Short Report

Novel c.191C>G (p.Pro64Arg) MPV17 mutation identified in two pairs of unrelated Polish siblings with mitochondrial hepatoencephalopathy


This study reports clinical, biochemical and histopathological findings associated with a novel homozygous MPV17 mutation in four patients with mitochondrial depletion syndrome. The severe course of the disease, which started in the first weeks of life, was dominated by a failure to thrive, hypotonia and liver dysfunction, with relatively mild neurological involvement. All affected infants died by 1 year of age. Laboratory findings included progressive liver failure (hypertransaminasaemia, icterus, and coagulopathy), recurrent hypoglycaemia, lactic acidaemia, hyperferritinaemia, and increased transferrin saturation. Histological and ultrastructural analyses uncovered significant lipid accumulation in hepatocytes and myocytes. A severe decrease in the mitochondrial/nuclear DNA (mtDNA/nDNA) ratio was found post-mortem in the livers (and in one muscle specimen) of both examined patients. Oxidative phosphorylation system (OXPHOS) Western blotting revealed low levels of complexes I, III and IV subunits. The highlights of our findings are as follows: (i) The novel p.Pro64Arg mutation is the second recurrent MPV17 mutation reported. The phenotype associated with the p.Pro64Arg mutation differs from the phenotype of the relatively common p.Arg50Gln mutation, suggesting the existence of a genotype–phenotype correlation. (ii) Tissues collected from patients during autopsy may be useful for both mtDNA/nDNA ratio assessment and OXPHOS Western blotting.

Conflict of interest

All authors declare no conflict of interest.
Recessive mutations in MPV17 are associated with hepatocerebral form of mitochondrial DNA depletion syndrome (MDS) (1) characterised by a severe reduction in mtDNA copy number. The exact function of MPV17 is still unknown. It is stated that its yeast ortholog, Sym1 is involved in the structural and functional stability of the inner mitochondrial membrane, control of oxidative phosphorylation system (OXPHOS) activity and mitochondrial morphology, as well as the maintenance and integrity of nucleoid structures (2).

In 31 MPV17-defective patients reported in the literature (3), 26 different mutations were found (HGMD Professional 2012.3). The disease manifests with hepatomegaly and progressive liver failure within the first weeks of life and leads to death in infancy or early childhood if not treated by liver transplantation (3).

Here, we describe four MPV17-mutated children from two unrelated families. This is the first report of mitochondrial depletion associated with MPV17 dysfunction in Polish patients.

Materials

Patients

Patient 1, a 9-month-old boy was referred to our gastroenterological ward with fulminant liver failure that developed after an upper respiratory infection. He died on the third day of hospitalisation, before qualifying for a liver transplant (LTx).

He was the first child of healthy consanguineous parents born after an uncomplicated pregnancy by caesarean section due to foetal asphyxia with birth weight 2980 g and Apgar scores 8/9/9. Weakness, impaired peripheral circulation, breathing problems with metabolic acidosis and hypoglycaemia appeared by the end of his first day of life. Neonatal screening for phenylketonuria was positive on the fourth day of life and normalised in 2 weeks. From the fifth week of life, recurrent vomiting, remarkable feeding problems with insufficient weight gain, truncal hypotonia and developmental delay were observed. At 9 months, there was severe clinical deterioration followed by progressive liver failure with hypoglycaemia and a high cerebrospinal fluid lactate concentration. The boy died from sudden cardiac arrest.

Patient 2, the younger sibling of Patient 1 was born at term (3230 g birth weight) with signs of foetal asphyxia, just prior to identification of the MPV17 mutation in his deceased brother. The neonatal dry blood spot tandem mass spectrometry (TMS) test was normal. When observed by us at the age of 6 weeks, the boy presented with hypotonia and severe failure to thrive. When he was 3 months old (after molecular confirmation of the c.191C>G MPV17 mutation), the parents decided to discontinue treatment. Significant deterioration had occurred; the patient’s status was severe, with hepatomegaly, ascites and haemorrhagic diathesis. He died at the age of 1 year due to progressive liver failure.

Patient 3 was a girl and the first child of healthy, unrelated parents. She was born with a weight of 3050 g and an Apgar score of 10. She presented at 2 months with severe failure to thrive, truncal hypotonia, weak tendon reflexes, strong fasciculations, cholestasis and a tendency to hypoglycaemia. At the age of 2–3 months, roving eye movements, nystagmus, episodes of unconsciousness and inspiratory stridor appeared. At 5 months, she developed liver failure with icterus, hepatomegaly, clotting disturbances, elevated transaminases and gamma-glutamyltranspeptidase (GGTP) activity, and increased bile acid and ferritin concentrations. An electrophysiological study showed neurogenic changes in muscles and signs of axonal—demyelination polyneuropathy. Evident neurological involvement disqualified LTx. The girl died at 12 months. MDS could not be verified at the time due to the unavailability of DNA analysis.

