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Mitochondrial DNA depletion syndrome due to mutations in the *RRM2B* gene ☆

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Abstract

Mitochondrial DNA depletion syndrome (MDS) is characterized by a reduction in mtDNA copy number and has been associated with mutations in eight nuclear genes, including enzymes involved in mitochondrial nucleotide metabolism (*POLG*, *TK2*, *DGUOK*, *SUCLA2*, *SUCLG1*, *PEO1*) and *MPV17*. Recently, mutations in the *RRM2B* gene, encoding the p53-controlled ribonucleotide reductase subunit, have been described in seven infants from four families, who presented with various combinations of hypotonia, tubulopathy, seizures, respiratory distress, diarrhea, and lactic acidosis. All children died before 4 months of age.

We sequenced the *RRM2B* gene in three unrelated cases with unexplained severe mtDNA depletion. The first patient developed intractable diarrhea, profound weakness, respiratory distress, and died at 3 months. The other two unrelated patients had a much milder phenotype and are still alive at ages 27 and 36 months.

All three patients had lactic acidosis and severe depletion of mtDNA in muscle. Muscle histochemistry showed RRF and COX deficiency. Sequencing the *RRM2B* gene revealed three missense mutations and two single nucleotide deletions in exons 6, 8, and 9, confirming that *RRM2B* mutations are important causes of MDS and that the clinical phenotype is heterogeneous and not invariably fatal in infancy.

Keywords: mtDNA depletion; Mitochondrial myopathy; RRM2B

1. Introduction

Within the past 6 years, mtDNA depletion syndrome (MDS) has been attributed to mutations in eight nuclear genes. Five of these (*TK2*, encoding mitochondrial thymidine kinase [1]; *DGUOK*, encoding deoxyguanosine kinase [2]; *POLG*, encoding the catalytic subunit of mitochondrial polymerase γ [3]; *SUCLA2*, encoding the β subunit of succinyl-CoA synthase (SCS-A) [4], and *SUCLG1*, encoding the α subunit of SCS-A [5]) are – directly or indirectly [6] – involved in the homeostasis of the mitochondrial nucleotide pool. Mutations in *PEO1*, encoding the T7-phage-like helicase (twinkle), typically cause autosomal dominant

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multiple deletions of mtDNA but can also cause autosomal recessive mtDNA depletion [7,8]. The seventh gene, *MPV17*, encodes an inner mitochondrial protein whose function remains elusive [9].

The latest gene to be associated with mtDNA depletion is *RRM2B*, which encodes the R2 subunit of a p53-controlled ribonucleotide reductase (p53R2). This enzyme catalyzes the biosynthesis of deoxyribonucleotides by storing organic free radicals required for catalysis in the R2 subunit [10].

Here, we describe three unrelated patients with five novel *RRM2B* mutations. One of the three had the fatal infantile presentation reported in the seven original patients [10], whereas the other two had milder clinical phenotypes and are alive at 27 and 36 months of age.

2. Case reports

2.1. Patient 1

This 8-week-old congenitally deaf infant girl, born to non-consanguineous parents after a normal pregnancy and delivery, was admitted with a 2-week history of watery diarrhea, persistent acidosis, progressive weakness, poor head control, and worsening respiratory distress requiring intubation. At admission, she was small for age and hypotonic, with bilateral central sensorineural hearing loss. The following laboratory tests were abnormal: repeat plasma lactate values ranged from 2.8 to 17.4 mmol/L (normal, <2.2); blood pyruvate was 0.434 mmol/L (normal, 0.03–0.107); plasma organic acids were normal except for increased lactate and pyruvate; CSF lactate was 5.2 mmol/L (normal, 0.5–2.8) and CSF protein was 164 (normal, 12–60). Notably, serum CK was normal (126; normal, <296). MRS of the brain at 7 weeks of age showed the presence of lactate in the left basal ganglia.

Various attempts to wean her from the ventilator failed and control of her metabolic and respiratory acidosis required ventilator adjustments and intravenous bicarbonate drip. She continued having diarrhea and required total parenteral nutrition. Her strength worsened progressively and at 8 months she had minimal spontaneous movements. After the parents decided to withdraw further care, a premortem muscle biopsy was obtained to confirm the diagnosis of mtDNA depletion obtained from a previous very small biopsy and to establish the molecular etiology.

2.2. Patient 2

This 4-year-old boy was born to non-consanguineous healthy parents after a normal pregnancy and delivery. He was normal at birth, but progressive failure to thrive rapidly ensued due to uncoordinated suck and swallow. He failed to gain developmental milestones and was hypotonic and microcephalic when first seen at 4 months of age. He developed respiratory failure, urinary infections, and intolerance to oral feeds: an immune deficient state was ruled out. At 7 months, a G-tube was placed, but he continued losing weight and developed electrolyte imbalance, with hyponatremia, hypochloremia, and hypokalemia, requiring supplementation. At 8 months, he required intubation and assisted ventilation, followed by tracheostomy at 10 months.

Laboratory tests at 8 months of age showed increased serum lactate (4.3) and normal CK (108 IU; normal, <296). Urinary organic acids, plasma amino acids and acylcarnitine profile were normal. There was mild generalized aminoaciduria but renal tubular function was not analyzed in detail. A low plasma carnitine level (21.1 nmol/ml; normal, 25–69) was attributed to malnutrition and corrected by carnitine supplementation. An MRI of the brain at 20 months showed bilateral and nearly symmetrical non-enhancing areas of abnormal signal and reduced diffusion in the white matter. Magnetic resonance spectroscopy (MRS) showed a lactate peak in the basal ganglia and an even higher peak in the CSF.

At 4 years of age, he is wheelchair-bound and is ventilated by tracheostomy. He has a stable encephalomyopathy, with microcephaly and global developmental delay. An ophthalmological exam has revealed peripheral pigmentary retinopathy and tunnel vision, but there is no evidence of optic atrophy. With a feeding tube and a Nissen fundoplication, he is growing well and has no overt liver or renal involvement. A recent electrocardiogram was normal.

2.3. Patient 3

This 27-month-old girl was born to non-consanguineous Hispanic parents after a normal pregnancy and delivery and developed normally until 6 months of age, when she was evaluated because of progressively worsening hypotonia, failure to thrive, and microcephaly. Abnormal laboratory tests included serum CK (318 IU/L; normal, <296), blood lactate (ranging from 4.1 to 7.2 mmol/L; normal, <2.2), and liver function tests (AST, 81 IU/L; normal, 12–27; ALT, 46 IU/L; normal, 7–28). She had multiple respiratory infections needing admissions to the hospital, but no major episodes of decompensation. After placement of a G-tube at 9 months of age and supplementation of vitamins and cofactors, including L-carnitine and coenzyme Q_{10}, she has maintained adequate weight gain and growth and has acquired normal head circumference. At 27 months, she can sit with assistance and walk with a pony walker, grabs, reaches, and has a 12-word vocabulary. Overall, she has continued to make progress.

3. Methods

3.1. Histochemistry and biochemistry

Histochemical studies of muscle using 8-μm-thick frozen sections were carried out as described [11]. Biochemical