A Homozygous Mutation in LYRM7/MZM1L Associated with Early Onset Encephalopathy, Lactic Acidosis, and Severe Reduction of Mitochondrial Complex III Activity

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ABSTRACT: Mutations in nuclear genes associated with defective complex III (cIII) of the mitochondrial respiratory chain are rare, having been found in only two cIII assembly factors and, as private changes in single families, three cIII structural subunits. Recently, human LYRM7/MZM1L, the ortholog of yeast MZM1, has been identified as a new assembly factor for cIII. In a baby patient with early onset, severe encephalopathy, lactic acidosis and profound, isolated cIII deficiency in skeletal muscle, we identified a disease-segregating homozygous mutation (c.73G>A) in LYRM7/MZM1L, predicting a drastic change in a highly conserved amino acid residue (p.Asp25Asn). In a yeast strain, the expression of a mzm1Δ yeast strain, the expression of a mzm1Δ mutant allele caused temperature-sensitive respiratory growth defect, decreased oxygen consumption, impaired maturation/stabilization of the Rieske Fe–S protein, and reduced complex III activity and amount. LYRM7/MZM1L is a novel disease gene, causing cIII-defective, early onset, severe mitochondrial encephalopathy.

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Mitochondrial complex III (cIII; ubiquinol–cytochrome c reductase, bc1 complex, E.C.1.10.2.2) catalyzes the transfer of electrons from coenzyme Q to cytochrome c (cyt c). The energy liberated by this reaction sustains the translocation of four protons across the inner mitochondrial membrane (IMM), which contributes to the formation of the electrochemical potential (ΔP).

Additional Supporting Information may be found in the online version of this article.

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Complex III is composed of 11 subunits. Cytochrome b (cyt b) is the only cIII subunit encoded by a mtDNA gene, whereas the remaining 10 are encoded by nuclear DNA genes. Several other nuclear genes are required for the proper formation and function of cIII [Crivellone et al., 1988]. The assembly of cIII in mammalian cells is a still poorly understood process, including the very mechanisms leading to the module-based, stepwise formation of the mature enzyme, the exact subunit composition of the assembly intermediates, and the complete set of specific cIII assembly factors.

We report here the identification of the first LYRM7/MZM1L pathogenic mutation in a patient with early onset, severe encephalopathy and lactic acidosis. LYRM7 (LYR motif containing 7) gene product is the human ortholog of the Saccharomyces cerevisiae protein MZM1 (Mitochondrial-Zinc-Maintenance 1); in both organisms, this protein is a cIII assembly factor that participates in the incorporation of the Rieske Fe–S protein into nascent cIII [Atkinson et al., 2011; Sánchez et al., 2013].

The proband was one of four siblings born from Moroccan first-cousin parents; two of them are both alive and well, whereas one has isolated, static mental delay. The patient was born at the 39th week of gestation after uncomplicated pregnancy and delivery. Weight at birth was 2.8 kg (5–10 percentile). Her development was normal during the first 20 months of life, when she manifested rapidly progressive weakness with reduced spontaneous motor activity and alertness, and polypnea. The laboratory tests showed anemia (hemoglobin 7.3 g/dl, n.v. 13–16) associated with low plasma iron (15 mg/dl, n.v. > 50), treated by iron supplementation. At 21 months, during an infectious episode with fever, the patient developed acute, severe dyspnea and stupor, requiring ICU treatment. The clinical examination showed generalized hypotonia, a central breathing pattern, and a fluctuating comatose state with hardly any reaction to external stimuli. Laboratory tests showed severe metabolic acidosis (pH 6.78, pCO2 16.5; O2 saturation 96%), with increased plasma lactate (11, 2 mg/dl, n.v. < 2) and ammonia (279 μM, n.v. < 80), and severe anemia (Hb 6.8). A blood culture was positive for Staphylococcus haemolyticus. The profiles of urinal organic acids, plasma amino acids, and acylcarnitines were normal. Heart and liver were normal as well. A CT scan showed marked bilateral hypodensity of the centrum semiovale, later confirmed by brain MRI, indicating severe demyelination and vacuolization of the white matter, and global atrophy with a thin corpus callosum (Fig. 1A and B). The patient was intubated for forced ventilation, and treated with blood transfusions and intravenous bicarbonates. In the next several days, the clinical features improved, with recovery...
Radiological, biochemical, and genetic features. (mzm1 and gene revealed a homozygous c.73G>A transition. The Asp25 residue is highly conserved in the phylogenesis (Fig. 1E). The mutation was present in heterozygosis in both parents and in all three unaffected siblings, according to autosomal recessive inheritance of the disease trait. Neither fibroblasts, nor other tissues of the proband were available. However, the LRYM7 gene is the human ortholog of MZM1 in Saccharomyces cerevisiae [Atkinson et al., 2011; Sánchez et al., 2013] and the Asp25 residue is conserved between the two species (Fig. 1E). We then introduced the change equivalent to the c.73G>A, p.Asp25Asn of human LRYM7 into the yeast MZM1 wild-type (wt) gene, cloned in a centromeric monocopy vector. The MZM1 and mzm1^{D25N} constructs were used to transform a mzm1Δ mutant, giving rise to mzm1Δ/MZM1 and mzm1Δ/mzm1^{D25N} yeast strains.

