SHORT REPORT

A novel mitochondrial tRNA Arg mutation resulting in an anticodon swap in a patient with mitochondrial encephalomyopathy

Sara Roos^{*1}, Niklas Darin², Gittan Kollberg³, Marita Andersson Grönlund⁴, Mar Tulinius², Elisabeth Holme³, Ali-Reza Moslemi¹ and Anders Oldfors¹

We report a mutation in the anticodon of the *tRNA^{Arg}* gene (m.10437 G>A), resulting in an anticodon swap from GCU to ACU, which is the anticodon of *tRNA^{Trp}*, in a boy with mitochondrial encephalomyopathy. Enzyme histochemical analysis of muscle tissue and biochemical analysis of isolated muscle mitochondria demonstrated cytochrome c oxidase (COX) deficiency. Restriction fragment length polymorphism analysis showed that 90% of muscle and 82% of urinary epithelium mtDNA harbored the mutation. The mutation was not identified in blood, fibroblasts, hair roots, or buccal epithelial cells and it was absent in the asymptomatic mother, suggesting that it was a *de novo* mutation. Single-fiber PCR analysis showed that the proportion of mutated mtDNA correlated with enzyme histochemical COX deficiency. This mutation adds to the three previously described disease-causing mutations in *tRNA^{Arg}*, but it is the first mutation occurring in the anticodon of *tRNA^{Arg}*.

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INTRODUCTION

Patients with mitochondrial disorders have molecular defects affecting the oxidative phosphorylation system. These disorders mostly target tissues with high-energy demand, such as skeletal muscle and the nervous system, but every tissue in the body can be affected, and mitochondrial diseases are often multisystemic.¹ More than 200 point mutations and rearrangements in the mitochondrial genome have been associated with mitochondrial disease, and more than half of these mutations have been located to *tRNA* genes.² The most commonly affected is *tRNA^{Leu}* and only three pathogenic mutations have been described in *tRNA^{Arg}*. The first is m.10406 G>A that was described in a 6-year-old boy with proximal myopathy and Asperger syndrome.³ The second mutation, m.10438 A>G, was found in an 8-year-old boy affected with mild cognitive impairment, ataxia, nystagmus, subnormal visual acuity, and muscle weakness.⁴ The third mutation, m.10450 A>G, was reported in an 8-month-old boy with visual dysfunction, psychomotor retardation, and generalized hypotonia.⁵

Mutations affecting the anticodon triplet of tRNAs, and thus resulting in an anticodon swap, are rare and only six previous mutations have been described.^{6–11}

In this study, we present a patient with mitochondrial myopathy harboring a novel m.10437 G>A mutation in the first base of the anticodon triplet, resulting in an anticodon swap (Arg to Trp) in the *tRNA^{Arg}* gene.

MATERIALS AND METHODS

Case report

This 16-year-old boy was the first child to non-consanguineous parents. His parents and two younger siblings were healthy. He was well until starting school at 7 years of age when he was found to be short in height, slightly myopic, astigmatic and to have subnormal visual acuity. Endocrinological investigations were performed that initially revealed borderline values for growth hormone (GH) deficiency and he was therefore treated for a period with GH without any beneficiary effect on his growth. At age 16 years, height and weight was –3 SD below the mean compared with a standardized Swedish growth chart. Ophthalmological investigations at 10 years of age showed pigmentary retinopathy and optic atrophy. An electroretinogram was performed and showed a severe rod cone dystrophy. He had decreased visual acuity, visual fields, color vision, and dark adaption. Nystagmus has been noted since 14 years of age. A year later, photophobia and bilateral cataracts were developed. The cataracts were operated on at 16 years of age. He is now blind. At 14 years of age, he was referred for neurological investigations. A clinical examination showed mild ataxia with tremor, dysmetria and gait instability together with mild to moderate muscle weakness, weak to absent tendon reflexes in the lower extremities, and exercise intolerance. Audiometric investigations revealed a mild bilateral sensorineural hearing impairment. At 15 years of age, he had an unprovoked epileptic seizure and he has since then been treated with levetiracetam. Decreased renal function was identified at 15 years of age, with increased serum creatinine to 145 µmol/l (reference interval 30–90 µmol/l), proteinuria with urine albumine at 244–538 mg/l (reference interval <20 mg/l), and urine albumine/creatinine ratio at 44–59 g/mol (reference interval <3 g/mol). A renal scintigraphy

