Mitochondrial Myopathy of Childhood Associated With Mitochondrial DNA Depletion and a Homozygous Mutation (T77M) in the TK2 Gene

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Background: The mitochondrial DNA depletion syndrome is an autosomal recessive disorder of infancy or childhood characterized by decreased mitochondrial DNA copy number in affected tissues. Mutations in 2 genes involved in deoxyribonucleotide metabolism, the deoxyguanosine kinase gene (DGK) and the thymidine kinase 2 gene (TK2), have been related to this syndrome.

Objective: To describe 3 siblings with the myopathic form of mitochondrial DNA depletion syndrome and a homozygous mutation in the TK2 gene.

Patients and Methods: These children developed normally until 12 to 16 months of age, when they started showing difficulty walking, which rapidly progressed to severe limb weakness. They died of respiratory failure between the ages of 23 and 40 months. Histochemical and biochemical studies of respiratory chain complexes were performed in muscle biopsy specimens. The whole coding region of the TK2 gene was sequenced.

Results: Muscle biopsy showed ragged-red cytochrome-c oxidase–negative fibers. All affected siblings had markedly decreased activities of respiratory chain complexes. Southern blot analysis showed severe reduction of the mitochondrial DNA–nuclear DNA ratio in muscle biopsy specimens from all patients, indicating 80% to 90% mitochondrial DNA depletion. Sequencing of the TK2 gene showed a homozygous C→T transition at nucleotide 228 in exon 5, which changes a threonine to a methionine at position 77 (T77M).

Conclusions: These results document the importance of screening the TK2 gene in patients with myopathic mitochondrial DNA depletion syndrome and confirm that exon 5 is a “hot spot” for TK2 mutations.

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MITOCHONDRIAL DNA (mtDNA) depletion syndrome (MDS) encompasses a clinically heterogeneous group of disorders characterized by severe reduction in mtDNA copy number.1 Primary mtDNA depletion is inherited as an autosomal recessive trait and may affect single organs, typically muscle or liver, or multiple tissues.

After the description of mutations in the thymidine kinase 2 gene (TK2) in patients with myopathic MDS2 and in deoxyguanosine kinase gene (DGK)3 in patients with hepatocerebral MDS, we documented that clinical expression of TK2 mutations is not limited to myopathy4 and that DGK mutations do not cause only liver or brain disease.5 Herein, we report a homoplasmic T77M mutation in 3 siblings with MDS and severe generalized myopathy causing respiratory failure and death before 4 years of age.

METHODS

Patients 1 and 2 have been described.6 Briefly, these infant boys were born at term after uneventful pregnancies to nonconsanguineous parents and developed normally until 12 to 14 months of age, when they showed difficulty walking and fell frequently. Both had increased levels of serum creatine kinase (386 and 905 U/L; reference value, <220 U/L); lactate and pyruvate levels were within the references ranges in patient 2. Both children developed respiratory distress and died at the ages of 40 and 36 months.

Patient 3, the third sibling, developed normally until the age of 16 months, when she started to walk with a waddle; by the age of 2 years she had lost the ability to walk. Examination at age 16 months showed normal intellect, cranial nerve function, sensation, and cerebellar function. She could walk only with assistance, was diffusely hypotonic, and had se-
vere limb weakness, more proximal than distal. Deep tendon reflexes were absent. She died of respiratory failure at the age of 23 months. Results of routine laboratory tests, including creatine kinase measurement, were normal. Serum lactate was not measured. A 16-year-old sister was healthy.

**HISTOCHEMICAL AND BIOCHEMICAL STUDIES**

Histochemical analyses of muscle biopsy specimens were performed as described previously. Respiratory chain enzyme activities were measured in muscle extracts as described.

**MOLECULAR GENETIC STUDIES**

Total DNA from patients’ muscle was extracted by means of standard protocols. Previously described methods were used for Southern blot analysis and quantification of mtDNA and for the screening of TK2.

**RESULTS**

In all 3 siblings, muscle biopsy specimens showed 40% to 60% cytochrome-c oxidase–negative fibers, which had increased staining for succinate dehydrogenase (Figure 1). Biochemical studies in muscle extracts showed markedly decreased cytochrome-c oxidase activity, whereas the activities of complexes I and III were normal or even increased when referred to wet tissue weight. However, as citrate synthase activity was greatly increased in all 3 patients, the activities of complexes I and III were also reduced when referred to citrate synthase, a good index of mitochondrial mass. Southern blot analysis showed reduction of the mtDNA–nuclear DNA ratio in muscle biopsy specimens from all patients, and the degree of mtDNA depletion ranged from 80% to 90%.

All affected siblings harbored a homozygous C→T transition at nucleotide 228 in exon 5, which changes a threonine to a methionine at position 77 (T77M) (Figure 2).

**COMMENT**

The MDS differs from other mitochondrial disorders because it is a quantitative rather than a qualitative defect. The low level of mtDNA in some tissues presumably causes insufficient synthesis of respiratory chain components. Depletion of mtDNA can occur as a secondary phenomenon, for example as a result of antiretroviral nucleoside analogue therapy. In MDS, however, mtDNA depletion is considered a primary process, and the degree of depletion is generally proportional to the severity of the phenotype.

Patients with the myopathic form of MDS usually present at or soon after birth with progressive weak-
ness, hypotonia, and areflexia, and die of respiratory failure before 1 year of age (congenital form) or before 10 years of age (juvenile form).

Primary mtDNA depletion is inherited as an autosomal recessive trait (as illustrated by this family, where 3 of 4 children were affected), but the genetic defect had remained elusive for more than a decade. In the past year, the same group of investigators identified mutations in the TK2 gene in patients with myopathic MDS and mutations in the DGK gene in patients with hepatocerebral MDS, confirming the concept that alterations of the mitochondrial nucleotide pool affect mtDNA maintenance and stability.

The TK2 gene is located on chromosome 16 and encodes a 234-amino acid polypeptide, which is synthesized in the cytoplasm, then imported into the mitochondrial matrix. It catalyzes the transfer of a phosphate group from adenosine triphosphate to thymidine or deoxycytidine,11 whereas deoxyguanosine kinase efficiently phosphorylates deoxyguanosine and deoxyadenosine to the corresponding deoxynucleotide monophosphates.12 The combined action of the 2 enzymes allows synthesis of all 4 deoxynucleotide triphosphates needed for mtDNA replication.11,12 Mutations in these 2 genes cause nucleotide pool imbalance, leading to inefficient replication and, therefore, depletion of mtDNA.4,5

This is the second description, to our knowledge, of a T77M mutation in the TK2 gene. Of the 5 pathogenic mutations described thus far, 3 are in exon 5 in patients with MDS. Similar to a previous patient, who was a compound heterozygote for T77M and H90N,4 the present patients developed isolated myopathy, confirming that muscle appears to be particularly vulnerable to TK2 mutations.2,4 As previously reported, the T77M mutation is near the active site, in the α4 helix of the protein, which is important for enzyme dimerization and nucleoside recognition. The change from threonine to a larger residue, such as methionine, at position 77 may disrupt packing or displace the α helix.

In summary, we identified the second T77M TK2 mutation in a family with severe, early-onset myopathy and MDS. This stresses the importance of screening the TK2 gene in patients with isolated or predominant muscle involvement and mtDNA depletion. Our data also confirm earlier suggestions that exon 5 is a “hot spot” for TK2 mutations. Knowledge of pathogenic mutations in the TK2 gene will improve genetic counseling and make prenatal diagnosis possible, thus, we hope, avoiding the recurrent early deaths experienced by this unfortunate family.

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