Case Report

A novel RRM2B mutation associated with mitochondrial DNA depletion syndrome

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ABSTRACT

Mitochondrial DNA (mtDNA) depletion syndromes are disorders characterized by infantile-onset, severe progression, and the drastic loss of mtDNA content in affected tissues. In a patient who showed severe hyponatia, proximal tubulopathy and sensorineural hearing loss after birth, we observed severe mtDNA depletion and impaired respiratory chain activity in muscle due to heterozygous variants c.686G>T and c.551-2A>G in RRM2B, encoding the p53R2 subunit of the ribonucleotide reductase.

1. Introduction

In mitochondrial DNA (mtDNA) depletion syndromes (MDDS), the loss of mtDNA content severely affects mitochondrial respiratory chain activity, thus impairing the energetic balance of either a specific tissue (most commonly muscle, liver and brain) or of multiple organs. [1]

MDDS usually display neonatal or infantile-onset and a rapid progression, resulting in death in the first years of life. [2] Loss of function mutations have been detected in several genes encoding for proteins involved in mtDNA maintenance. [2] In proliferating tissues, mtDNA replication mainly relies on cytosolic deoxyribonucleotides (dNTPs) metabolism, which is cell-cycle dependent. Instead, in postmitotic cells, dNTPs supply is guaranteed by a dedicated set of mitochondrial enzymes belonging to the dNTPs salvage pathway. [3] The regulatory subunit of the p53-inducible ribonucleotide reductase (p53R2), encoded by the RRM2B gene, takes part in the cytoplasmic de novo conversion of ribonucleoside diphosphates into the corresponding deoxyribonucleoside diphosphates. [4] Biallelic RRM2B mutations have been mainly associated with infantile-onset myopathic MDDS with renal proximal tubulopathy, sensorineural deafness and neurological deterioration. [5,6] An adult case presenting mitochondrial neuro-gastrointestinal encephalopathy (MNGIE) was also associated to recessive RRM2B variants. [7] Heterozygous RRM2B mutations have been also described in adult patients showing a mild myopathic phenotype and an accumulation of multiple mtDNA deletions in skeletal muscles. [8]

Here we present the clinical, molecular, and biochemical findings of an Italian patient, who presented at birth with hyponatia, progressive...
birthweight was 3530 g. As a 13% weight loss was recorded on the third day after birth, an emergency cesarean section was performed at 42 weeks of pregnancy, and cardiotocography monitoring showed trace alterations, with no signs of impending fetal distress. The infant was uneventful gestation, the mother developed mild fever at the end of pregnancy, and both parents were healthy with no history of neurological disorders. After an uncomplicated delivery, the neonate was transferred to our unit at day 12 after birth. Glycosuria and severe metabolic acidosis; with glycerol levels of 22 mmol/L were reported. Metabolic acidosis was corrected after 27 days after birth, showed diffuse white matter hyperintensity on T2-weighted imaging, reduced subarachnoid spaces and normal ventricles. At the age of 3 months, the patient was discharged home to thrive. Seizures and non-neurological manifestations are often reported in patients with RRM2B deficiency. In our case, the infant presented with severe hypotonia, generalized weakness, and early failure to thrive. Seizures and non-neurological manifestations are often observed, including respiratory distress, renal tubulopathy, sensory-neural hearing loss and gastrointestinal disturbances. In our case, the diagnostic process was supported by several factors in addition to the clinical picture: severe loss of COX activity at COX/SDH double staining, impaired activity of mtDNA-encoded respiratory chain complexes, severe mtDNA depletion (levels <2% of age-matched controls). The p.Gly229Val mutation has been previously detected in two Sudanese siblings, who died in infancy, and in a Caucasian patient, affected by severe hypotonia, seizures and sensorineural deafness. In all cases, massive mtDNA loss was observed in muscle. On the contrary, the c.551-2A>G mutation is novel and results in exon 6 skipping. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, the c.551-2A>G variant, meeting pathogenicity criteria PVS1, PM2 and PM3, can be classified as pathogenic (ACMG Class 5).

In RRM2B-mutated patients, the residual mtDNA levels, which reflect the ribonucleotide reductase activity, have been correlated with the clinical phenotype. Specifically, null alleles are associated with severe mtDNA depletion in muscle and early-onset presentation with multisystemic signs and symptoms, which lead to death soon after birth. Missense biallelic mutations, otherwise, have been detected in childhood-onset cases (mean age at onset, 7 years) with severe and multisystem disorders, longer post-onset survival and accumulation of multiple mtDNA deletions at muscle biopsy. In a single adult case, a MNGIE-like presentation was associated to recessive RRM2B missense mutations causing moderate muscle mtDNA depletion. Finally, dominantly-inherited heterozygous variants have been linked to late-onset (mean age at onset 46 years) milder forms of myopathy, characterized by PEO, ptosis, proximal muscle weakness, bulbar dysfunction, seizures and sensorineural deafness. Overall, we suggest considering RRM2B screening when the clinical suspicion is high, even prior to muscle biopsy. Indeed,
Fig. 1. (A) Proband's daily plasma lactate levels from birth. (B) T2-weighted (T2W) turbo spin-echo (TSE) MRI images performed at 41 weeks from birth reveal diffuse white matter hyperintensity likely due to vasogenic edema. (C) Oil Red O staining in patient shows a marked lipids accumulation (insert: Oil Red O staining on age-matched control muscle section). (D) Double staining for SDH and COX activities showed the complete absence of COX activity in the patient (insert: double staining for SDH and COX activities on age-matched control biopsy). Magnification 20×. Scale bar 50 μm. (E) RT-PCR analysis of muscle-derived RRM2B cDNA encompassing Exons 5 and 8. Patient's lane (P) shows a shorter band compared to controls (C). Sequence electropherograms of the amplicons disclose the physiological (top) and abnormal (down) splicing event. (F) Quantitative PCR analysis of muscle-extracted DNA by using two independent duplex assays for the simultaneous detection of mitochondrial and nuclear DNA (CYTB/APP and ND1/RNASEP). The bars indicate Relative Quantification levels of mitochondrial DNA content normalized to nuclear DNA. Age matched healthy controls (n = 10) and TK2-mutated patients (n = 3) are shown for comparison. (G) Respiratory chain complex activities normalized to the matrix enzyme Citrate Synthase. Values are expressed as pmol/min/mg proteins. Control values are indicated as mean ± standard deviation.
molecular testing might precede (and often make unnecessary) invasive procedures, especially in pediatric patients. On the other hand, we stress the relevance of muscle biopsy to sustain the diagnosis of primary mitochondrial dysfunction and, in cases like ours, to provide material for biochemical and molecular studies (including a reliable assessment of mtDNA content) required to support the pathogenicity of novel RMR2B variants. In this scenario, a targeted NGS gene panel approach can be used to investigate simultaneously multiple mtDNA maintenance-related genes, including TK2, for which a disease-modifying therapy is currently under investigation. [17]

Ethics statement

The “Comitato Etico Milano Area 2 Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico” (Milan, Italy) approved the study, and the parents of the proband provided written informed consent.

Data availability statement

Data that support the findings of this study are available upon request.

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