Abstract  

Objects: We hoped to itemize the clinical and neuroradiological features of six neonates with mitochondrial disorders.  

Methods: We examined a case series of six neonates. The diagnosis of mitochondrial cytopathy was made on the basis of spectrophotometric measurements of respiratory chain enzyme activities in skeletal muscle biopsy specimens. Magnetic resonance (MR) imaging was performed in all cases. Conclusions: The antenatal onset in five cases and the lack of any symptom-free interval are suggestive of fetal expression of the disease. No specific symptoms were found: arthrogryposis congenita multiplex in one, progressive hepatocellular dysfunction in three, encephalomyelopathy and cardiomyopathy in four. Complex I deficiency was found in three patients, while one patient had a defect of complex IV and the last a combined defect of complexes I and IV. Neuroradiological findings were either cerebral atrophy or white matter abnormalities of the brain stem in all cases but one and gave additional information, because clinical symptoms are not quite specific. The combination of clinical and MRI findings in neonatal cases can rule out hypoxic isch-emic encephalopathy, which suggests an additional screening method to look for mitochondrial disorder.  

Keywords  
Mitochondrial disease · Respiratory chain · MR imaging · White matter changes

Introduction  
Oxidative phosphorylation, i.e. the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP, occurs in almost all tissues. Consequently, a disorder of oxidative phosphorylation can induce a variety of symptoms in any organ or tissue at any time from conception onward and throughout fetal and postnatal development [12]. In addition, any mode of inheritance is possible, owing to the mixed genetic origin of mitochondrial respiratory chain enzymes, which are encoded both by the nuclear genome and by mitochondrial DNA (mtDNA), which is maternally inherited, except for complex II, which is entirely of nuclear origin [17]. Oxidative phosphorylation occurs via five multienzymatic complexes: NADH-coenzyme Q (CoQ) reductase (complex I), succinate CoQ reductase (complex II), CoQ-H2 cytochrome-c reductase (complex III), cytochrome-c oxidase (complex IV), and ATP synthase (complex V).
The onset of oxidative phosphorylation disorders is frequently in the neonatal period (36% of cases in a paediatric study) [11]. In general, the prevalent symptoms are either isolated myopathy or multisystemic disorders with encephalomyopathy, nephropathy, hepatic involvement and cardiomyopathy. These symptoms are not specific in neonates. Encephalopathy, acute renal failure and pulmonary hypertension mimicking cardiomyopathy can be caused by birth asphyxia, making the differential diagnosis difficult.

The diagnosis of a mitochondrial disorder is based on the presence of clusters of abnormal mitochondria in muscle cells (ragged red fibres) and/or a biochemically defined defect in the respiratory chain enzymes and/or mutations in nuclear or mitochondrial DNA [11]. However, ragged red fibres are rarely encountered in neonates.

Neuroimaging has proved useful in the diagnosis of mitochondrial syndromes. Kearns-Sayre syndrome and mitochondrial encephalomyelopathy lactic acidosis and stroke-like episodes syndrome (MELAS) show characteristic lesions on computed tomography (CT) and magnetic resonance (MR) imaging studies [20]; but these syndromes are never encountered in newborns. Typical imaging findings are the hallmark of Leigh syndrome, which is rare in the neonatal period. So far, very little is known about the value of neuroradiological studies in neonates presenting with mitochondrial disorders.

We report the clinical features and the neuroradiological findings in six cases of neonatal mitochondrial disorder.

Patients and methods

Patients

This study describes a case series of 6 neonates seen with mitochondrial disorders over a period of 4 years (1996–1999). They were acutely sick newborns who required intensive care.

Methods

Onset, symptoms and outcome of the disease during the perinatal period was given in all. Diagnosis of mitochondrial cytopathy was based on the biochemical demonstration of a defect in the respiratory chain enzymes and/or a mutation of the mitochondrial DNA in muscle cells. Muscle samples were obtained by biopsy of the quadriceps muscle under general anaesthesia after birth and always after 40 weeks of age, corrected for prematurity.

Histology

For histochemical studies, frozen skeletal muscle sections were used. Modified Gomori trichrome staining was used on adjoining sections, while lipid staining with Sudan black was performed on frozen tissue. Autopsies and histopathological studies were carried out on patients 3 and 4.

Biochemistry

Spectrophotometric measurements of enzyme activities were performed on muscle homogenate prepared from about 30 mg of frozen muscle. All optical measurements were performed at 30°C and initiated by substrate addition (except for cytochrome c oxidase [38°C, enzyme addition]). Substrate conversion proceeded linearly with time and amount of enzyme. Citrate synthase was measured according to the methodology described by Mousson et al. [10]. For complex I (NADH ubiquinone reductase), the oxidation of NADH by rotenone-sensitive complex I was recorded using decylubiquinone (UQ2Sigma) as the electron acceptor. Mitochondria were disrupted by freeze thawing three times in liquid nitrogen. For complex II (succinate ubiquinone reductase), the oxidation of succinate was recorded using decylubiquinone (UQ2 Sigma) as the electron acceptor. Succinate dehydrogenase was measured in one case using phenazine methosulfate and dichloro-phenol-indophenol as the electron acceptor. For complex III (ubiquinol cytochrome-c oxidase) measurement of the reduction of cytochrome-c was recorded using decylubiquinol (UQ2H2) as the electron donor. Complex IV (cytochrome-c oxidase) was measured by the reoxidation of the cytochrome-c reduced by NaBH4. Because mitochondrial enrichment will vary between muscle homogenates, different ages and different levels of muscular activity, results were expressed as specific activities (nanomoles per minute and milligram of noncollagenic proteins compared with the citrate synthase activity) and were presented as both absolute values and ratios [14].

Molecular analysis of mtDNA

Five micrograms of total DNA was extracted from frozen muscle and circulating leukocytes, digested with Pvull (nt 2656), electrophoresed in a 0.8% agarose gel and transferred onto nylon membranes as previously described [4]. The filters were hybridised simultaneously with two probes. One probe was pGT 10/12.2 containing a 14032 bp EcoRI mtDNA insert cloned into pBB3 vector. The other probe contained nuclear encoded 18S rDNA sequences. The nylon filters were prehybridised, hybridised, washed and exposed according to the supplier (Amersham, U.K.). We compared samples from patients and controls on the same membrane.

MR imaging

MR imaging was performed in all cases on a 1-T magnet (Siemens, Magnetom SP 40) when the babies between 35 weeks and 49 weeks of age corrected for prematurity. Gradient echoT1-weighted and turbo spin echo T2-weighted images were obtained in all cases. T1-weighted images were obtained following the three planes of the head, while axial and coronal T2-weighted images were acquired.

Results

Clinical characteristics and outcome

This study was conducted in a series of four full-term newborn (patients 1, 4, 5, 6) and two preterm newborns born at 29 weeks, who were twins born following a monochorionic biamniotic pregnancy (patients 2, 3) (Table 1).

The disease had an antenatal onset for five of the six patients: hydrops fetalis in four (patients 1, 2, 3, 4) and...