¹Department of Pathology, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; ²Department of Pediatrics, Institute of Clinical Sciences, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; ³Department of Clinical Chemistry, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; ⁴Department of Pediatric Ophthalmology, Institute of Neuroscience and Physiology/Ophthalmology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

*Correspondence: Dr S Roos, Department of Pathology, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, Gula Stråket 8, SE-413 45 Gothenburg, Sweden. Tel: +46 (0)31 342 8548; Fax: +46 (0)31 342 2886; E-mail: sara.roos@gu.se

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Figure 1 Enzyme histochemical analyses of muscle. (a) COX-succinate dehydrogenase double staining showing numerous fibers with COX deficiency (blue fibers). (b) Trichrome staining illustrating mitochondrial proliferation and ragged red fibers.

demonstrated reduced kidney size. Cr⁵¹-EDTA clearance showed a decreased glomerular filtration rate of 42% of normal. The filtration rate decreased over time to 29% of normal at 16 years of age. Since 14 years of age, cardiac investigations have demonstrated hypertrophy of the walls of the left ventricle (+3 SD compared with normal) with normal systolic and diastolic function and without obstruction of the outflow.

Mitochondrial investigations were performed at 14 years of age. Blood levels of lactate and pyruvate were 1.6 and 0.096 mmol/l, respectively, leading to a ratio of 33 (reference interval <20), while cerebrospinal fluid (CSF) levels of lactate and pyruvate were 3.6 and 0.137 mmol/l, respectively, leading to a lactate to pyruvate ratio of 26. The urinary lactate excretion was normal. The CSF albumin was increased to 258 mg/l (reference level <225 mg/l) and he also had an increased CSF/plasma albumin ratio of 7.4 (reference level <5). The serum acyl carnitine profile and muscle carnitine levels were normal. The serum creatine kinase activity was mildly increased to 5.4 μ kat/l (reference interval <3.5 μ kat/l).

Morphological and biochemical analysis

Morphological and histochemical analyses of fresh-frozen muscle tissue were performed as described previously.¹² Isolation of skeletal muscle mitochondria, oximetric measurements of fresh mitochondria and spectrophotometric analyses were performed as described previously.¹³

Molecular analysis

Total DNA was extracted from peripheral blood leukocytes, muscle, hair roots, buccal mucosa, urinary epithelial cells, and cultured skin fibroblasts using the DNeasy Blood and Tissue Kit, the DNA Blood Mini Kit, or the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) as described in the protocols provided by the manufacturer. Direct sequencing of the 22 mitochondrial *tRNA* genes was performed in a 3730 DNA Analyzer using Big Dye Terminator v3.1 Cycle Sequencing Kits (Life Technologies, Carlsbad, CA, USA). The major arc of mtDNA was amplified by long-range PCR to rule out large-scale mtDNA rearrangements.

Single-muscle fibers with normal and deficient cytochrome *c* oxidase (COX) activity were isolated with a tungsten needle as described previously.¹² Briefly, 15- μ m thick frozen muscle sections were double stained for COX and succinate dehydrogenase, and blue fibers were regarded as COX-negative while brown fibers were regarded as COX-positive. A total of 22 fibers were investigated.

For analysis of the proportion of mutated mtDNA, a 239-bp fragment was amplified by PCR using a FAM-labeled forward primer at nt 10302 (5'-ACT AAC CTG CCA CTA ATA GT-3') and a reverse primer at nt 10540 (5'-GAG CGA TAT ACT AGT ATT CC-3'). The amplified wild-type fragment contains a unique restriction site for the endonuclease *TaqI* (New England Biolabs, Ipswich, MA, USA) and digestion results in two products of 134 and 105 bp, whereas the fragment with the mutation remains uncut. After digestion, results were visualized using a 3730 DNA Analyzer and analyzed using the Gene Mapper software (Life Technologies).

Table 1 Respiratory rate and enzyme activity in isolated skeletal muscle mitochondria

	Patient	Controls (n = 18)
<i>Polarography (nmol/min/mg protein)</i>		
Pyruvate + malate	107	92–194
Glutamate + malate	109	91–213
Palmitoyl-carnitine + malate	39	26–74
Succinate + rotenone	92	82–176
Ascorbate + TPMD	201	278–446
<i>Spectrophotometry</i>		
NADH ferricyanide reductase (nmol/min/mg protein)	5316	5096–9692
Succinate cytochrome-c reductase (nmol/min/mg protein)	390	218–518
Cytochrome-c oxidase (per min $\times$ per protein)	4.5	7.7–21.1
Mg ²⁺ ATPase (nmol/min/mg protein)	998	577–1237
Citrate synthase (μ mol/min/mg protein)	2.9	2.2–3.2

Reference values are from age-matched patients without mitochondrial disease. The reference intervals are mean $\pm$ 2 SD.

