Clinical Spectrum of Mitochondrial DNA Depletion Due to Mutations in the Thymidine Kinase 2 Gene

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Background: Mitochondrial DNA depletion syndrome is an autosomal recessive disorder characterized by decreased mitochondrial DNA copy numbers in affected tissues. It has been linked to 4 genes involved in deoxyribonucleotide triphosphate metabolism: thymidine kinase 2 (*TK2*), deoxyguanosine kinase (*DGUOK*), polymerase gamma (*POLG*), and *SUCLA2*, the gene encoding the β-subunit of the adenosine diphosphate–forming succinyl coenzyme A synthetase ligase.

Objective: To highlight the variability in the clinical spectrum of *TK2*-related mitochondrial DNA depletion syndrome.

Design: Review of patients and the literature.

Setting: Tertiary care university.

Patients: Four patients with mitochondrial DNA depletion syndrome and mutations in the *TK2* gene.

Main Outcome Measures: Definition of clinical variability.

Results: Patient 1 had evidence of lower motoneuron disease and was initially diagnosed as having spinal muscular atrophy type 3. Patient 2, who is alive and ambulatory at age 9 years, presented at age 2 years with a slowly progressive mitochondrial myopathy. Patient 3 had a more severe myopathy, with onset in infancy and death at age 6 years of respiratory failure. Patient 4 had a rapidly progressive congenital myopathy with rigid spine syndrome and he died at age 19 months.

Conclusion: The clinical spectrum of *TK2* mutations is not limited to severe infantile myopathy with motor regression and early death but includes spinal muscular atrophy type 3–like presentation, rigid spine syndrome, and subacute myopathy without motor regression and with longer survival.

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MITOCHONDRIAL DNA (mtDNA) depletion syndrome is an autosomal recessive disorder first described in 1991.[1] It is a quantitative rather than a qualitative defect, characterized by decreased mtDNA copy numbers in affected tissues, which in turn impairs the synthesis of mtDNA-encoded respiratory chain components. The mtDNA depletion syndrome has been linked to 4 genes involved in deoxyribonucleotide triphosphate metabolism: (1) thymidine kinase 2 (*TK2*), associated with the myopathic form of the disease[2,3]; (2) deoxyguanosine kinase (*DGUOK*), associated with the hepatocerebral form of the disease[4,5]; (3) polymerase gamma (*POLG*), also associated with the hepatocerebral form of the disease and, more specifically, to Alpers syndrome[6,7]; and (4) *SUCLA2*, the gene encoding the β-subunit of the adenosine diphosphate–forming succinyl coenzyme A synthetase ligase, associated with a more generalized encephalomyopathic form of the disease.[8] The first described children with *TK2* mutations had severe infantile myopathy with motor regression and early death from respiratory insufficiency. However, more recent case reports have expanded the clinical phenotype to include a spinal muscular atrophy–like presentation[9] and milder forms with longer survival.[10,11] In this article, we describe 4 patients with *TK2* mutations, who illustrate the variability in the clinical spectrum of mtDNA depletion syndrome, and review all cases reported to date.

METHODS

PATIENTS

Patient 1 was described before the molecular basis of mtDNA depletion syndrome was known,[9] and we recently identified *TK2* mu-

tations in this child. The boy was born to consanguineous Ashkenazi Jewish parents after a normal-term pregnancy and delivery. He sat unsupported at age 6 months, walked at age 11 months, and had normal early development. At 2 years of age, he developed in-turning of 1 foot, with the inability to run and decreased physical stamina. At age 2½ years, evaluation showed elevated venous lactate levels (33.3 mg/dL [3.7 mmol/L]; reference range, 4.5-19.8 mg/dL [0.5-2.2 mmol/L]) and serum creatine kinase (CK) levels (577 U/L; reference range, <225 U/L); electromyography (EMG) suggested denervation. By 3 years of age, he could no longer walk independently. On physical examination at age 3½ years, he was alert and cognitively healthy with a normal head circumference. He had full extraocular movements, without ptosis, and a normal-appearing tongue. He was diffusely hypotonic and weak, with poor head control, and could not stand without support. Tendon reflexes were trace or absent, with reduced muscle bulk throughout. Repeated venous lactate and CK measurements were normal. The EMG showed myopathic features with no spontaneous activity, and findings from nerve conduction studies were normal. By 9 years of age, he required assisted ventilation. By 12 years of age, he developed mild ptosis, with normal extraocular movements, diffuse muscle wasting, and contractures, and required gastrostomy tube feeding. He remained alert and cognitively intact. He died at 16½ years of age after bleeding from a lesion at his tracheostomy site.

