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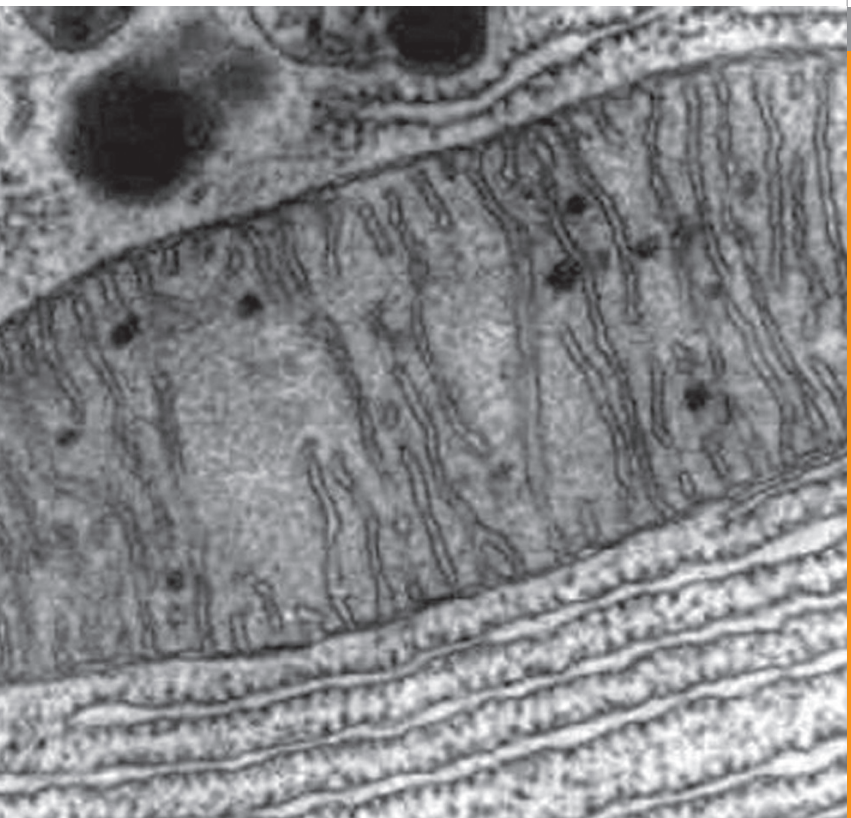
Persistent pulmonary hypertension and congenital intestinal dysmotility in a newborn with mitochondrial respiratory chain disease



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INTRODUCTION

Mitochondrial respiratory chain (RC) diseases can affect multiple organs including the nervous system, heart, skeletal muscle, liver and gastrointestinal system. Antenatal manifestations include growth retardation, cardiomyopathy and congenital malformations (1). The most common neonatal presentations are neurologic, hepatic and intestinal, followed by hypoglycemia, cardiac and hematologic manifestations, and failure to thrive (2). The neonatal presentation of RC disease is associated with high mortality. No effective medications are known and treatment is supportive. Because of their variable and unspecific manifestations, RC disorders represent a diagnostic challenge which requires a high level of suspicion. We present a critically ill newborn with a previously unreported association of two rare manifestations of mitochondrial RC disease.

CASE REPORT

A female patient was born at 38 weeks of gestation to a G1/P1 mother. Apgar scores were 9, 10 and 10 at 1, 5 and 10 minutes, respectively. Umbilical cord pH was 7.11 (arterial) and 7.22 (venous). The patient was symmetrically growth restricted with a birth weight of 2050 g (< P3), a length of 45 cm (P 3-10) and a head circumference of 32 cm (P 3-10). Physical examination and vital signs were unremarkable.

At 30 hours of life, the pediatrician was called because the infant had persistent vomiting. There was profound cyanosis with a SaO2 of 35% and poor peripheral perfusion. Heart rate was 170/min and respiratory

rate was 70/min with mild retractions. SaO2 remained at 60% despite intubation and mechanical ventilation with 100% oxygen. Arterial blood gas showed marked hypoxemia and lactic acidosis (pH 7.06, PaO2 16 mmHg, PaCO2 32 mmHg, HCO3 8.5 mmol/L, lactate 19 mmol/L). Chest radiography showed a normal cardiac silhouette with clear lung fields and decreased vascular markings (Fig. 1). Echocardiography revealed suprasystemic pulmonary hypertension with right to left shunting through the foramen ovale. She immediately responded to 20 ppm of inhaled nitric oxide (iNO) with a PaO2 increasing to 364 mmHg.

