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SHORT REPORT

Unusual clinical expression and long survival of a pseudouridylate synthase (*PUS1*) mutation into adulthood

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A homozygote missense mutation of the pseudouridylate synthase gene was found in an adult patient with chronic sideroblastic anemia, diarrhea, microcephaly and failure to thrive. Moderate muscle weakness occurred in adulthood (6-min walk distance at 26 years: 240 m, control range 380–782 m) but a profound deficiency of mitochondrial respiratory chain complexes I and IV were found in her skeletal muscle. This, to our knowledge, is the first example of long survival of this usually fatal mitochondrial deficiency into adulthood. We suggest giving consideration to mitochondrial translation deficiency in unexplained syndromic sideroblastic anemia in adulthood.
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INTRODUCTION

Congenital sideroblastic anemia is a rare genetic disease caused by defects of heme biosynthesis and mitochondrial energy production. The most commonly recognized congenital sideroblastic anemias are X-linked sideroblastic anemia due to mutations in the gene encoding delta amino levulinic acid synthase (*ALAS2*)[1] and the Pearson marrow-pancreas syndrome, caused by sporadic, large-scale deletions of mitochondrial DNA.[2] Recently, nuclear-encoded disorders of mitochondrial translation have emerged as a novel cause of syndromic sideroblastic anemia with myopathy and lactic acidosis. Indeed, two disease genes affecting mitochondrial translation have been shown to cause syndromic sideroblastic anemia, namely the mitochondrial tyrosyl-tRNA synthetase 2 gene (*YARS2*)[3] and the pseudouridylate synthase gene (*PUS1*).[4]

On the other hand, the myopathy, lactic acidosis and sideroblastic anemia syndrome (MLASA) is a rare condition that combines early-onset myopathy with lactic acidosis and sideroblastic anemia. MLASA has been ascribed to mutations in PUS1, an enzyme involved in post transcriptional modification of multiple cytoplasmic and mitochondrial tRNAs (pseudouridylation). We identified a homozygous *PUS1* mutation in an adult patient with congenital sideroblastic anemia, diarrhea, microcephaly, short stature, late-onset moderate muscle weakness and a rather favorable clinical outcome at 26 years of age. This report illustrates the unusual expression and occasionally favorable outcome of this condition in adulthood.

SUBJECTS AND METHODS

The patient, the fourth child of healthy first cousin Turkish parents, was born at term after an uncomplicated pregnancy. Three elder sibs are healthy. Aged 1 year, following episodes of pallor and jaundice, she was diagnosed with congenital sideroblastic anemia. Bone marrow aspirate showed dyserythropoiesis and supported the diagnosis of transfusion-dependent congenital sideroblastic anemia (hemoglobin, 6.3 g/l; reticulocytes, 62 000/mm^3; white blood cells, 7500/mm^3; platelets, 345 000/mm^3). She had no muscle, heart or liver involvement, but chronic diarrhea prompted to consider the diagnosis of Pearson marrow-pancreas syndrome.[5] Skeletal muscle biopsy at 4 years ruled out mitochondrial DNA deletion, but detected a profound deficiency of mitochondrial respiratory chain complexes I and IV (complex IV/II+III = 0.9, control mean ± 1 SD: 3.1 ± 0.4; complex IV/III: 0.6, control mean ± 1 SD: 1.5 ± 0.4). Iterative blood transfusions were well tolerated and she only complained about diarrhea, abdominal pain and occasional headache. She failed to thrive normally; her muscle bulk was reduced but no major muscle weakness, lid ptosis or fatigue was reported. Histological examination of muscle revealed few ragged red fibers (Figure 1a). Neither acute episodes of metabolic acidosis nor neurological symptoms were noted. Plasma lactate consistently remained into normal range. She needed special schooling, but she was an otherwise friendly and outgoing adolescent and young adult. She is now a 26-year-old female, with mild intellectual disability and microcephaly (OFC: 49 cm, − 3 SD; weight: 43 kg, − 2 SD; height: 153 cm, − 1 SD). She can walk unaided with no muscle pain but she recently developed a late-onset, moderate muscle weakness (6-min walk distance at 26 years: 240 m, control range 380–782 m[6]). She has no cardiac involvement and normal heart ultrasound. Apart for monthly blood transfusions and prevention of hemochromatosis, her major concern is her social and professional future.

