A Distinct Mitochondrial Myopathy, Lactic Acidosis and Sideroblastic Anemia (MLASA) Phenotype Associates with YARS2 Mutations

Rojeen Shahni, Yehani Wedatilake, Maureen A. Cleary, Keith J. Lindley, Keith R. Sibson, and Shamima Rahman

1Mitochondrial Research Group, Clinical and Molecular Genetics Unit, UCL Institute of Child Health, London, UK
2Metabolic Unit, Great Ormond Street Hospital, London, UK
3Gastroenterology Unit, Great Ormond Street Hospital, London, UK
4Haematology Unit, Great Ormond Street Hospital, London, UK

Manuscript Received: 19 March 2013; Manuscript Accepted: 2 May 2013

Nuclear-encoded disorders of mitochondrial translation are clinically and genetically heterogeneous. Genetic causes include defects of mitochondrial aminoacyl-tRNA synthetases, and factors required for initiation, elongation and termination of protein synthesis as well as ribosome recycling. We report on a new case of myopathy, lactic acidosis and sideroblastic anemia (MLASA) syndrome caused by defective mitochondrial tyrosyl aminoacylation. The patient presented at 1 year with anemia initially attributed to iron deficiency. Bone marrow aspirate at 5 years revealed ringed sideroblasts but transfusion dependency did not occur until 11 years. Other clinical features included lactic acidosis, poor weight gain, hypertrophic cardiomyopathy and severe myopathy leading to respiratory failure necessitating ventilatory support. Long-range PCR excluded mitochondrial DNA rearrangements. Clinical diagnosis of MLASA prompted direct sequence analysis of the YARS2 gene encoding the mitochondrial tyrosyl-tRNA synthetase, which revealed homozygosity for a known pathogenic mutation, c.156C>G; p.F52L. Comparison with four previously reported cases demonstrated remarkable clinical homogeneity. First line investigation of MLASA should include direct sequence analysis of YARS2 and PUS1 (encoding a tRNA modification factor) rather than muscle biopsy. Early genetic diagnosis is essential for counseling and to facilitate appropriate supportive therapy. Reasons for segregation of specific clinical phenotypes with particular mitochondrial aminoacyl tRNA-synthetase defects remain unknown.

How to Cite this Article:
Shahni R, Wedatilake Y, Cleary MA, Lindley KJ, Sibson KR, Rahman S. 2013. A distinct mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA) phenotype associates with YARS2 mutations.
Am J Med Genet Part A 161A:2334–2338.

INTRODUCTION
The congenital sideroblastic anemias are rare inborn errors of metabolism caused by defects of mitochondrial energy production, heme biosynthesis and iron–sulfur cluster biogenesis [Bergmann et al., 2010; Fleming, 2011]. The most commonly recognized congenital sideroblastic anemias are X-linked sideroblastic anemia (XLSA) secondary to mutations in ALAS2 encoding delta amino levulinic acid synthetase, and the Pearson marrow-pancreas syndrome caused by sporadically arising large-scale rearrangements of the mitochondrial genome. Mitochondrial DNA (mtDNA) encodes 13 polypeptide subunits of the oxidative phosphorylation
(OXPHOS) system, together with 22 transfer RNA (tRNA) and two ribosomal RNA (rRNA) molecules required for intramitochondrial synthesis of these 13 polypeptides. The mtDNA rearrangements in Pearson syndrome typically delete several tRNA genes, leading to global impairment of mitochondrial translation [Rotig et al., 1995]. More recently, nuclear-encoded disorders of mitochondrial translation are emerging including two gene defects (PUS1 and YARS2 mutations) that appear to specifically associate with a syndrome of myopathy, lactic acidosis and sideroblastic anemia (MLASA) [Bykhovskaya et al., 2004; Riley et al., 2010; Rotig, 2011].

XLSA and Pearson syndrome can be easily distinguished clinically since the former causes a pure sideroblastic anemia phenotype (which may be late-onset and not require regular transfusions), whilst the latter causes an infantile-onset transfusion-dependent sideroblastic anemia associated with lactic acidosis and multisystem disturbances. Clinical manifestations of Pearson syndrome include exocrine and endocrine pancreatic insufficiency, renal tubulopathy and faltering growth [Rotig et al., 1995]. Death in infancy or early childhood frequently follows overwhelming lactic acidosis or acute liver failure [Rotig et al., 1995]. Survivors develop a progressive neurological disorder including ophthalmoplegia, cardiac conduction defects and cerebellar ataxia, within the Kearns–Sayre syndrome spectrum [McShane et al., 1991; Rahman and Leonard, 2000].