Patient 2 is an African American girl born to nonconsanguineous parents after a normal-term pregnancy and vaginal delivery. Early developmental milestones were normal. She walked independently at age 11 months and was able to run in the second year of life. By 2 years of age, she fatigued easily and developed proximal weakness. At 3 years of age, she was evaluated at another institution. Her serum CK levels were elevated (823 U/L; reference range, <225 U/L), and her EMG findings were neuropathic; spinal muscular atrophy type 3 was suspected, but no deletion in the survival motor neuron 1 (*SMN1*) gene was found. At 7 years of age, she was evaluated at Columbia University Medical Center, New York, NY. She was alert and cognitively intact. Head circumference was 50.5 cm, and general physical examination findings were normal. Cranial nerves were intact, with no ophthalmoparesis, ptosis, or tongue fasciculations. Muscle bulk was significantly decreased throughout, with diffuse hypotonia and weakness. She could walk independently but showed pronounced lumbar lordosis, toe walking, and Gowers sign. Tendon reflexes were trace. Her venous lactate level was elevated (51.4 mg/dL [5.7 mmol/L]. At 9 years of age, she walks independently and has facial diplegia and normal pulmonary function.

Patient 3 is a Brazilian girl born to nonconsanguineous parents after a normal-term pregnancy and delivery. Motor developmental milestones were delayed in the first year of life. She sat unsupported at age 12 months and walked independently by 18 months of age. Social and cognitive development were normal. At 2 years of age, she developed gait abnormalities and progressive proximal weakness. At 5 years of age, physical examination showed normal cranial nerves, proximal limb weakness with a myopathic gait, and Gowers sign. Muscle bulk was significantly diminished throughout, with hypoactive tendon reflexes. Laboratory investigations showed normal serum lactate levels (15.3 mg/dL [1.7 mmol/L]; reference range, <18.9 mg/dL [<2.1 mmol/L]) and elevated serum CK levels (790 U/L; reference range, 225 U/L). The EMG showed a myopathic pattern. She died at 6 years of age of respiratory failure after pneumonia.

Patient 4 is a Chilean boy born to nonconsanguineous parents by cesarean delivery due to fetal macrosomia. He could grasp objects at age 3 months and sit with support by age 6 months. Starting at age 7 months, there was regression in acquired motor skills. He could no longer hold objects or sit, and he lost head control. At 11 months of age, he was alert and aware of his surroundings, with significant head lag, rigid spine syndrome, decreased spontaneous movements, proximal weakness, and preserved tendon reflexes. Laboratory studies showed elevated serum CK levels (1724 U/L), normal serum and cerebrospinal fluid lactate levels, and normal brain magnetic resonance imaging findings. By 12 months of age, he depended on mechanical ventilation and developed distal limb weakness. A gastrostomy tube was inserted by means of a Nissen fundoplication procedure. By 13 months of age, he developed facial diplegia and global hypotonia. A tracheostomy was performed. His tendon reflexes became difficult to elicit at age 16 months and were absent by 17 months of age. He continued to depend on mechanical ventilation and was fed entirely by gastrostomy tube. Echocardiographic findings were normal, and EMG findings were consistent with myopathy. At age 19 months, after the decision was made to provide only palliative care (supported by the hospital ethics committee), he developed sepsis and died.

MORPHOLOGY, BIOCHEMISTRY, AND MOLECULAR GENETICS

Routine muscle histologic examination and histochemical analysis for cytochrome-*c* oxidase and succinate dehydrogenase were performed as previously described.[12] Respiratory chain enzyme activities in muscle were measured as previously described.[13] Total DNA samples from patients' muscle were extracted using standard protocols.[14] Southern blot analysis and quantification of mtDNA were performed as previously described.[1,15] The *TK2* gene was amplified with polymerase chain reaction using intronic primers. Mutations were detected by means of direct sequencing.

RESULTS

BIOCHEMICAL ANALYSIS

Respiratory chain activities in muscle homogenate are given in **Table 1**. In patients 1, 2, and 3, succinate dehydrogenase and citrate synthase activities were increased, whereas cytochrome-*c* oxidase and succinate–cytochrome-*c* reductase activities were decreased. Biochemical analysis could not be performed in patient 4 owing to the limited size of the muscle specimen.