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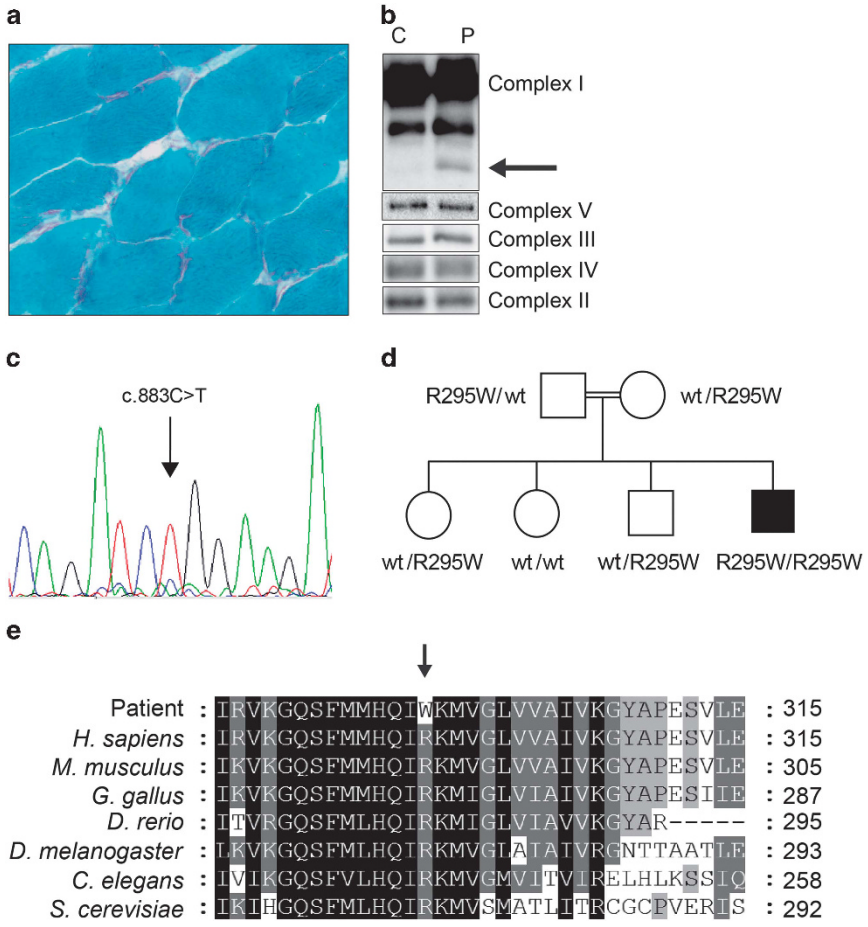

Figure 1 (**a**) Gomori trichrome staining of muscle biopsy from the patient. Note the presence of few ragged-red fibers. (**b**) BN-PAGE analysis of mitochondria from cultured skin fibroblasts of patient (P) and control (C) using specific antibodies. The arrow indicates a putative subcomplex of complex I. (**c**) Molecular analysis of the *PUS1* gene in the patient. The arrows indicate the nucleotide variation. (**d**) Segregation of the PUS1 mutation in the family. (**e**) Evolutionary conservation of human Arg294 in PUS1 of various species. Position of the Arg294 is indicated by an arrow.

All samples from the patient were collected in accordance with regulations from the Ethical Committee of the Necker Hospital. Informed consent was given before sample collection.

Blue-Native polyacrylamide gel (BN-PAGE) analysis was carried out on mitoplasts isolated from control and patient cells grown in DMEM GlutaMax media containing 10% FBS, 100 μg/ml streptomycin and 100 U/ml penicillin (Life Technologies, Saint Aubin, France). Cells from a single 75 cm^2 flask were grown to ~90% confluence, trypsinized, collected by centrifugation, washed once with PBS and resuspended in 100 μl cold PBS (Life Technologies). Digitonin (4 mg/ml) was added to a final concentration 2 mg/ml. After incubation on ice for 10 min, samples were diluted by addition of 1 ml cold PBS. Mitoplasts were recovered by a centrifugation at 16 000 *g* for 10 min at 4°C and resuspended in 30 μl ACBT buffer (1.5 M aminocaproic acid, 75 mM Bis-Tris, pH 7.0) containing 1% w/v N-Dodecyl-β-D-maltoside. Protein concentration was measured using Bradford reagent (BioRad, Marnes-la-Coquette, France) and 20 μg of mitoplast protein were fractionated through a 4–16% NativePage gel (Life Technologies) according to manufacturer's recommendations. Finally, OXPHOS complexes were electro blotted on PVDF membranes (GE Healthcare, Aulnay sous Bois, France) and detected by immunostaining with antibodies directed against proteins from individual OXPHOS complexes (Abcam, Cambridge, UK): anti-GRIM19 for complex I (CI), anti-SDHA for complex II (CII), anti -core II for complex III (CIII), anti-COXII for complex IV (CIV) and anti-$F_1\beta$ subunits for complex V (CV).

Patient's genomic DNA (1 μg) was isolated from blood leukocytes. Exons were captured by an in-solution enrichment methodology (SureSelect Human All Exon Kits Version 3, Agilent, Les Ulis, France) using a biotinylated oligonucleotide probe library (Human All Exon v3 50 Mb, Agilent).

Gomori trichome staining was performed as previously described.[7]

RESULTS

BN-PAGE on cultured skin fibroblasts revealed qualitative anomalies in assembly of complex I respiratory enzyme (Figure 1b), reminiscent of anomalies observed in fibroblasts of patients with mitochondrial translation deficiencies.[8,9]

To identify the causative nuclear gene mutation, we sequenced the exome of this patient. From the 22 697 SNPs and Indels identified, the pathogenic variant was selected after filtering against known SNPs reported in dbSNP, 1000 genomes, Exome Variant Server, Intergenic variants and in house polymorphisms. Considering the consanguinity of the parents, only homozygous variations were considered.