YARS2 mutations have previously been reported in four patients from three apparently unrelated families [Riley et al., 2010; Sasarman et al., 2012]. Here we describe another case of MLASA caused by YARS2 mutation and show that this is a clinically distinct phenotype that can be diagnosed without recourse to muscle biopsy, the “gold-standard” method for diagnosing mitochondrial disease.

PATIENT AND METHODS

The patient, the second child of healthy unrelated Lebanese parents, was born at term after an uncomplicated pregnancy. Two siblings aged 7 and 16 years are healthy. Aged 1 year, following an episode of pallor and jaundice, the patient was diagnosed with iron deficiency anemia and was prescribed iron supplements. At 5 years, the family migrated to the UK and he was diagnosed with congenital sideroblastic anemia following a bone marrow aspirate (Fig. 1a). Pyridoxine was commenced and his hemoglobin remained stable (9–11 g/dl) until 11.5 years. At 10 years he presented with abdominal pain, poor weight gain and lethargy. Upper gastrointestinal (GI) endoscopy revealed reflux esophagitis and patchy duodenal erythema with mild focal lymphocyte infiltration. Poor growth and intermittent vomiting persisted despite serial interventions including gluten-free diet and nasogastric, gastrostomy and (eventually) parenteral feeding. Repeat upper GI endoscopy and video capsule endoscopy revealed multiple ileal ulcers, and an atrophic duodenal bulb, with absent villi and duodenal ulcers. A trial of azathioprine and prednisolone was commenced for presumed inflammatory bowel disease.

At 11.5 years he developed transfusion-dependent anemia; repeat bone marrow aspiration and trephine biopsy appearances were unchanged. The worsening anemia was initially attributed to chronic low-grade gastrointestinal bleeding. He also required twice-weekly granulocyte colony stimulating factor infusions for neutropenia. Nine months later he developed shortness of breath and was found to have a pericardial effusion, necessitating pericardiocentesis. Transthoracic echocardiogram also showed severe biventricular hypertrophy with preserved biventricular systolic function. Due to recurrent episodes of dizziness he underwent 24 hr Holter monitoring which showed sinus rhythm.

At 12.5 years he became increasingly lethargic, with declining weight despite parenteral nutrition and regular transfusions every 2–3 weeks. Examination showed myopathic facies, bilateral ptosis, palor and cachexia, (weight 23.6 kg, 0.4th centile and height 139.9 cm, 2nd centile), with generalized muscle weakness. Electroencephalography demonstrated severely myopathic changes. Sleep study revealed severe hypoventilation with evidence of CO2 retention and he was commenced on nocturnal BiPAP ventilation. Blood investigations showed anemia (Hb 6.2–8.4 g/dl), neutropenia (neutrophils 0.26 × 109/L), elevated lactate and respiratory acidosis (venous blood gas pH 7.3, pCO2 9.9 kPa, HCO3 37 mmol/L). Plasma amino acids demonstrated raised alanine (1113 μmol/L, reference range 150–450) and low arginine (28 μmol/L, reference range 40–120). Acylcarnitine analysis revealed low free carnitine levels. Urine organic acid analysis demonstrated strongly raised lactate and pyruvate with moderately raised 2-hydroxybutyrate and mildly raised 2-hydroxyisovalerate, secondary to disturbed lactate metabolism. Stool elastase was normal (>500 μg/g, normal >200).

All genetic studies were performed with informed parental consent and ethical approval for the study was obtained from the National Research Ethics Committee London Bloomsbury, UK. Long-range PCR of mitochondrial DNA was used to screen for large-scale mtDNA rearrangements. Because of a clinical suspicion of MLASA syndrome, and the Lebanese origin of the patient, the YARS2 gene was sequenced in the patient and both parents. PCR amplification was performed using primers and conditions as previously reported, and PCR products were directly sequenced on an ABI PRISM 3730 automated sequencer (Source Biosciences) [Riley et al., 2010]. Sequence comparisons and analysis were performed using the Sequencher program, V.4.9.