Patient 4, the younger sister of Patient 3 was born at term after an uneventful pregnancy, with a weight of 3980 g and an Apgar score of 10. The neonatal TMS amino acid profile was normal. The urine organic acid profile showed an excess of lactate and dicarboxylic acids. Her development was otherwise normal until 4 months, when she developed failure to thrive and a slight liver enlargement with raised transaminase activities. By 6 months the girl deteriorated rapidly, with recurrent episodes of vomiting, dehydration, hypoglycaemia, severe lactic acidosis, progressive liver failure with jaundice, elevated liver enzymes, hepatomegaly, ascites, coagulopathy, and bradycardia. She was treated at a regional hospital and was referred to our centre when her status was very severe, with cardiopulmonary insufficiency, sepsis and anuria. She died 9 days later.

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. All parents or authorized tutors of the patients gave their informed consent for the study, and this protocol was approved by the CMHI Bioethics Commission.

Methods

Histological, histochemical and spectrophotometric examinations of available tissues were performed according to routine protocols.

Levels of individual mitochondrial respiratory chain subunits were assessed with the MitoProfile® Total OXPHOS Human WB Antibody Cocktail (MitoSciences, Eugene, OR) and antibodies against subunits of the individual respiratory chain complexes followed by secondary AP-conjugated antibodies (Bio-Rad Laboratories, Marnes-la Coquette, France).

Total DNA was isolated by standard phenol/chloroform extraction. A modified real-time polymerase chain reaction (PCR) protocol was used, as published by Walker (4).

Coding regions of the DGUOK, POLG and MPV17 genes were amplified, directly sequenced in 3130 Genetic Analyzer, and analysed with Sequencing Analysis Software v.5.4 (Applied Biosystems/Life Technologies, Foster City, CA). All available family members and 100 control subjects were tested for the molecular variant found in the patients.
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Fig. 1. Electropherograms of the MPV17 exon 3 fragment containing the c.191C>G mutation (reverse direction). (a) Arrow indicates mutation. (b) Wild-type sequence.

Long-range PCR employing a pair of primers (nucleotides: 6841–6860 and 16526–16546), and the Expand Long Template PCR System (Roche Applied Science, Mannheim, Germany) were used to amplify a ~9.8 kb product across the major arc of wild-type mtDNA to screen for mtDNA rearrangements.

Results

The novel c.191C>G (p.Pro64Arg) mutation in MPV17 exon 3 was revealed in two alleles in all patients studied (Fig. 1). The mutation was identified in the heterozygous form in parents from both families. Due to the absence of a functional assay for this protein, its pathogenicity was determined by the clinical phenotype, amino acid conservation, the screening of 200 control alleles, and prediction algorithms (Alamut and SIFTBlink).

Severe mtDNA depletion was detected in the liver of the two patients who were examined: Patient 1 (autopsy: 0.01% and 9.6%) and Patient 4 (autopsy: 3.6% and 16.8%). mtDNA depletion was also found in muscle samples of Patient 4 (biopsy: 0.06%, autopsy: 15.8%), but not in Patient 1 (autopsy: 101.5%). There was no mtDNA depletion in the remaining tissues of Patient 1: brain (106.1%) and heart (119.6%) or Patient 4: kidney (60.4%) and heart (91.5%). Additionally, a pattern of multiple mtDNA deletions was observed in the liver samples of Patients 1 and 4 (Fig. 2).

The iron/TIBC ratio in Patient 2 (at 3 months), Patient 3 (at 3 months), and Patient 4 (at 6 months) was 80.3% (90/112 μg/dl), 34.3% (72/210 μg/dl), and 100% (43/42 μg/dl), respectively. Ferritin was elevated to 927, 949.4 and 546 ng/ml (control 12–327 ng/ml) when measured in Patients 2, 3, and 4, respectively. The methionine concentration, which was measured several times during follow-up appointments with the patients reported in this study, did not differ from normal values.