This filtering resulted in a list of 14 genes, only one of which encoding a known mitochondrial protein, *PUS1*. Sanger sequencing confirmed this homozygous missense mutation in exon 5 of the *PUS1* gene (c.883 C>T, p.Arg295Trp, Figure 1c). The parents were heterozygous for this mutation affecting a conserved codon (Figures 1d and e), which was predicted to be deleterious by various prediction softwares (SIFT: deleterious (score: 0.00, median: 3.02); MutationTaster: disease causing (*P*-value: 1.0); Polyphen2: probably damaging, score 1.000[10,11]).

DISCUSSION

PUS1 mutations have been rarely reported. Systematic analysis of *PUS1* in a large series of 60 patients with sideroblastic anemia allowed to exclude this gene in all patients but two who had MLASA.[12] Moreover, *PUS1* mutations have also been excluded as a cause of acquired sideroblastic anemia in a series of 37 patients.[13] Until now, all patients with *PUS1* mutations have MLASA.[4,12,14,15] Our rather unusual observation emphasizes the clinical variability of the disease and the occasionally long survival of congenital mitochondrial translation deficiency. The variable expressivity and relatively favorable outcome of this case of *PUS1* mutation, compared with the severity of most reported cases, remains unexplained. Trying to elucidate the underlying mechanisms of this favorable outcome should certainly help improving the long-term prognosis of respiratory chain deficiency into adulthood. *PUS1* encodes a mitochondrial tRNA modification factor and mutations of this gene have been showed to result in abnormal mitochondrial translation.[14] Mitochondrial translation deficiencies often result in quantitative and/or qualitative respiratory chain assembly defects in fibroblasts but this feature has never been described for *PUS1* mutations to our knowledge. Therefore, we suggest to test *PUS1* in patients with isolated or syndromic sideroblastic anemia associated with abnormal respiratory chain assembly pattern.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Cotter PD, Rucknagel DL, Bishop DF: X-linked sideroblastic anemia: identification of the mutation in the erythroid-specific delta-aminolevulinate synthase gene (ALAS2) in the original family described by Cooley. *Blood* 1994; **84**: 3915–3924.
2 Rotig A, Colonna M, Bonnefont JP *et al*: Mitochondrial DNA deletion in Pearson's marrow/pancreas syndrome. *Lancet* 1989; **1**: 902–903.
3 Riley LG, Cooper S, Hickey P *et al*: Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia—MLASA syndrome. *Am J Hum Genet* 2010; **87**: 52–59.
4 Bykhovskaya Y, Casas K, Mengesha E, Inbal A, Fischel-Ghodsian N: Missense mutation in pseudouridine synthase 1 (PUS1) causes mitochondrial myopathy and sideroblastic anemia (MLASA). *Am J Hum Genet* 2004; **74**: 1303–1308.
5 Bader-Meunier B, Rotig A, Mielot F *et al*: Refractory anaemia and mitochondrial cytopathy in childhood. *Br J Haematol* 1994; **87**: 381–385.
6 Casanova C, Celli BR, Barria P *et al*: The 6-min walk distance in healthy subjects: reference standards from seven countries. *Eur Respir J* 2011; **37**: 150–156.
7 Gomori G: A rapid one-step trichrome stain. *Am J Clin Pathol* 1950; **20**: 661–664.
8 Galmiche L, Serre V, Beinat M *et al*: Exome sequencing identifies MRPL3 mutation in mitochondrial cardiomyopathy. *Hum Mutat* 2011; **32**: 1225–1231.
9 Vedrenne V, Galmiche L, Chretien D, de Lonlay P, Munnich A, Rotig A: Mutation in the mitochondrial translation elongation factor EFTs results in severe infantile liver failure. *J Hepatol* 2012; **56**: 294–297.
10 Kumar P, Henikoff S, Ng PC: Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009; **4**: 1073–1081.
11 Adzhubei IA, Schmidt S, Peshkin L *et al*: A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7**: 248–249.
12 Bergmann AK, Campagna DR, McLoughlin EM *et al*: Systematic molecular genetic analysis of congenital sideroblastic anemia: evidence for genetic heterogeneity and identification of novel mutations. *Pediatric blood & cancer* 2010; **54**: 273–278.
13 Steensma DP, Hecksel KA, Porcher JC, Lasho TL: Candidate gene mutation analysis in idiopathic acquired sideroblastic anemia (refractory anemia with ringed sideroblasts). *Leuk Res* 2007; **31**: 623–628.
14 Fernandez-Vizarra E, Berardinelli A, Valente L, Tiranti V, Zeviani M: Nonsense mutation in pseudouridylate synthase 1 (PUS1) in two brothers affected by myopathy, lactic acidosis and sideroblastic anaemia (MLASA). *J Med Genet* 2007; **44**: 173–180.
15 Zeharia A, Fischel-Ghodsian N, Casas K *et al*: Mitochondrial myopathy, sideroblastic anemia, and lactic acidosis: an autosomal recessive syndrome in Persian Jews caused by a mutation in the PUS1 gene. *J Child Neurol* 2005; **20**: 449–452.