RESULTS

Long range PCR of genomic DNA extracted from peripheral blood leucocytes did not detect any mtDNA rearrangements, excluding Pearson syndrome. On the basis of phenotype similarities between our patient and previously reported patients we sequenced the YARS2 gene, which encodes the human mitochondrial tyrosyl-tRNA synthetase, and identified a homozygous mutation, c.156C > G/p.F52L (Fig. 1b), which affects a highly conserved amino acid residue and has previously been reported to be pathogenic [Riley et al., 2010].

DISCUSSION

The combination of sideroblastic anemia and lactic acidosis is strongly suspicious of a mitochondrial disorder. In infantile-onset sideroblastic anemia with lactic acidosis, the most frequent cause is Pearson marrow-pancreas syndrome associated with single large-scale mtDNA rearrangements. Several lines of evidence argued...
against Pearson syndrome in our patient: continuing transfusion dependency at 13 years (in Pearson syndrome transfusion dependency typically resolves by the age of 2 years, as mutant mtDNA is progressively cleared from rapidly dividing hemopoietic cells), absence of pancreatic insufficiency (normal stool elastase levels), lack of neurological symptoms other than myopathy by teenage years (in particular, no evidence of cerebellar ataxia), and absence of renal tubulopathy, pigmentary retinopathy and sensorineural hearing loss (which are all typical of Pearson and Kearns–Sayre syndromes) [Pitceathly et al., 2012]. The bone marrow appearances were also atypical for Pearson syndrome, in which there is usually vacuolization of early erythroid and myeloid progenitors in addition to the presence of ringed sideroblasts, whereas in our patient no significant vacuolization was seen. Finally, Pearson syndrome was definitively excluded in our patient by the absence of mtDNA rearrangements in leukocyte DNA.

A clinically more fitting diagnosis in our patient was MLASA, that is, mitochondrial myopathy, lactic acidosis and sideroblastic anemia syndrome. This was confirmed by YARS2 analysis, which revealed homozygosity for the known pathogenic mutation c.156C>G;p.F52L. Parents were both heterozygous for the mutation, although they are not known to be related, lending weight to the hypothesis that this is a Lebanese founder mutation [Riley et al., 2010]. Two different homozygous mutations have been reported in YARS2, c.156C>G;p.F52L (as in our patient) and c.137G>A;p.G46D, in three apparently unrelated families of Lebanese descent. Our patient is the fifth with YARS2 mutations reported to date. All five patients had MLASA.

A characteristic clinical phenotype of YARS2-related MLASA syndrome appears to be emerging (Table I). Gastro-intestinal disturbance and feeding difficulties appear to occur frequently in MLASA, whereas exocrine pancreatic insufficiency (a cardinal feature of Pearson syndrome) has never been reported. Respiratory failure requiring ventilatory support and cardiomyopathy were present in two cases including ours. In Pearson and Kearns–Sayre syndromes cardiomyopathy may occur, but the most significant cardiac problem is usually heart block, which has not been reported in MLASA. Compared to Pearson syndrome, hearing and vision appear to be relatively spared in YARS2-associated MLASA. One case had asymmetric ptosis and limitation of lateral gaze bilaterally in his thirties [Sasarman et al., 2002], while no hearing loss was reported in any of the five cases.

There appears to be a common pathogenic mechanism in Pearson syndrome and MLASA, namely global impairment of mitochondrial translation, manifest histologically by a ragged-red fiber myopathy with subsarcolemmal mitochondrial proliferation. In Pearson syndrome multiple mitochondrial tRNA genes are deleted, whilst in MLASA caused by PUS1 mutations there is a defect in post-transcriptional modification (pseudouridylation) of multiple mitochondrial and cytosolic tRNAs [Patton et al., 2005]. YARS2 mutations lead to failure of aminoacylation of the mitochondrial tRNA tyrosine. Interestingly the common 4,977 bp mtDNA deletion causing Pearson syndrome does not include the tRNA for tyrosine [Rotig et al., 1995]. Only two mutations have previously been reported in the MT-TY gene encoding the mitochondrial tRNA tyrosine molecule, and neither of these was
associated sideroblastic anemia, providing further evidence that impaired translation of tyrosine residues is not the cause of sideroblastic anemia [Pulkes et al., 2000; Scaglia et al., 2003]. However, the two patients did have some clinical features in common with our patient with YARS2 mutations, namely myopathy and lactic acidosis in the first case and cardiomyopathy in the second, arguing for a role for impaired tyrosine aminoacylation in causation of some of the features of MLASA [Pulkes et al., 2000; Scaglia et al., 2003]. The mechanistic link between YARS2 and PUS1 mutations is also unclear. PUS1 has been shown to modify bases 27 and/or 28 of several mitochondrial tRNA species [Suzuki et al., 2011], but the pseudouridylation status of mitochondrial tRNA tyrosine has not yet been reported.