The patient was weaned off iNO on day of life 3 and was extubated the following day. She progressively deteriorated within a few hours with decreased SaO2, agitation and lactic acidosis. Treatment with CPAP and iNO improved oxygenation (PaO2 250 mmHg) and lactate decreased from 8 to 5 mmol/L. Several attempts to wean iNO failed, even under sildenafil therapy.

After 2 weeks of iNO and oxygen, intravenous L-arginine (2 mmol/kg/day) and oral tetrahydrobiopterin (BH4) (10 mg/kg/day) were introduced and allowed successful weaning off iNO and CPAP. Plasma arginine levels were maintained between 100 and 160 µmol/L and urinary pterin analyses confirmed adequate BH4 absorption.

The patient stabilized for several weeks under this

treatment with oxygen requirements of less than 30%. However, hypoxic crises replapsed in the 4th month of life. A trial with coenzyme Q10, riboflavin, thiamin and L-carnitine did not influence lactic acidosis.

Since admission, the patient had abundant gastric residuals. The abdomen was soft with normal bowel sounds. Gastrointestinal tract contrast studies ruled out obstruction but revealed an increased transit time consistent with intestinal dysmotility. The patient could not tolerate enteral nutrition despite prokinetic treatments (metoclopramide, erythromycin, cisapride) and feeding through an oroduodenal tube. Therefore, she remained dependent on total parenteral nutrition. She developed mild cholestasis but liver function was stable with good synthetic parameters and transaminases within the normal range. No neurologic abnormalities were detected on clinical examination. Brain MRI was normal, and brain MRS showed a normal metabolite profile including lactate.

On day of life 83, echocardiography revealed suprasystemic pulmonary hypertension with right ventricular hypertrophy and dilatation. In agreement with the parents and because of the poor prognosis, it was decided not to restart iNO treatment and to provide palliative care. The patient developed hypoxic crises of increasing frequency and expired at 4.5 months of age. Autopsy showed marked cardiac hypertrophy affecting primarily the right ventricle. Microscopic

examination revealed concentric hypertrophy of the pulmonary vasculature with abnormal extension of smooth muscle into intra-acinar arterioles (Fig. 2). Intestinal smooth muscle had a normal histologic appearance. Ganglionic cells were identified in myenteric and submucosal plexuses. The liver was enlarged with signs of periportal fibrosis. Other organs including the brain were unremarkable.

