α -12 helix, which plays a crucial role in the interaction with the anticodon of cognate tRNA^{Tyr} (Bonnefond et al. 2007). In the homologous TyrRS from the bacterium *T. thermophilus*, where Asp259 is the equivalent to human Asp311, Yaremchuk et al. demonstrated a specific interaction between the Asp carboxyl group and G34 in the anticodon of tRNA^{Tyr} (Yaremchuk et al. 2002). This essential function is further supported by the complete observation of this Asp residue throughout evolution, while other putative anticodon-binding residues are not conserved (Bonnefond et al. 2007).

The *MSYI*-defective yeast strain (*msy1Δ*) displays an OXPHOS phenotype characterized by failure to grow in media containing ethanol, an obligatory respiratory compound as the only carbon source. The expression in the *msy1Δ* strain of *msyI^{D333E}* variant, equivalent to *YARS2* D311E, gave a clearly defective OXPHOS phenotype, but less drastic than that of the *msy1Δ* null strain, indicating the preservation of some functional competency. Likewise, the decrease of two components of mtDNA-related respiratory chain complexes, cytochrome aa3, part of CIV, and cytochrome b, part of CIII, was marked but milder than in the *msy1Δ* strain (Fig. 2). In no case cytochrome c was decreased, as expected in mtDNA-specific translation defects.

The patient described by Nakajima et al. showed the most severe clinical phenotype among those previously reported in association with *YARS2* mutations, characterized

Table 1 Clinical features of reported patients with *YARS2* mutations

	pt1 ^a	pt 2 ^a	pt 3 ^a	pt 4 ^b	pt 5 ^c	pt 6 ^d	pt 7 ^d	pt 8 ^d	pt 9 ^e	pt 10 ^f	pt 11 ^f
Anemia	2 months	First months	7 years	31 years	1 years	2 months	23 years	<1 years	1–2 months	2 months	1 month
Transfusion	Yes (2 months-n.a.)	Yes (onset-n.a.)	No (parents' refusal)	No	Yes (11.5 years)	Yes (2 months-n.a.)	No	Yes (onset-1 year)	Yes (2 months)	Yes (2–12 months)	Yes (1 month-currently)
Myopathy	Yes (7 years)	Yes	Yes (n.a.)	Yes	Yes	n.a.	Yes (mild 23 years)	Yes (6 years)	? (axial hypotonia)	No	No
Lactic acidosis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hypertrophic cardiomyopathy	Yes (3 months)	n.a.	n.a.	n.a.	Yes (11.5 years)	Yes (2 months)	No	n.a.	Yes	No	No
Outcome	Deceased (18 years, respiratory failure)	Last observation 16 years	Last observation 24 years	Last observation 34 years	Last observation 16 years	Deceased (3 months, cardiomyopathy)	Last observation 28 years	Last observation 6 years	Deceased (3 months, cardiopulmonary arrest)	Last observation 6 years, good clinical evolution	Last observation 13 months, periodic transfusion
Muscle RC	Reduced I–IV	Reduced I–III–IV	Reduced I–IV	Reduced I–III–IV	n.a.	n.a.	n.a.	n.a.	n.a.	Reduced I–III–IV	n.a.
Fibroblast RC	Normal	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Reduced I–III–IV–V	n.a.	Normal	n.a.
<i>YARS2</i> mutations	c.156C>G p.Phe52Leu	c.156C>G p.Phe52Leu	c.156C>G p.Phe52Leu	c.137G>A p.Gly46Asp	c.156C>G p.Phe52Leu	c.156C>G p.Phe52Leu	c.156C>G p.Phe52Leu	c.572G>A p.Gly191Asp c.1078C>T	c.1303A>G p.Ser435Gly	c.933C>G p.Asp311Glu	c.933C>G p.Asp311Glu
Mutations location	Catalytic domain	Catalytic domain	Catalytic domain	Catalytic domain	Catalytic domain	Catalytic domain	Catalytic domain	Catalytic domain	S4-like domain	αACB domain	αACB domain