| Reference | Riley et al. [2010] | Sasarman et al. [2012] a | This report |
|-----------|---------------------|------------------------|-------------|
| Patient   | P1                  | P2                     | P3          |
| Sex       | M                   | F                      | F           |
| Age at onset | 10 weeks          | Infancy                | 7y          |
| Age at death | 18y                | Alive at 16y           | Alive at 24y|
| Consanguinity | Yes               | Yes                    | Yes         |
| Family history | Sibling [P2]     | Sibling [P1]           | Maternal aunt died of SA at 26y b |
| Ethnicity | Lebanese            | Lebanese               | Lebanese    |
| Sideroblastic anemia | Yes              | Yes                    | Yes         |
| Transfusion-dependent | Yes—initially 2–3 monthly, later every 3–4 weeks | Yes   | Yes         |
| Faltering growth | Yes               | —                      | —           |
| Dysphagia/feeding difficulties | Yes—eventually permanent vocal cord paresis | —       | —           |
| Hypertrophic cardiomyopathy | Yes               | —                      | —           |
| Exercise intolerance | Yes               | Yes                    | Yes         |
| Respiratory failure | 17y               | —                      | No          |
| Blood lactate [mmol/L] | 3–13.7          | 2.5–8.4                 | 4.1         |
| Muscle histology | "Incipient, reduced COX staining" | Lipid vacuoles | Severe reduced COX staining and increased SDH; EMMsubsarcolemmal aggregation of peripheral mitochondria, many containing crystalline inclusions |
| Muscle respiratory chain enzyme activities (% of control) | CI                | CI                     | CI (<6%) |
| YARS2 mutations | Homozygous         | Homozygous             | Homozygous  |
|               | c.156C > G.p.52L   | c.156C > G.p.52L       | c.156C > G.p.52L |
|               | c.156C > G.p.52L   | c.156C > G.p.52L       | c.156C > G.p.52L |
| Key: CI, complex I; CII + III, complexes II + III; CIII, complex III; CIV, complex IV; COX, cytochrome c oxidase; ND, not determined; RRF, ragged red fibres; —, information not provided in original report. aClinical features of P4 initially reported in Sasarman et al. [2002]. bThese individuals have not been tested for YARS2 mutations. |
roblastic anemia [Rotig, 2011]. The reasons for the tissue specificity associated with \textit{YARS2} and \textit{PUS1} mutations are currently not understood, but it is possible that the \textit{YARS2} protein has a second function necessary for hematopoiesis that may be unrelated to its role in mitochondrial protein synthesis. Moonlighting functions are recognised for other mitochondrial proteins, for example one subunit of respiratory chain complex I is a tumour suppressor and another is involved in caspase-independent cell death, whilst various complex I assembly factors have been implicated in immune pathways and to enhance tumour growth in some cancers [Rahman and Thorburn, 2013].

In conclusion, patients with congenital sideroblastic anemia should be assessed for features of MLASA and consideration should be given to screening the \textit{YARS2} gene in patients with lactic acidosis and sideroblastic anemia persisting beyond early childhood, particularly if mtDNA deletions have been excluded. Although no specific curative therapies are available, early diagnosis and initiation of appropriate supportive therapy is likely to be important in the prevention of the longterm complications of this disorder. Direct targeting of the \textit{YARS2} and \textit{PUS1} genes in MLASA patients can avoid invasive muscle biopsy for diagnosis of this subgroup of inborn errors of mitochondrial metabolism.