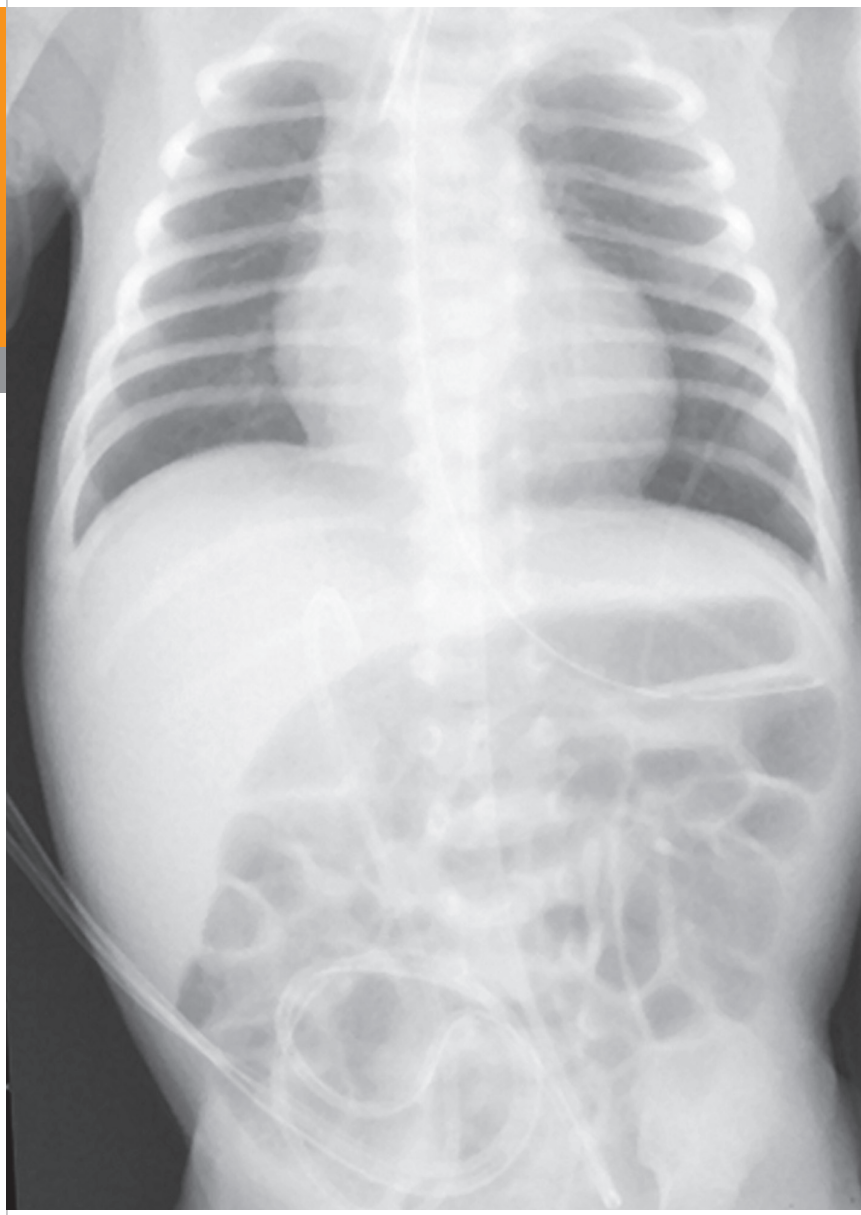
A metabolic work-up initiated in the first week of life revealed persistent lactic acidosis (plasma lactate 16-21 mmol/l) with elevated lactate/pyruvate ratio in several samples (20-28 ; normal values <20) and increased plasma alanine (496-2600 µmol/L ; normal range 100-310). Urinary organic acids revealed massive excretion of lactate (14800 mmol/mol creatinine), pyruvate (1700 mmol/mol creatinine), and Krebs cycle intermediates (succinate 241 mmol/mol creatinine, fumarate 57 mmol/mol creatinine, malate 137 mmol/mol creatinine) with ketosis (3-hydroxybutyrate 911 mmol/mol creatinine).

As the biochemical pattern was suggestive of mitochondrial dysfunction, muscle and skin biopsies were performed. A defect in oxidative phosphorylation was found both in fresh-frozen muscle tissue and cultured skin fibroblasts (Table 1). In our patient, the activity of complex IV was reduced to 43% and 37% of normal values in muscle tissue and skin fibroblasts, respectively. Western blot analysis of complex I-IV in muscle

tissue showed lower complex IV protein levels in our patient compared to controls. Mitochondrial DNA quantification in skeletal and cardiac muscle ruled out mitochondrial DNA depletion. Neither structural anomalies, nor point mutations were found by complete screening of mtDNA in several tissues (skeletal and cardiac muscle, lungs, jejunum, ileum, colon). Because of its role in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome, the thymidine phosphorylase gene (TYMP) was analyzed by direct sequencing of all exons and intron-exon boundaries, but no mutation was found. SCO1, SCO2, COX10 and COX15 (nuclear genes involved in complex IV deficiency) were tested in genomic DNA, but no mutation was found.

Comparative genomic hybridization array, performed with an Agilent oligoNT array 244K (50 Kb resolution), revealed a maternally inherited heterozygous duplicated region of 453-479 Kb on chromosome 6q22.31 (bp 118'774'552 to bp 119'228'361, human genome build 36). This genomic rearrangement does not correspond to known copy number variations (CNVs) (3) and encompasses the whole phospholamban (PLN) gene (OMIM 172405), which has been implicated in the pathogenesis of cardiomyopathies (4). However, the same duplication was found in the asymptomatic mother, indicating that it was unlikely to be the cause of the disease.

Fig. 1



Radiography at 30 hours of life showing a normal cardiac silhouette with clear lung fields and decreased vascular markings.

