

Mitochondrial DNA Depletion

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Abstract

The mtDNA depletion syndrome (MDS) is a clinically heterogeneous group of mitochondrial disorders characterized by a reduction of the mtDNA copy number in affected tissues without mutations or rearrangements in the mtDNA. Therefore, MDS is thought to be a primary process. Depletion of mtDNA can also be secondary (as seen in *inclusion body myositis*) or iatrogenic (as seen in patients treated with nucleoside analogs). MDS may be a relatively common neurogenetic disorder of infancy and childhood. In one report, 11% of young children (<2 years old) referred for weakness, hypotonia, and developmental delay had mtDNA depletion. MDS is phenotypically heterogeneous, manifesting either as a hepatocerebral form, a myopathic form, a benign "later-onset" myopathic form or a cardiomyopathic form. Children usually present in infancy with hypotonia, lactic acidosis, and elevated serum creatin kinase (CK). Some of them also have severe, often fatal hepatopathy or renal involvement mimicking the de Toni-Fanconi syndrome. A reduced activity of the respiratory chain, and, more importantly, a low mtDNA/nDNA ratio in affected tissues confirm clinical diagnosis. MDS often strikes recurrently in families with a possible autosomal recessive inheritance, suggesting a genetic defect in the nuclear DNA. Actually, mutations in the nuclear-encoded mitochondrial deoxyguanosine kinase (DGUOK) and deoxythymidine kinase (TK2) genes have been associated with the hepatocerebral and myopathic forms of MDS, respectively. These two genes encode mitochondrial salvage pathway enzymes, which are involved in mtDNA synthesis via supply of deoxyribonucleotides (dNTPs). Muscle weakness and exercise intolerance may be responsive to coenzyme Q supplementation.

Keywords

Mitochondrial DNA depletion syndrome; mitochondrial deoxythymidine kinase (TK2); mitochondrial deoxyguanosine kinase gene (DGUOK); mutation screening.

Disease name

Mitochondrial DNA depletion syndrome (MDS)

Definition/diagnostic criteria

MDS is a clinically heterogeneous group of mitochondrial disorders characterized by a

reduction of the mtDNA copy number in affected tissues without mutations or rearrangements in the mtDNA (Hirano *et al.*, 2001).

After the initial report of MDS (Moraes *et al.*, 1991), additional patients have been described (Tritschler *et al.*, 1992; Mazziotta *et al.*, 1992; Telerman-Toppet *et al.*, 1992; Larsson *et al.*, 1994; Mariotti *et al.*, 1995; Poulton *et al.*, 1995; Paquis-Flucklinger *et al.*, 1995; Pons *et al.*, 1996; Kirches *et al.*, 1998), all exhibiting variable levels of mtDNA depletion in affected tissues, whereas unaffected tissues show invariable amount of mtDNA.

A reduced activity of the respiratory chain, and, more importantly, a low mtDNA/nDNA ratio in affected tissues confirm clinical diagnosis.

MDS is transmitted as an autosomal recessive trait.

Differential diagnosis

MDS must be differentiated from all the other mitochondrial respiratory chain disorders that share similar presentation symptoms. It must be suspected especially when the biochemical tests show multiple deficiencies of the respiratory chain complexes with sparing of complex II enzyme activity. Southern blot analysis allows ruling out the diagnosis of mtDNA depletion.

Etiology

Synthesis of mtDNA is not cell cycle-regulated and a constant supply of deoxyribonucleoside triphosphates (dNTPs) is crucial for the maintenance of mitochondrial integrity. Since there is apparently no *de novo* nucleotide synthesis in the mitochondria, and the mitochondrial inner membrane is impermeable to charged molecules, the mitochondrial dNTP pool is maintained either by import of cytosolic dNTPs through specific transporters or by salvaging deoxynucleosides within the mitochondria. In non-replicating cells, where cytosolic dNTP synthesis is downregulated and the import of nucleotides from the cytosol to the mitochondria is not possible, mtDNA synthesis depends solely on the mitochondrial salvage pathway enzymes, the deoxyribonucleoside kinases. Two of them, the deoxyguanosine kinase (DGUOK) and the deoxythymidine kinase (TK2), are expressed in human mitochondria. DGUOK phosphorylates deoxyguanosine and deoxyadenosine, whereas TK2 phosphorylates deoxythymidine, deoxycytidine, and deoxyuridine. In the second half of 2001, mutations in *DGUOK* (Mandel *et al.*, 2001) and in *TK2* (Saada *et al.*, 2001) were first associated with the hepatocerebral and the myopathic forms of MDS, respectively. These findings have been subsequently confirmed (Taanman *et al.*, 2002; Salviati *et al.*, 2002; Mancuso *et al.*, 2002; Vila *et al.*, 2003; Carozzo