In order to reveal a possible respiratory growth defect, serial dilutions of the strains were incubated on minimum medium supplemented with either dextrose or glycerol, at 28°C and 37°C. A clear growth defect of the mzm1Δ/mzm1^{D25N} was observed in glycerol-containing plates incubated at 37°C but not at 28°C (Fig. 2A), similar to, albeit less pronounced than, the mzm1Δ mutant [Atkinson et al., 2010]. To further analyze the respiratory deficiency, we measured oxygen consumption, cIII activity and cyt spectra. The O₂ consumption rates of mzm1Δ/mzm1^{D25N} and mzm1Δ were 56% and 54% less than that of mzm1Δ/MZM1, respectively (Fig. 2B).

Likewise, the cIII activity was reduced in mzm1Δ/mzm1^{D25N} (65% residual activity) and mzm1Δ (50%) mitochondria (Fig. 2C). Moreover, the mzm1Δ/mzm1^{D25N} strain showed marked reduction of the peak at 570 nm, corresponding to respiratory cyt b (not shown). These results are consistent with the temperature-sensitive respiratory growth defect observed in the mzm1Δ/mzm1^{D25N} serial dilution spot test.

Mzm1p plays a role in the last steps of cIII assembly, being involved in the maturation of the Rieske iron-sulfur protein (Rip1p in yeast), an essential catalytic subunit of cII [Atkinson et al., 2011; Cui et al., 2012]. Before incorporation into nascent cII, the Rip1p precursor undergoes two proteolytic cleavages, which remove a 30-amino-acid import signal at the N-terminus. The mitochondrial matrix peptidase removes the first 22 N-terminal residues, giving rise to a partially processed, immature form of Rip1p (i-ISP). Next, eight additional N-terminal residues are removed by Oct1p, to generate the mature form (m-ISP), which is eventually incorporated in the nascent cII [Graham et al., 1994; Nett and Trumpower, 1999]. MZM1 deletion impairs Rip1p maturation and causes marked decrease in its steady state level. In order to evaluate the effects of the mzm1^{D25N} mutation on Rip1p biosynthesis and cIII function, we studied mitochondria purified from mzm1Δ/MZM1, mzm1Δ/mzm1^{D25N}, and mzm1Δ strains, cultured at 37°C in YNB medium. Mitochondrial proteins were resolved by SDS-page and both i-ISP and m-ISP were immunovisualized by Western-blot analysis. As shown in Figure 2D, m-ISP and i-ISP levels were strongly reduced in the mzm1^{D25N} compared with the MZM1 parental strain, resembling the mzm1Δ strain defects [Cui et al. 2012]. The reduced level of Rip1p was associated with a clear reduction of cIII holocomplex (Fig. 2E), with no trace of stable subassembly intermediates (Fig. 2F). These data indicate that the Mzm1^{D25N} mutant protein impairs the stabilization/maturation of ISP and, as a consequence, the assembly of the cIII holoenzyme, thus supporting the pathogenic role of its human ortholog.