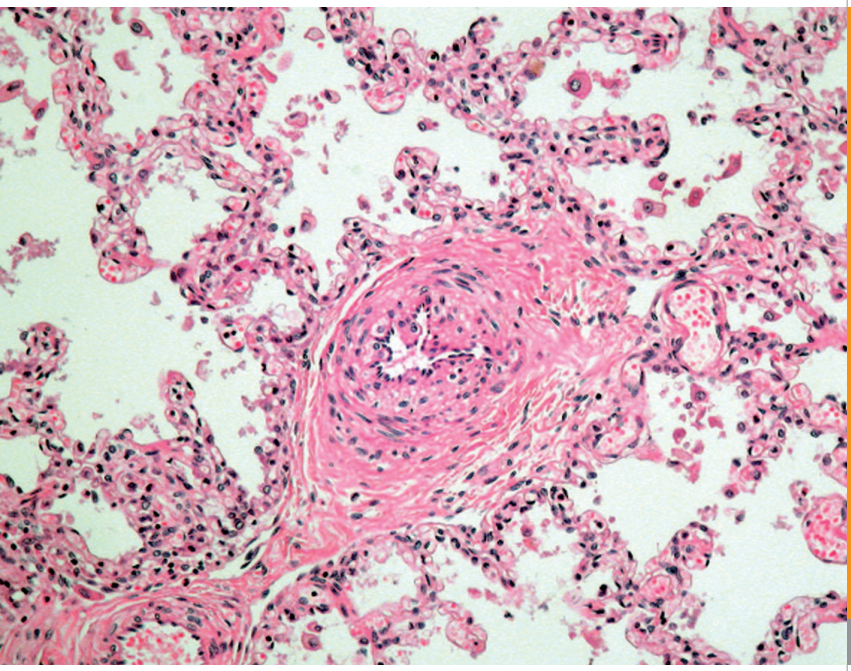


Fig. 2

Lung section showing concentric hypertrophy of the pulmonary vasculature with abnormal extension of smooth muscle into the intra-acinar arterioles (H&E, original magnification x 200).

Table 1

Oxidative phosphorylation (OXPHOS) enzyme activities in skeletal muscle and cultured skin fibroblasts.

OXPHOS enzyme	Skeletal muscle (mU/mU CS)		Fibroblasts (mU/mU CS)	
	Patient (% controls)	Controls (n = 31) Range (Mean ± SD)	Patient (% controls)	Controls (n = 22) Range (Mean ± SD)
Complex I	0.17 (89)	0.14-0.28 (0.19 ± 0.09)	0.19 (70)	0.19-0.46 (0.27 ± 0.06)
Complex II	0.23 (109)	0.14-0.36 (0.21 ± 0.05)	0.23 (70)	0.17-0.52 (0.33 ± 0.09)
Complex III	0.80 (102)	0.50-1.11 (0.78 ± 0.15)	0.45 (75)	0.35-0.87 (0.60 ± 0.15)
Complex IV	**0.51 (43)**	0.57-1.76 (1.16 ± 0.28)	**0.28 (37)**	0.42-1.11 (0.75 ± 0.18)
Complex V	0.37 (95)	0.17-0.66 (0.39 ± 0.13)	0.16 (80)	0.12-0.38 (0.20 ± 0.08)
Citrate synthase (CS) (mU/mg prot)synthase (CS) (mU/mg prot)	70 (67)	70-173 (105 ± 25)	211 (116)	134-228 (181 ± 29)

Persistent pulmonary hypertension of the newborn (PPHN) is characterized by failure to achieve or sustain the normal decrease of pulmonary vascular resistance at birth and is associated with right to left shunting of blood across the patent ductus arteriosus and/or the foramen ovale. PPHN usually presents within 12 hours of birth with cyanosis but relatively mild respiratory distress. PPHN can be primary or secondary to a variety of disorders including fetal or perinatal hypoxia, infection, meconium aspiration, congenital diaphragmatic hernia, pulmonary hypoplasia, alveolar capillary dysplasia and congenital heart disease. Our patient presented at 30 hours of life with PPHN and lactic acidosis. Most secondary causes of PPHN were ruled out.

