Sideroblastic anemia with myopathy secondary to novel, pathogenic missense variants in the *YARS2* gene

Sideroblastic anemias (SA), both hereditary and acquired, are characterized by ring sideroblasts (RS), which are bone marrow erythroid precursors (erythroblasts) with iron loaded mitochondria visualized as a perinuclear ring by Perl's stain. Acquired SA, myelodysplastic syndrome with RS (MDS-RS), is characterized by recurrent somatic mutations in spliceosomal complex gene *SF3B1* and usually portends a good prognosis.[1] It is also important to exclude toxin (lead) or ethanol as reversible causes of SA.[2]

Mutations in several genes have been associated with inherited SA, primarily in genes involved in synthesis and transport of mitochondrial proteins, the production of heme and iron-sulphur cluster biosynthesis. Pathogenic variants in *ABCB7, ALAS2, GLRX5, YARS2, PUS1, SLC25A38, TRNT1* and *SLC19A2* as well as sporadic, large-scale single mitochondrial DNA (mtDNA) deletion have all been associated with SA;[3] however, the phenotype is variable with overlapping clinical presentations, including syndromic diseases and variable responsiveness to pyridoxine.

The *YARS2* (12p11.21) gene encodes the mitochondrial tyrosyl tRNA synthetase, a key enzyme in mitochondrial protein synthesis. Pathogenic mutations in the *YARS2* gene causes a clinical triad of Myopathy, Lactic Acidosis and Sideroblastic Anemia (MLASA, OMIM 613561).[4-6] Patients manifest multiple mitochondrial respiratory chain defects in affected tissues such as skeletal muscle, often demonstrated by the severe loss of cytochrome c oxidase (COX) activity. *YARS2*-related mitochondrial disease is inherited in an autosomal recessive manner.[7,8,9]

Our patient is 54-year-old female born to non-consanguineous Caucasian parents. She describes marked lethargy from childhood, fatigued easily during participation in recreational sports, and was first found to be anemic at the age of 10. She was diagnosed with SA aged 17 years when she collapsed with a haemoglobin of 70g/L. She did not respond to high dose pyridoxine, folic acid, Danazol and oxymetholone. She needed intermittent transfusion support during all of her 3 pregnancies, but has been on a regular transfusion program for the last 10 years. She was recently referred to our center in view of her SA, iron overload, hepatomegaly and portal hypertension. She had macrocytic anemia (Haemoglobin 73 g/l, MCV 120 fl) with high ferritin, 4219 ug/l (normal range 20-200ug/L), marginally elevated LDH, and normal bilirubin. Bone marrow demonstrated 45% ring sideroblasts, reversed myeloid: erythroid ratio, with normal metaphase cytogenetics and SNP-A karyotype (*Online Supplementary Figure S1A*). Sanger sequencing of the *SF3B1* gene did not reveal any pathogenic variants and a 33-gene panel did not detect any acquired mutations associated with myeloid malignancies. She did not respond to exogenous erythropoietin or lenalidomide, and was intolerant to various iron chelators. Her symptoms of extreme fatigue, poor exercise tolerance (less than 100 yards), and bone pain gradually worsened. She also developed gastrointestinal symptoms with episodic vomiting, abdominal bloating, urge incontinence and increased frequency of stools. Although she had two recent periods (12 months and 8 months) of remaining free of transfusions, she remained extremely symptomatic with lethargy. Examination showed evidence of proximal muscle weakness (Medical Research Council grade 4/5) post exercise, brisk tendon reflexes, and flexor plantar reflexes. An exercise field test with concomitant lactate testing was performed.