Whilst numerous mutations of the gene encoding cyt b, a mtDNA-encoded cIII subunit, have been associated with slowly progressive, usually adult onset, mitochondrial myopathy, mutations in nuclear genes associated with cIII deficiency are much rarer, and characterized by severe clinical course. In fact, only a handful of nucleus-encoded proteins related to cIII biogenesis have been linked...
to disease in humans: three small structural subunits, Ubiquinol cyt c-reductase binding protein (UQCRB), Ubiquinol-cyt c-reductase subunit VII (UQCRQ), and Ubiquinol-cyt c-reductase core 2 protein (UQRCR2), and two cIII assembly factors, BC-synthesis-1 like (BCS1L) and tetra-trico-peptide repeat domain 19 (TTC19). Only one mutation in each of the cIII encoding subunits UQCRB, UQCRQ, and UQRCR2 has been reported, each in single families, and with clinical presentations of variable severity and features [Ghezzi and Zeviani, 2011]. However, several mutations in BCS1L are known, involving a clinical spectrum ranging from Biornstad syndrome [Miyake et al., 2013; Ghezzi and Zeviani, 2011]. Only one mutation in each of the cIII encoding subunits UQCRB, UQCRQ, and UQRCR2 has been reported, each in single families, and with clinical presentations of variable severity and features [Ghezzi and Zeviani, 2011]. However, several mutations in BCS1L are known, involving a clinical spectrum ranging from Biornstad syndrome (nerve deafness and piti torti) (MIM #209900), to isolated early onset encephalopathy (MIM #124000), to multisystem GRACILE syndrome (MIM #603358), linked to a founder mutation that is part of the Finnish disease heritage. TTC19-mutant patients are characterized by slowly progressive neurodegenerative disorder, usually starting in infancy or childhood, although a late-onset rapidly progressive neurological syndrome has also been described, resembling Jacob-Creutzfeldt Disease [Ghezzi et al., 2011]. LYRM7 is a novel human cIII assembly factor containing a LRY motif. LYRM7 is involved in the maturation of the Rieske iron–sulfur protein, an essential catalytic subunit containing a (2Fe–2S) cluster, which is incorporated late during cIII assembly [Cui et al., 2012]. Interestingly, small LRY proteins are deemed to be involved in the biogenesis of iron–sulfur cluster containing structures, including respiratory chain complex I, where several LRY proteins are present as ancillary subunits, and complex II, for which a LRY-containing assembly factor, SDHAf1, was recently identified [Ghezzi and Zeviani, 2011]. The role of LYRM7 in maturation/incorporation of the Rieske protein in cIII explains the specific biochemical phenotype and the severe, early onset encephalopathy with lactic acidosis found in our patient, who carried a disease-segregating homozygous missense mutation in LYRM7, affecting a highly conserved amino-acid residue. In the yeast Saccharomyces cerevisiae, the ortholog of LYRM7 is MZM1, which plays the same role on cIII assembly. We took advantage of the phylogenetic conservation of both gene and mutant residue, to demonstrate the pathogenic role of the human variant in a yeast recombinant model carrying the equivalent mutant allele. We clearly showed that the mutant yeast strain has impaired oxidative growth, low cIII activity, reduced amount of cyt b and cIII holocomplex; the mature Rieske protein steady state level is also strongly decreased. Taken together, these results confirm the pathogenicity of the mutation, according to a recessive trait, and provide genetic evidence for a role of LYRM7 in a terminal step of cIII assembly, relevant to warrant OXPHOS proficiency in cells and tissues.

Figure 2. Phenotypes of the yeast model (A) oxidative growth.
Mzm1Δ strains harboring a wild-type MZM1 allele, the mzm1ΔD25N allele or the empty vector were serially diluted from 10^2 to 10^6 cells/mL. Five microliters of each dilution was spotted on YNB minimum agar plates minus uracil with dextrose or glycerol. Plates were incubated at 37°C for 4 days. (B) Oxygen consumption rate was recorded on cells grown at 37°C in YNB medium without uracil, supplemented with 0.6% dextrose. Values were normalized to wild-type rate of oxygen consumption (35 nmol O_2·min^-1·mg^-1) and represented as the mean of at least three values ± SD. One-tail, unpaired Student’s t-test was applied for statistical significance (P < 0.05). (C) Complex I–III specific activity was recorded on purified mitochondria from cells grown at 37°C as in B. Values were normalized to that of MZM1 parental strain (413.5 units per mg of mitochondrial proteins) and represented as the mean of at least two independent experiments (D) ISP immunoblot analysis. Fifteen micrograms of total purified mitochondrial proteins was separated by a 12% SDS-PAGE and electroblotted onto a nitrocellulose membrane; the ISP protein was immunovisualized by a specific antibody. (E) Western-blot immunovisualization of first dimension (1D) blue-native gel electrophoresis. An antibody against Core1 and 2 was used to detect complex II (cIII); an antibody against CoxII (cytochrome c oxidase, subunit II) was used for complex IV (cIV). Signal quantification and normalization were carried out using QuantityOne (Bio-Rad). The signal of the mzm1Δ/MZM1 (wild-type) strain, taken as 1.00, was used to normalize those of the other samples. (F) Western-blot immunovisualization of second dimension (2D) blue-native gel electrophoresis. Antibodies against Core1 and 2 were used to detect complex III (cIII).