et al., 2003; Mancuso *et al.*, 2003). Subsequently, in patients with encephalomyopathy and mtDNA depletion, it was shown that a deleterious mutation in *SUCLA2*, the gene encoding the beta subunit of succinyl-CoA synthetase ligase, led to a marked decrease in succinyl-CoA synthetase activity in muscle mitochondria. Given that succinyl-CoA synthetase is tightly associated in a complex with mitochondrial nucleotide diphosphate kinase (NDPK), a defect in the last step of mitochondrial dNTP salvage was proposed as a novel cause of the mtDNA depletion syndrome (Elpeleg *et al.*, 2005).

The association of MDS with mutations in *DGUOK* and *TK2* nuclear genes suggest that the maintenance of a balanced mitochondrial dNTP pool plays a crucial role in mtDNA replication and integrity, and ultimately in the mtDNA content. A similar hypothesis has been evocated for another mtDNA syndrome, the [mitochondrial neurogastrointestinal encephalomyopathy \(MNGIE\) syndrome](#). Mutation screening in *DGUOK* and *TK2* revealed that these two genes are not commonly involved in MDS, suggesting that additional genes are likely to be responsible for mtDNA depletion syndrome. Moreover, the initial association of *TK2* mutations with the late-onset muscle-specific variant of the disease (Saada *et al.*, 2001) was confirmed only in 1 out of 4 patients by Mancuso and colleagues (2002) and was not confirmed by others (Carozzo *et al.*, 2003). This limits the possibility of an easy genotype-phenotype correlation.

Clinical description

MDS is phenotypically heterogeneous and manifests either as a hepatocerebral form, a myopathic form, a benign "later-onset" myopathy form and a form with cardiomyopathy (see below). Children usually present in infancy with hypotonia, lactic acidosis and elevated serum creatin kinase (CK) (in the myopathic forms). Some also have severe, often fatal hepatopathy (in the hepatocerebral form) or renal involvement (in the myopathic form) mimicking the Toni-Fanconi syndrome. In some other cases, a later onset, a longer survival, and a multisystem involvement have been observed (Campos *et al.*, 1998; Vu *et al.*, 1998). mtDNA depletion has been described in a patient with Alpers' syndrome (Naviaux *et al.*, 1999) and in patients with Navajo neurohepatopathy (Vu *et al.*, 2001), but the role of mtDNA in these diseases remains to be elucidated.

- **Hepatocerebral form**

Onset: birth to 6 months

Early progressive liver failure: hepatocellular liver disease

Neurological abnormalities: muscle hypotonia, hyperreflexia, irritability
 Death: 1st year of age due to hepatic failure

- **Myopathic form**

Onset: birth to 24 months
 Hypotonia
 Progressive muscle weakness, high CK
 Some cases with renal dysfunction
 Death in childhood due to respiratory failure

- **Benign "later-onset" form**

Onset: 1 week-5 years
 Hypotonia
 Non-progressive weakness, high CK
 Neuropathy (40%) may be subclinical
 Survival: less than 15 years; respiratory failure is common

- **Cardiomyopathic form**

Onset: in the 1st year of life
 No muscular symptoms, normal CK
 Survival: for 2-3 years from diagnosis unless treatment with heart transplantation

Frequency

MDS may be a relatively common mitochondrial disorder of infancy and childhood. In one report, 11% (6/54) of young children (<2 years old) classified as being affected by a mitochondrial disorder had mtDNA depletion (Macmillan and Shoubridge, 1996).