Pulmonary hypertension is a known manifestation of RC disorders, but it is rare, especially when it occurs without concomitant cardiomyopathy. Few cases have been described and only two were newborns (5-7). Deficiencies in cytochrome-c oxidase, RC complex I, II, III and IV have been identified, suggesting that pulmonary hypertension is not associated with a specific enzymatic RC deficit. Following the diagnostic score for mitochondrial disease proposed by Morava et al (8) that takes into account clinical and metabolic parameters, our patient reached a score of 6 (2 points for involvement of gastrointestinal tract and heart, 4 points for lactic acidosis, elevated L/P ratio, elevated alanine and urinary Krebs cycle intermediate excretion), which corresponds to a "probable" mito-

chondrial disease. Both enzymatic and Western blot analyses confirmed the presence of a significant impairment of oxidative phosphorylation with a quantitative deficiency of complex IV. The similarity of the RC profile in both muscle tissue and fibroblast culture confirms the significance of this finding. Despite extensive molecular investigations, we could not identify a genetic mutation responsible for the RC disease in our patient. This is not unusual in mitochondrial disorders and indicates that important genes for mitochondrial function remain to be identified.

Management of newborns with PPHN involves oxygen therapy, mechanical ventilation, hemodynamic support and iNO. Sildenafil, a phosphodiesterase inhibitor, has recently been used to improve oxygenation in PPHN (9). Likewise, L-arginine, a NO donor, has been administered to increase oxygenation in PPHN (10). L-arginine has also been used for treatment of mitochondrial diseases. It improved endothelial function and decreased symptoms of stroke in patients with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke) (11). The cofactor tetrahydrobiopterin (BH4) is an important regulator of nitric oxide synthase (NOS) enzymatic function and a reactive oxygen species (ROS) scavenger. BH4 promotes coupling of NOS reaction in the presence of ROS and therefore prevents further formation of peroxynitrites. In mice, BH4 deficiency caused pulmonary hypertension (12) and induction of BH4 synthesis in the endothelium prevented hypoxia-

induced pulmonary hypertension. Since sildenafil and L-arginine supplementation did not improve oxygenation and echocardiographic signs of pulmonary hypertension in our patient, we added BH4 in order to further promote endogenous NO synthesis. This treatment allowed complete weaning off iNO and stabilization of oxygenation for several weeks, suggesting an additive or synergistic effect of L-arginine (substrate) and BH4 (cofactor) on the endothelial NO synthase. Unfortunately, the effect of this treatment was transient and pulmonary hypertension relapsed in the 4th month of life. The worsening of gastro-intestinal function may have impaired BH4 enteral absorption.

The pathophysiological link between mitochondrial disturbance and pulmonary hypertension is unknown. Oxidative phosphorylation supplies most organs with energy. In consequence, abnormal oxidative phosphorylation induced by RC disease can theoretically affect any tissue, including the pulmonary vasculature. Mitochondria are a major source of ROS which can regulate vascular tone. Thus, abnormal ROS production by pulmonary endothelial cells may have caused pulmonary hypertension.

Intestinal dysmotility or intestinal pseudo-obstruction is a rare disorder characterized by signs and symptoms of intestinal obstruction in the absence of a mechanical obstructive lesion. A wide spectrum of pathologic disorders can alter bowel motility. Treatment involves

parenteral nutrition, prokinetics and surgical decompression. Intestinal dysmotility has been described in patients with RC disease (13). The onset of symptoms is typically in the second or third decade and it is most commonly a manifestation of MNGIE syndrome. Intestinal dysmotility due to RC disease rarely presents during childhood. However, it has been described in six newborns with RC disease who required long-term nutritional support and subsequently developed neurologic symptoms (14).

The unique association of PPHN and intestinal dysmotility in our patient adds to the large clinical spectrum of RC disorders. Remarkably, there was no clinical involvement of liver, kidney, muscle and brain, which are commonly affected in neonatal RC disorders. Recognition of mitochondrial dysfunction as the underlying cause of pulmonary hypertension may indicate poor prognosis, as all the few cases reported so far have had a fatal outcome (5, 6). Management of pulmonary hypertension was complicated by an unusually long-lasting iNO dependence. L-arginine and BH4 seemed to be helpful, at least transiently, in reducing iNO dependence and their combined effect may therefore be considered for the treatment of milder cases or in cases of isolated PPHN, with or without underlying mitochondrial dysfunction.

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