References
Atkinson A, Khamisonchuk O, Smith P, Sabic H, Eide D, Winge DR. 2010. Mzm1 influences a labile fraction of mitochondrial zinc important for respiratory function. J Biol Chem 285:19450–19459.
Atkinson A, Smith P, Fox JL, Cui TZ, Khamisonchuk O, Winge DR. 2011. The LRY protein Mzm1 functions in the insertion of the Rieske Fe/S protein in yeast mitochondria. Mol Cell Biol 31:3998–3998.
Baruffini E, Ferrero I, Foury F. 2010. In vivo analysis of mtDNA replication defects in yeast. Methods 51:426–436.
Buigiani M, Invernizzi F, Albertosi S, Briel I, Laamanen E, Carrara F, Moroni I, Farina L, Spada M, Donati MA, Uziel G, Zeviani M. 2004. Clinical and molecular findings in children with complex I deficiency. Biochim Biophys Acta 1659:136–147.
Crivellone MD, Wu MA, Tragoulof A. 1988. Assembly of the mitochondrial membrane complex system. Analysis of structural mutants of the yeast coenzyme QH2-cytochrome c reductase complex. J Biol Chem 263:14323–14333.
Cui TZ, Smith PM, Fox JL, Khamisonchuk O, Winge DR. 2012. Late-stage maturation of the Rieske Fe/S protein: Mzm1 stabilizes Rip1 but does not facilitate its translocation by the AAA ATPase Bci1. Mol Cell Biol 32:4400–4409.
Ghezzi D, Arezzi F, Zordan M, Peroni B, Lamporti E, Binni E, Cattaneo P, D’Adamo P, d’Adamo P, Diodato D, Costa R, Mariotti C, Uziel G, Smidler C, Zeviani M. 2011. Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies. Nat Genet 43:259–263.
Ghezzi D, Zeviani M. 2011. Mitochondrial disorders: Nuclear gene mutations. In Encyclopedia of life sciences (ELS). Chichester: John Wiley & Sons, Ltd. DOI: 10.1002/9780470015902.a0005540.pub2.
Goffrini P, Ercolino T, Panizza E, Giaché V, Cavone L, Chiarugi A, Dima V, Ferrero I, Mannelli M. 2009. Functional study in a yeast model of a novel succinate-dehydrogenase subunit B gene germline missense mutation (C191Y) diagnosed in a patient affected by a glomus tumor. Hum Mol Genet 18:1860–1868.

Graham LA, Brandt U, Trumpower BL. 1994. Protease maturation of the Rieske iron–sulphur protein after its insertion into the mitochondrial cytochrome bcl complex of Saccharomyces cerevisiae. Biochem Soc Trans 22:188–191.

Heckmatt JZ, Dubowitz V. 1984. Needle biopsy of skeletal muscle. Muscle Nerve 7:594.

Lodi T, Ferrero I. 1993. Isolation of the DLD gene of Saccharomyces cerevisiae encoding the mitochondrial enzyme d-lactate ferricytochrome c oxidoreductase. Mol Gen Genet 238:315–324.

Miyake N, Yano S, Sakai C, Hatakeyama H, Matsushima Y, Shiina M, Watanabe Y, Bartley J, Abdenur JE, Wang RY, Chang R, Tsuruaki Y, et al. 2013. Mitochondrial complex III deficiency caused by a homozygous UQRC2 mutation presenting with neonatal-onset recurrent metabolic decompensation. Hum Mutat 34:446–452.

Nett JH, Trumpower BL. 1999. Intermediate length Rieske iron–sulfur protein is present and functionally active in the cytochrome bcl complex of Saccharomyces cerevisiae. J Biol Chem 274:9253–9257.

Sanchez E, Lobo T, Fox JI, Zeviani M, Winge DR, Fernandez-Vizarra E. 2013. LYRM7/MZM1L is a UQCRFS1 chaperone involved in the last steps of mitochondrial Complex III assembly in human cells. Biochim Biophys Acta 1827:285–293.

Schiestl RH, Gietz RD. 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr Genet 16:339–346.

Sciacco M, Bonilla E. 1996. Cytochemistry and immunocytochemistry of mitochondria in tissue sections. Methods Enzymol 264:509–521.