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSES

Muscle biopsy specimens were compatible with mitochondrial myopathy in all 4 patients. There were multiple ragged red fibers and cytochrome-*c* oxidase–negative fibers (**Figure**, A), which had increased staining for succinate dehydrogenase (ragged blue fibers, Figure, B).

DNA ANALYSIS

Quantitation of mtDNA by means of Southern blot analysis showed marked reduction of the mtDNA/nuclear DNA ratio in muscle from all 4 patients. The degree of mtDNA depletion ranged from 75% to 82%. In patient 1, immunostaining with anti-DNA antibodies showed a proliferation of mitochondria virtually devoid of mtDNA, the pathologic hallmark of mtDNA depletion. However, be-

Table 1. Mitochondrial Enzyme Activities in Muscle Homogenate*

Enzyme, μmol/min per Gram of Muscle	Patient 1	Patient 2	Patient 3	Patient 4	Controls
NADH-dehydrogenase (complex I)	38.71	31.02	20.13	NE	35.48 ± 7.07
NADH–cytochrome-*c* reductase (complexes I + III)	1.10	0.73	3.94	NE	1.02 ± 0.38
SDH (complex II)	2.51	1.51	2.23	NE	1.00 ± 0.53
Succinate–cytochrome-*c* reductase (complexes II + III)	0.41	0.68	0.40	NE	0.70 ± 0.23
COX (complex IV)	1.41	0.91	1.98	NE	2.80 ± 0.52
Citrate synthase	17.65	8.06	23.23	NE	9.88 ± 2.55
mtDNA depletion, %	NE	79	75	82	NA

Abbreviations: COX, cytochrome-*c* oxidase; NADH, reduced nicotinamide adenine dinucleotide; mtDNA, mitochondrial DNA; NA, not applicable; NE, not evaluated; SDH, succinate dehydrogenase.
*Data are given as single values for patients 1 to 4 and as mean ± SD for controls.

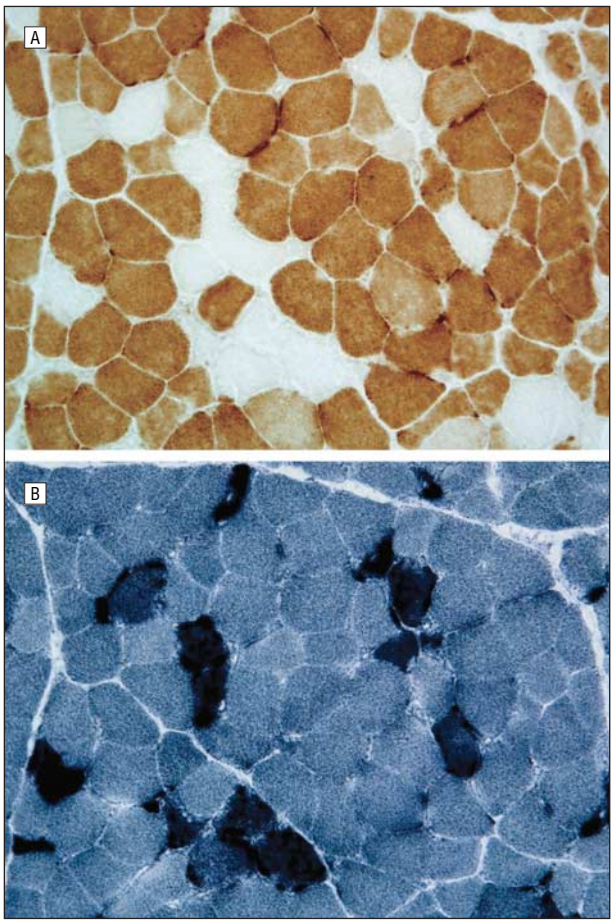

Figure. Muscle histochemical analysis in patient 3. A, Cytochrome-*c* oxidase staining shows reduced enzymatic activity in several myofibers. B, A serial section stained with succinate dehydrogenase illustrates that most cytochrome *c* oxidase–negative fibers are "ragged blue" (for both, original magnification ×120).

cause this method does not quantify mtDNA, the degree of mtDNA depletion is not available for this patient.[9]