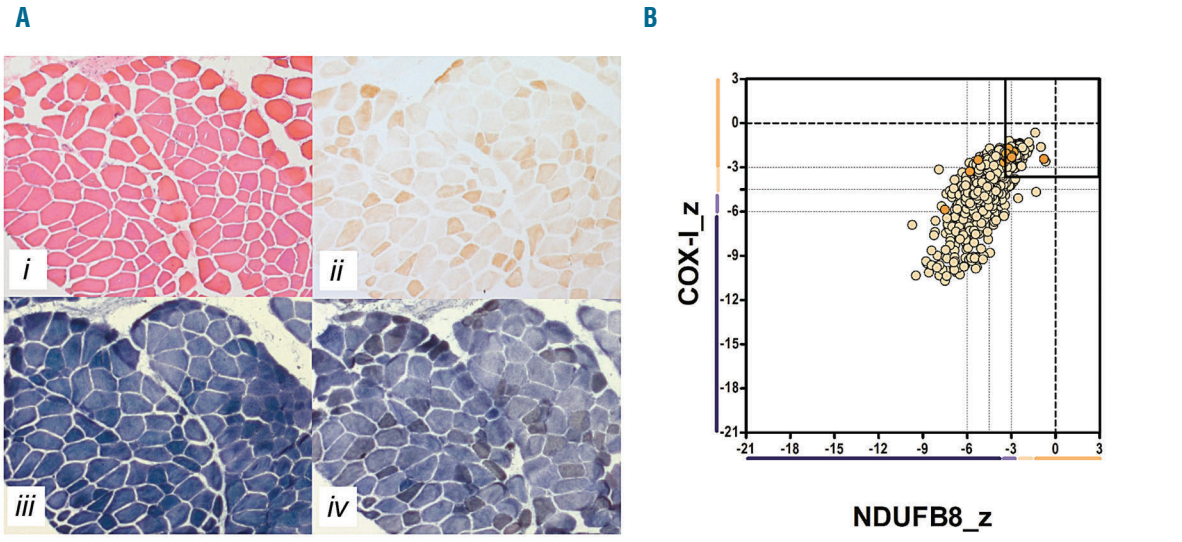

Figure 1. Histopathological and biochemical characterization of the patient's muscle biopsy. (A) Sequential H&E (i), cytochrome c oxidase (COX) (ii), succinate dehydrogenase (SDH) (iii) and COX-SDH histochemistry (iv) revealed global COX deficiency across the biopsy section with strong SDH activity. (B) Quadruple OXPHOS immunofluorescence analysis showed the presence of individual muscle fibers lacking both complex I (NDUFB8) and complex IV (COX-1) expression, confirming multiple respiratory chain defects in keeping with a generalized disorder of mitochondrial translation. The respiratory chain profile illustrated demonstrates complex I, complex IV and porin levels in patient muscle fibers, with each dot representing an individual muscle fiber colour-coded according to its mitochondrial mass (very low: blue, low: light blue, normal: light orange, high: orange and very high: red). Thin black dashed lines indicate the SD limits for the classification of fibers, lines next to the x and y axes indicate the levels of NDUFB8 and COX-I respectively (beige: normal, light beige: intermediate(+), light purple: intermediate(-) and dark purple: negative), bold dashed lines indicate the mean expression level of normal fibers.[14-16]

There was no evidence of lactic acidosis (plasma lactate 1.2mmol/L and arterial blood lactate 1.4mmol/L). Her exercise tolerance was significantly reduced (performed less than 40 seconds of exercise) and was unable to generate enough activity to precipitate a rise in lactate and stopped due to leg pain. Cardiac MRI revealed mild LV dysfunction with no evidence of hypertrophic cardiomyopathy. Vital capacity, flow volume loops, and forced expiratory volume were normal, but nocturnal hypoxemia was demonstrated. Comprehensive gastrointestinal workup including endoscopy, capsule enteroscopy, CT colonoscopy and MRI did not reveal any abnormality.

Next-generation sequencing of 10 genes associated with SA identified two heterozygous variants in the *YARS2* (NM_001040436) gene; c.365C>G; p.Pro122Arg and c.623T>G; p.Leu208Arg. Both variants affect highly conserved amino acids, are of low population frequency (absent from the ExAC database[10]), and had not been previously reported in SA patients. The NGS reads confirmed that the variants were *in trans* and were most likely inherited from unaffected parents. Cascade testing of other family members is being undertaken although parental samples are not available.

A skeletal muscle biopsy was subjected to histopathological investigations, including assessment of oxidative enzymes and OXPHOS function immunohistochemically. The patient's muscle biopsy was morphologically normal (Figure 2A). Oxidative enzyme histochemistry showed intense SDH activity and a generalized decrease in COX activity across the biopsy, confirmed by the sequential COX-SDH histochemical reaction (Figure 1A). Quadruple OXPHOS immunofluorescence revealed many fibers lacking both complex I (NDUFB8) and complex IV (COX-1) expression, confirming a multiple respiratory chain defect (Figure 1B).

We used the budding yeast, *Saccharomyces cerevisiae*, to assess the pathogenicity of the novel variants thanks to the presence of the *YARS2* orthologous gene named *MSY1*. The human residues Pro122 and Leu208 are conserved from yeast to human and correspond respectively to the yeast residues Pro134 and Leu226 (Figure 2A). The yeast mutant alleles msy1P134R and msy1L226R, corresponding to the human variants, were cloned into the pFL39 vector and expressed in the *msy1*Δ null mutant, analyzing oxidative growth and respiratory activity. Oxidative growth of the strain expressing $msy1^{P134R}$ was completely abolished similarly to the strain lacking the gene, whereas the $msy1^{L226R}$ variant led to a mild growth reduction on oxidative carbon sources (Figure 2B). In agreement with the oxidative growth defect, the strain expressing $msy1^{P134R}$ was unable to consume oxygen like the null mutant. Expression of the $msy1^{L226R}$ allele induces about 40% reduction of the respiratory rate compared to the wild-type strain (Figure 2C), confirming pathogenicity of both mutant alleles.