^a Riley et al. (2010), ^b Sasarman et al. (2012), ^c Shahmi et al. (2013), ^d Riley et al. (2013), ^e Nakajima et al. (2014), ^f this report; n.a. not achieved

by a rapidly progressive anemia and recurrent metabolic decompensation leading to fatal outcome at 3 months of age. Disease onset was in the first months in our patients, who became transfusion-dependent, similar to the six previously reported patients whose disease onset was during the first year. An additional patient was characterized by later onset, at 7 years of age, and her parents refused transfusion. She was treated with multivitamin cocktails and her hemoglobin maintained between 8.0 and 11.0 g/dl (Riley et al. 2010). Finally, two patients presented an adult onset and did not require transfusion. These observations suggest that early onset of anemia is a prognostic factor for transfusion dependence.

In contrast with most of the patients previously reported in the literature, our children had no clinical evidence of muscle involvement, although the muscle biopsy in patient 1 showed mild myopathic features. Measurement of the respiratory chain complexes confirmed a combined defect of CI-III-IV in muscle of patient 1 but not in his fibroblasts. The absence of clinical involvement of the CNS of MLASA patients is confirmed also in our cases: the neurological examination has been normal and the brain MRI findings in patient 1 can be considered as unspecific. Likewise, a brain MRI in a previously reported child showed a thin corpus callosum which was not associated with any clinical sign of neurological impairment (Nakajima et al. 2014).

Lactic acidosis is confirmed in our patients, and it is a constant feature in MLASA2. Interestingly, in our patient 1, the recovery of anemia coincided with normalization of lactic acidemia.

Hypertrophic cardiomyopathy was observed in four patients reported in the literature but was absent in our siblings.

In summary, we report a new mutation in *YARS2* gene and confirm that MLASA must be considered in patients with congenital sideroblastic anemia: early diagnosis and supportive therapy may be important to prevent severe complications.

Anemia and lactic acidosis are constant features, CNS is spared, and myopathy is a variable finding probably appearing later in life. The severity of the clinical course is quite variable, considering the small number of patients and the prevalence of the same mutations in the original Lebanese patients (Riley et al. 2010, 2013; Sasarman et al. 2012; Shahni et al. 2013). The prognosis is relatively good in most of the cases but somehow unpredictable; two of them had a fatal course in the first year of life (Riley et al. 2013; Nakajima et al. 2014), and one with a severe form of myopathy and cardiomyopathy died suddenly because of respiratory failure at the age of 18 years (Riley et al. 2010).

The only patient with a mutation in the S4-like domain in the anticodon-binding region (Nakajima et al. 2014) had a very poor clinical outcome, suggesting a possible

correlation between the severity of the phenotype and specific mutations, since all the other previously described patients carried mutations in the catalytic domain. Our siblings also harbor a mutation involving the anticodon-binding region, but in the α ACB domain, their clinical phenotype is mild. Thus, definite conclusions about genotype-phenotype correlation are difficult to draw due to the exiguity of the cohort of patients so far reported in the literature. Increased awareness of the existence of this rare disorder will hopefully lead to the identification of additional cases, which will help to achieve a better definition of both clinical spectrum and prognostic clues.

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Take-Home Message

MLASA must be considered in patients with congenital sideroblastic anemia: early diagnosis and supportive therapy may be important to prevent severe complications.

Compliance with Ethic Guidelines

Conflict of Interest statements

Anna Ardisson, Eleonora Lamantea, Jade Quartararo, Cristina Dallabona, Franco Carrara, Isabella Moroni, Claudia Donnini, Barbara Garavaglia, Massimo Zeviani, and Graziella Uziel declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Animal Rights

This article does not contain any studies with animal subjects performed by the any of the authors.

Contributions of Individual Authors

AA, IM, and GU evaluated the patients and wrote the case report. FC performed biochemical and molecular analyses.

EL monitored biochemical and genetic analyses. JQ and CDa performed experiments in yeast model. CDo coordinated the yeast studies. AA and EL wrote the manuscript; MZ, BG, and GU critically revised the manuscript for important intellectual content. All authors read and approved the manuscript.

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