**ACKNOWLEDGMENTS**

S.R. is supported by Great Ormond Street Hospital Children’s Charity and Y.W. by a Wellcome Trust Research Training Fellowship. We gratefully acknowledge funding from the Muscular Dystrophy Campaign.

**REFERENCES**

Bergmann AK, Campagna DR, McLoughlin EM, Agarwal S, Fleming MD, Bottomley SS, Neufeld EJ. 2010. Systematic molecular genetic analysis of congenital sideroblastic anemia: evidence for genetic heterogeneity and identification of novel mutations. Pediatr Blood Cancer 54:273–278.

Bykhovskaya Y, Casas K, Mengesha E, Inbal A, Fischel-Ghodsian N. 2004. Missense mutation in pseudouridine synthase 1 (PUS1) causes mitochondrial myopathy and sideroblastic anemia (MLASA). Am J Hum Genet 74:1303–1308.

Fleming MD. 2011. Congenital sideroblastic anemias: Iron and heme lost in mitochondrial translation. Hematology Am Soc Hematol Educ Program 2011:525–531.

Konovalova S, Tyynismaa H. 2013. Mitochondrial aminoacyl-tRNA synthetases in human disease. Mol Genet Metab 108:206–211.

McShane MA, Hammans SR, Sweeney M, Holt JJ, Beattie TJ, Brett EM, Harding AE. 1991. Pearson syndrome and mitochondrial encephalomyopathy in a patient with a deletion of mtDNA. Am J Hum Genet 48:39–42.

Patton JR, Bykhovskaya Y, Mengesha E, Bertolotto C, Fischel-Ghodsian N. 2005. Mitochondrial myopathy and sideroblastic anemia (MLASA): missense mutation in the pseudouridine synthase 1 (PUS1) gene is associated with the loss of tRNA pseudouridylation. J Biol Chem 280:19823–19828.

Pitceathly RD, Rahman S, Hanna MG. 2012. Single deletions in mitochondrial DNA–molecular mechanisms and disease phenotypes in clinical practice. Neuromuscul Disord 22:577–586.

Pulkos T, Siddiqui A, Morgan-Hughes JA, Hanna MG. 2000. A novel mutation in the mitochondrial tRNA(Tyr) gene associated with exercise intolerance. Neurology 55:1210–1212.

Rahman S, Leonard JV. 2000. Early onset of complete heart block in Pearson syndrome. J Inherit Metab Dis 23:753–754.

Rahman S, Thorburn DR. 2013. 189th ENMC International workshop Complex I deficiency: Diagnosis and treatment 20–22 April 2012, Naarden, The Netherlands. Neuromuscul Disord 23:506–515.

Riley LG, Cooper S, Hickey P, Rudinger-Thirion J, Mckenzie M, Compton A, Lim SC, Thorburn D, Ryan MT, Giege R, Bahlo M, Christodoulou J. 2010. Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia–MLASA syndrome. Am J Hum Genet 87:52–59.

Rotig A. 2011. Human diseases with impaired mitochondrial protein synthesis. Biochim Biophys Acta 1807:1198–1205.

Rotig A, Bourgeron T, Chretien D, Rustin P, Munnich A. 1995. Spectrum of mitochondrial DNA rearrangements in the Pearson marrow-pancreas syndrome. Hum Mol Genet 4:1327–1330.

Sarasman F, Karpati G, Shoubridge EA. 2002. Nuclear genetic control of mitochondrial translation in skeletal muscle revealed in patients with mitochondrial myopathy. Hum Mol Genet 11:1669–1681.

Sarasman F, Nishimura T, Thiffault I, Shoubridge EA. 2012. A novel mutation in \textit{YARS2} causes myopathy with lactic acidosis and sideroblastic anemia. Hum Mutat 33:1201–1206.

Scaglia F, Vogel H, Hawkins EP, Vladutiu GD, Liu LL, Wong L J. 2003. Novel homoplasmic mutation in the mitochondrial tRNA(Tyr) gene associated with atypical mitochondrial cytopathy presenting with focal segmental glomerulosclerosis. Am J Med Genet Part A 123A:172–178.

Suzuki T, Nagao A, Suzuki T. 2011. Human mitochondrial tRNAs: Biogenesis, function, structural aspects, and diseases. Annu Rev Genet 45:299–329.