All reported cases of MLASA2 syndrome due to pathogenic *YARS2* variants have presented with neurological symptoms with subtle and variable levels of anemia.[7]

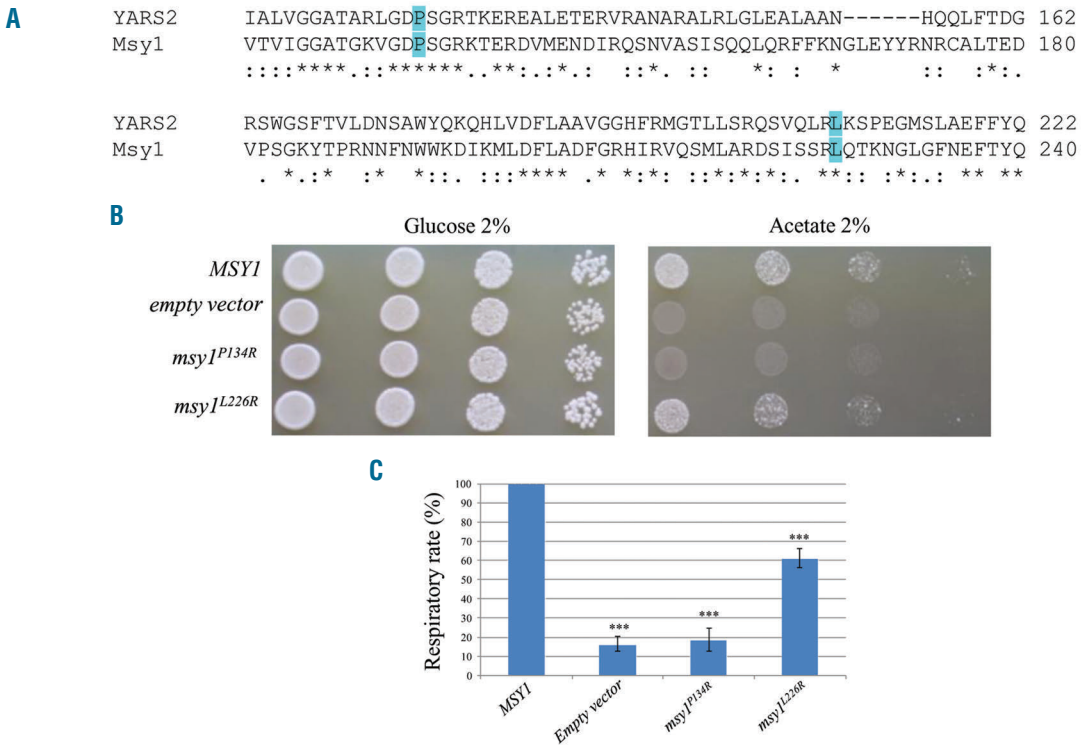

Figure 2. Modelling the novel *YARS2* mutations in *Saccharomyces cerevisiae*. (A) Protein sequence alignment (Clustal Omega) of the human *YARS2* and the yeast Msy1 proteins. The residues examined in this study are conserved between species and are highlighted in blue. (B) Oxidative growth experiments: msy1Δ strains harbouring the *MSY1* wild-type allele, the mutant alleles or the empty vector were serially diluted and spotted on SC agar plates (without tryptophan) supplemented with 2% glucose or 2% acetate. Plates were incubated at 28°C. (C) Oxygen consumption rates: cells were grown at 28°C in SC medium without tryptophan supplemented with 0.6% glucose. Values were normalized to the *MSY1* wild type strain and represented as the mean of at least three values ± SD. Statistical analysis was performed by paired, two-tail Student's *t*-test: ***P<0.001.

Contrarily, our case presented with severe anemia and progressive lethargy, which was incorrectly attributed to the underlying anemia. The detection of significant myopathy with global loss of COX activity and lack of improvement of fatigue following transfusion or during periods of transfusion independency indicates an MLASA-like phenotype. The early onset myopathy which was unrecognized during adolescence was unmasked by pregnancy and advancing age.

Episodic vomiting and urge incontinence could be related to mitochondrial myopathy, but has only been described in one previous case.[7] Our patient is also the second oldest surviving patient. Spontaneous recovery of anemia and fluctuating/intermittent need for transfusions, with unmasking of transfusion requirements during all 3 pregnancies, remain largely unexplained. The variable penetrance and clinical heterogeneity of MLASA syndromes is probably due to multiple factors, including the type of variant and its effect on expression, the *YARS2* genotype, mitochondrial DNA haplotype,[11] and other contributing genetic loci.

The Phase 2 data using TGFβ ligand traps as pharmacological agents and improving erythropoiesis in patients with acquired SA (MDS) is promising.[12] It is tempting to speculate that such agents, Luspatercept and Sotatarcept, could potentially be effective in inherited SA, improving haemopoiesis and possibly improving muscle strength by trapping other TGFβ ligands like myostatin and BMP-11.[13]

Our data highlight the importance of re-evaluating young patients with SA for the presence of rare causes of inherited anaemia, especially in the presence of myopathy. This brings to the fore the utility of unbiased genomic screening tools for evaluating rare anemias and inherited haematological diseases and underpins the need for functional studies to prove pathogenicity of VUS.

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