RESULTS

Morphology and Biochemistry

Histopathological and enzyme histochemical investigations of the muscle biopsy showed mitochondrial proliferation with ragged red fibers and numerous COX-negative fibers (Figure 1).

Biochemical analyses revealed a COX deficiency and the NADH ferricyanide reductase activity was lower than expected in relation to the high succinate cytochrome-c reductase activity (Table 1).

Molecular analysis

Sequencing of all mitochondrial *tRNA* genes revealed one alteration, a novel G-to-A mutation at nucleotide position 10437 in the mitochondrial *tRNA*^{Arg} gene. The mutation results in an anticodon swap changing the anticodon GCU of *tRNA*^{Arg} to ACU, which is a tryptophan anticodon (Figure 2a).

The proportion of mutated mtDNA as determined by PCR and restriction fragment length polymorphism analyses was 90% in muscle homogenate and 82% in urinary epithelial cells. The mutation load in COX-deficient muscle fibers was significantly higher ($P < 0.05$, Mann–Whitney *U* test) than in COX-positive cells (Figure 2b). The mutation was not detected in fibroblasts, hair roots, buccal mucosa, or in blood of the patient. It was neither identified in blood, hair roots, buccal mucosa, or urinary epithelial cells of the patient's mother nor in 100 controls.

Figure 2 (a) Schematic illustration of the cloverleaf structure of human mitochondrial *tRNA*^{Arg} showing the position of the 10437G>A mutation in the anticodon. (b) Single-fiber analysis and mutational loads determined in COX-positive and COX-negative fibers demonstrate segregation of the 10437 A mutant genotype with respiratory chain dysfunction. The horizontal lines indicate the mean mutational load.

DISCUSSION

We present a novel mutation in the anticodon of the mitochondrial *tRNA*^{Arg} resulting in an anticodon swap (Arg to Trp). This is the fourth *tRNA*^{Arg} mutation and the seventh tRNA mutation in the anticodon region. The pathogenic nature of this mutation is confirmed by fulfilling the following accepted criteria: It is not present in 100 control subjects and has not previously been described as a neutral polymorphism. Second, it is heteroplasmic and there is a higher percentage of mutated mtDNA in COX-negative fibers than in COX-positive, confirming segregation of the 10437A mutant genotype with respiratory chain dysfunction. Third, the first base of the anticodon is a highly conserved residue and a functionally important site. Finally, the mutation is consistent with the histochemical observation of COX-negative ragged red fibers and respiratory chain deficiencies. Using the pathogenicity scoring system developed by Yarham *et al*¹⁴, this mutation would score 11, thus being classified as 'definitely pathogenic'.

The patient displayed a multisystemic disease with symptoms and signs very typical of a mitochondrial encephalomyopathy due to an mtDNA mutation. The rapid segregation of the mutation is indicated by the highly variable proportion of mutant mtDNA with very high levels of mutant mtDNA in muscle and urinary sediment but undetectable levels in blood, fibroblasts, buccal mucosa, and hair roots. As the mutation was not identified in blood, hair roots, buccal mucosa, or urinary epithelial cells of the patient's mother it had probably occurred *de novo* in the germ line or early during embryogenesis. In this case the rapid segregation may explain the early onset and major involvement of some tissues.

Only one of the six previously described mutations in the anticodon of a mitochondrial tRNA appears to be functionally dominant.⁹ A C>T mutation at nt 5545 in the anticodon of *tRNA*^{Trp} was demonstrated to cause biochemical deficiency at very low levels of mutant load (below 10%). Although inappropriate amino-acid insertion was not directly proven, the finding of abnormal mitochondrial translation products favored this hypothesis as the cause of the functionally dominant effect of the mutation in this case.

The m.10437 G>A *tRNA*^{Arg} mutation in our patient resulting in an anticodon swap acts functionally as a recessive mutation indicated by the high level of mutant mtDNA in muscle and results from single-fiber analysis. The effect of the mutation is probably impaired aminoacylation, resulting in a lack of functional *tRNA*^{Arg} needed for protein synthesis and consequently causing an oxidative phosphorylation deficiency in tissues with high-mutant load.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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