Diagnostic methods

MDS diagnosis is currently based on clinical criteria and is confirmed by spectrophotometric measurement of the activities of the respiratory chain complexes (laboratory findings show multiple deficiencies of activities of the respiratory chain complexes sparing complex II) and, more importantly, by the identification of a low mtDNA/nDNA ratio (genetic testing). Finally about 10% of all the patients with a MDS can be definitively diagnosed by the finding of a mutation in the *DGUOK* and *TK2* genes (Mancuso *et al.*, 2002; Salviati *et al.*, 2002; Carrozzo *et al.*, 2003).

Laboratory findings

- **Hepatocerebral form**

-*Histochemical studies*: mosaic pattern of normal and deficient cytochrome-c oxidase fibers in muscle and liver; ragged red fibers in muscle are not a consistent feature
 -*Biochemical studies*: Hypoglycemia; lactic acidosis; low activity of respiratory complexes I, III, IV; normal value of respiratory complex II
 -*Residual mtDNA in muscle*: from 20%-50% of control levels

-*Residual mtDNA in liver*: from 11%-30% of control levels

- **Myopathic form**

-*Histochemical studies*: reduced COX and SDH staining; presence of COX positive and ragged red fibers. COXVIIa, b are selectively reduced in severe mtDNA depletion
 -*Biochemical studies*: high serum CK; plasma lactate normal or elevated; low activity of respiratory complexes I, III, IV; normal value of respiratory complex II
 -*Residual mtDNA in muscle*: from 5% to 60% of control levels

- **Benign "later-onset" myopathy form**

-*Histochemical studies*: presence of ragged red fibers and at earlier stage also COX(-) fibers; some of them later become COX(+). COXVIIa,b and COXII are selectively reduced
 -*Biochemical studies*: lactic acidosis (60%); high serum CK in 60%; in early stage cytochrome-c oxidase activity ranges from 6% to 66% of the control's activity, returning towards normal in later stage.

- **Cardiomyopathic form**

-*Histochemical studies*: presence of marked mitochondrial proliferation (SDH) and reduced COX only in the heart specimen (endomyocardial biopsy). Muscles appear normal.
 -*Biochemical studies*: lactic acidosis; normal serum CK; in heart cytochrome-c oxidase activity is significantly low with respect to the control's activity; in muscles the activities are normal.

Genetic testing

A low mtDNA/nDNA ratio in affected tissues confirms clinical diagnosis. In order to quantify mtDNA in affected tissues, Southern blot analysis is performed using the entire human mitochondrial genome and the 18S rRNA as a probe (Moraes *et al.*, 1991). Sequence analysis of *TK2* and *DGUOK* genes for the myopathic and hepatopathic form is indicated.

Genetic counselling

TK2 and *DGUOK* genes screening is only likely to be useful in a very few MDS families (10-14% in 3 series), since additional genes may be involved in MDS.

Prenatal diagnosis

Prenatal diagnosis is possible either by Southern blot and/or by analysis of *DGUOK* or *TK2* genes, when mutations are identified.

Management

Organ transplantation or gene therapy are among the possible therapeutic approaches but they may not be available to all affected individuals. Therapies that stimulate nucleotide biosynthesis, either via the *de novo* or the salvage pathway to increase the cellular dNTP pools, may increase the chance to delay disease development. In accordance with this assumption, it has been recently reported that mtDNA depletion due to deoxyguanosine kinase (dGK) deficiency could be partially reversed by supplementing deoxyadenosine monophosphate (dAMP) or deoxyguanosine monophosphate (dGMP) in a resting culture of dGK-deficient fibroblasts (Taanman *et al.*, 2003).

Some patients are responsive to therapy with Coenzyme Q10.

Unresolved questions

Based on the phenotypic heterogeneity associated with mtDNA depletion, it is clear that this molecular abnormality is the end result of defects in any of several proteins, most of which are yet to be identified. The recent findings of mutations in the *DGUOK* and *TK2* genes underscored the importance of the deoxyribonucleoside salvage pathway in mtDNA synthesis. The exact sequence of events leading to mtDNA depletion remains to be established.

Given the genetic complexity of the dNTP system and the even more complex machinery regulating mitochondrial replication and copy number, further investigation on molecular heterogeneity is warranted.

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