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

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Supplementary Data:

Materials and Methods

Patient

Examination showed no ptosis or eye movement abnormality. Findings from fundal examination were normal. She had no pyramidal signs or extrapyramidal features. She had 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. Her exercise tolerance was so reduced (performed less than 40 seconds of exercise) she was unable to generate enough activity to precipitate a rise in lactate (or sufficient increase in heart rate) and stopped due to leg pain.

Genetic investigation

Germline DNA was extracted from whole blood in EDTA using the Qiasymphony DSP1000 kit according to the manufacturer's instructions (Qiagen, Germany). Next generation sequencing was carried out using the Agilent XT library preparation kit according to the manufacturer's instructions, shearing DNA into 500bp fragments (Agilent Technologies, USA). In solution bait capture with a custom designed capture library targeting ~200 genes associated with inherited red blood cell disorders (supplementary table 1 for gene list). The library was sequenced on an Illumina MiSeq with paired-end 300bp reads according to the manufacturer's instructions (Illumina, USA). Sequencing data was analysed using a bespoke bioinformatics pipeline.[1] Common genetic variants (population frequency ≥10%) were filtered out of the analysis, sequence variants in genes associated with sideroblastic anemia (*ABCB6, ABCB7, ALAS1, ALAS2, GLRX5, PUS1, SF3B1, SLC19A2, SLC25A38* and *YARS2)* were classified for pathogenicity according to the Association for Clinical Genomic Science (ACGS) best practice guidelines (http://www.acgs.uk.com/quality/best-practice-guidelines/). Variants

were confirmed by M13 tagged Sanger DNA sequencing. (supplementary table 2 for oligonucleotide sequences)

Muscle biopsy analysis

A diagnostic skeletal muscle biopsy was taken with informed consent and subjected to a standard range of histological and histochemical investigations, including oxidative enzymes, particularly sequential cytochrome *c* oxidase (COX)/succinate dehydrogenase (SDH) histochemistry.[2] A further biochemical assessment of OXPHOS function was undertaken using a quadruple immunohistochemical (IHC) assay to assess mitochondrial complex I (NDUFB8) and complex IV (COX-1) protein expression, exactly as described.[3]

Yeast strains, plasmids and media

The W303-1B genotype is Matα ade2-1 leu2-3, 112 ura3-1 trp1-1 his3-11, 15 can1-100. All experiments except transformation were performed in synthetic complete (SC) media (0.69% YNB without amino acids powder, ForMedium) supplemented with 1g/L dropout mix without amino acids or bases necessary to keep plasmids (i.e. uracil for pFL38 and tryptophan for the pFL39) (Baruffini et al., 2010). Media were supplemented with various carbon sources at 2% (w/v) (Carlo Erba Reagents), in liquid phase or after solidification with 20g/L agar (ForMedium).

Transformation was performed according to Gietz and Schiestl (2007) after growth in YPAD medium (1% Yeast extract, 2% Peptone, 40 mg/l adenine base and 2% glucose).

Strains were constructed as previously described.[4,5] Mutagenesis was performed using appropriate primers (supplementary table 3) to obtain the mutant alleles msy1P134R and msyL226R that were then cloned into the pFL39 vector and expressed in the msy1Δ null mutant.

Mitochondrial respiration

Oxygen consumption rate was measured at 30°C from yeast cell suspensions cultured for 18 hrs at 28˚C in SC medium supplemented with 0.6% glucose until exhaustion using a Clark-type oxygen electrode (Oxygraph System Hansatech Instruments England) with 1 ml of air-saturated respiration buffer (0.1 M phthalate–KOH, pH 5.0), 0.5% glucose

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Supplementary Figure 1: Identification of novel YARS2 variants and bone marrow aspirate. A, Prussian blue staining of bone marrow aspirate showing ring sideroblasts (x 100). **B,** The two novel missense variants identified in our patient are highlighted on the YARS2 protein sequence, along with previously-reported variants described in the literature (adapted and modified from Sommerville et al)[5].