Liver and skeletal muscle contained a variable degree of lipid accumulation, from moderate to severe (Figs S1 and S2), resembling in Patient 4 (at the age of 7 months) a lipid storage myopathy. This pattern was accompanied by the unquestionably pathological microvascular lipid degeneration of cardiomyocytes and most likely also renal tubular cells found in autopsy both in the light and electron microscopy (not shown).

The cytochrome c oxidase (COX) deficiency was not a salient feature in the investigated MPV17-mutated tissues. However, the study revealed decreased expression of complex I subunit and the occurrence of selective degradation products of the complex III subunit (Fig. 3) that may be related to a specific mitochondrial membrane destruction and merit further study.

Discussion

MPV17-related MDS is extremely rare, with the exception of a Navajo tribe living in southern North America (5). Most of single-affected families identified all over the world carry ‘private’, usually homozygous mutations. Only six compound heterozygotes have been reported thus far (1, 6–8). Interestingly, both Polish families reported in this study, although not related,
have their roots in a central part of the country characterised by low population mobility.

Recent reports have shown that mutations in \textit{MPV17} are not exclusively found in infantile hepatocerebral MDS but can be associated with various clinical presentations and result in either quantitative or qualitative mtDNA abnormalities. \textit{MPV17} causative mutations and multiple mtDNA deletions were found in adult patients with neuropathy and leukoencephalopathy (9) and in those with multisystemic disorder (10). The simultaneous occurrence of both mtDNA depletion and deletions found in the reported patients is not frequently identified.

From the clinical point of view, the four Polish infants with the novel p.Pro64Arg \textit{MPV17} mutation displayed a strong clinical resemblance to the majority of patients reported in the literature (1, 6, 8). Some features that consistently occurred in the reported cases should be emphasised.

First, increased transferrin saturation (much above the upper limit control values of 30\%) developed in all four patients. We can speculate that increased ROS and impaired antioxidant defences associated with abnormal iron metabolism may lead not only to liver failure but also to neuropathic pathology. Decreasing transferrin oversaturation should be considered as a supportive treatment in patients with MDS.

Second, our observations do not confirm the previously documented elevated methionine levels in a laboratory profile of the \textit{MPV17} defect (11).

Finally, severe microvesicular steatosis (90\% and 80\%) was found not only in both investigated livers but was also present in other tissues apparently not involved in the disease process.

Our observations emphasize the need for extension of the standard procedure by the storage of the frozen tissue sections in each case of unexplained death of an infant, particularly at the emergency. Identification of mtDNA depletion or deletions in such collected tissues may be of significant importance for the family with reproductive perspectives. In Patient 1, the initial misdiagnosis was an acute liver failure caused by a viral
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infection; a mitochondrial defect was not considered in this family until severe mtDNA depletion was detected in the liver tissue gathered at autopsy. In Patient 3, MDS was clinically diagnosed, but could not be confirmed until a proper storage procedure was applied at the autopsy of the younger affected sibling.

Supporting Information

The following Supporting information is available for this article:

Fig. S1. Histological and histochemical studies of livers and skeletal muscle of the patients with c.191C>G MPV17 mutation. (a) Muscle biopsy from Patient 4 (at 7 months) shows severe lipid accumulation consistent with a lipid storage myopathy. Oil red O, ×100 original magnification. (b) Liver section from Patient 1 shows severe microvesicular steatosis, hepatocyte damage, necrosis (30%), and fibrosis with early nodular transformation. Oil red O, ×200 original magnification. (c) Post-mortem examination of liver of Patient 1 revealed severe microvesicular steatosis (90%), hepatocyte damage and degeneration, no evident necrosis (10–20%) or cholestasis, and moderate fibrosis with early nodular transformation. H and E, ×200 original magnification. (d) A core needle biopsy of Patient 3 showed severe microvesicular steatosis (80%), hepatocyte damage and degeneration, no evident necrosis (30%) or cholestasis, and mild periportal fibrosis. PAS staining, ×400 original magnification.

Fig. S2. Ultrastructural findings in MPV17 deficiency. Muscle of Patient 4: (a) Muscle cells with dispersed and accumulated LBs. ×9000 magnification. (b) Lipid body with dense granular cap (arrow). ×60,000 magnification. (c) Affected nucleus occupied by altered mitochondria and a large number of LBs. ×22,000 magnification. Liver of Patient 3: (d) Accumulated LBs. ×9000 magnification. (e) Two enlarged mitochondria with electron dense matrix and unstructured cristae consisting vesicle like structures. ×80,000 magnification. (f) Large lipid-like droplets within mitochondrial matrix (asterix). ×60,000 magnification.

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

Acknowledgements

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References