The DNA sequencing analysis of the *TK2* gene (numbering according to GenBank accession No. NM_004614 for the messenger RNA sequence) from all 4 patients showed several mutations. In patient 1, we found 2 heterozygous changes. The first was a novel AT insertion at nucleotide 337 (exon 5), resulting in a premature stop codon 20 amino acids after the insertion. The second was a previously reported T→A transition at nucleotide 645 (exon 9), resulting in the substitution of an isoleucine by an asparagine at residue 212 (I212N). Patient 2 harbored the same AT insertion in exon 5 as patient 1, as well as an A→G transition at nucleotide 278 (exon 4) substituting an asparagine with a serine (N93S). Sequencing from patient 3 revealed 2 homozygous and previously reported changes (GC→AA) in exon 5. The first was a homonymous polymorphism; that is, the substitution of G→A at nucleotide 360, resulting in R120R. The second was a C→A change at nucleotide 361, changing histidine to asparagine at residue 121 (H121N). Patient 4 had 2 novel heterozygous mutations in exon 5: (1) a C→T nucleotide change at position 323, resulting in a threonine-to-methionine replacement at residue 108 (T108M), and (2) a stop codon mutation resulting from a C→T nucleotide transition at position 373 that replaces a glutamine with a stop codon at residue 125 (Q125X). This mutation is also associated with an adjacent homonymous polymorphism, T→C at nucleotide 372, resulting in P124P.

COMMENT

We describe 4 children with mtDNA depletion syndrome and *TK2* mutations and compare their clinical (**Table 2**), laboratory, and molecular (**Table 3**) features with those of 16 children in the literature. The mean age at onset was 11.4 months, and the mean age at death in 14 (70%) of 20 patients was 42.1 months. The cause of death in most patients (12/14; 86%) was respiratory failure. Proximal weakness and hypotonia were common to all patients. Less common features included facial diplegia, ptosis, ophthalmoplegia, exercise intolerance, and feeding difficulties that required gastrostomy.

The *TK2* gene phosphorylates deoxythymidine and deoxycytidine, thereby participating in the salvage pathway of deoxynucleotide synthesis in the mitochondria. In nonreplicating tissues, such as the liver, brain, muscle, heart, and skin, *TK2* is one of the indispensable enzymes for mtDNA maintenance. It remains unclear why patients with *TK2* mutations have selective muscle involvement with sparing of other nonreplicating tissues. To answer this question, a recent study[19] investigated the expression of mitochondrial deoxynucleotide carrier, the amount of mtDNA, and the activity of *TK2* in the mitochondria of various tissues. The results suggest that a higher requirement for mi-

Table 2. Clinical Features of 20 Patients Described in the Present Study and in the Literature

Sex	Age at Onset, mo	Age at Death and Cause	Weakness and Hypotonia	CN	GT	Ventilator	Source
M	2	10 mo; RF	+	–	NA	NA	Carrozzo et al,[16] 2003
F	5	15 mo; RF	+	–	NA	NA	Carrozzo et al,[16] 2003
M	12	40 mo; RF	+	–	NA	NA	Mancuso et al,[3] 2003
M	14	36 mo; RF	+	–	NA	NA	Mancuso et al,[3] 2003
F	16	23 mo; RF	+	–	NA	NA	Mancuso et al,[3] 2003
M	12	40 mo; RF	+	–	NA	+	Mancuso et al,[10] 2002
F	16	Alive at 4 y	+	–	NA	NA	Mancuso et al,[10] 2002
F	1	24 mo	+	–	NA	+	Mancuso et al,[10] 2002
M	15	Alive at 2 y	+	–	NA	NA	Mancuso et al,[10] 2002
F	8	Alive at 3 y	+	–	+	+	Saada et al,[2] 2001, and, Nevo et al,[17] 2002
F	8	19 mo; RF	+	–	NA	NA	Saada et al,[2] 2001, and, Nevo et al,[17] 2002
M	6	Alive at 3 y	+	Ptosis and PEO	+	+	Saada et al,[2] 2001, and, Nevo et al,[17] 2002
F	Birth	4 y	+	Ptosis and PEO	NA	+	Saada et al,[2] 200l, and Nevo et al,[17] 2002
M	28	Alive at 12 y	+	–	–	–	Wang et al,[11] 2005
F	11	24 mo; RF	+	–	NA	NA	Tulinius et al,[18] 2005
F	12	28 mo; RF	+	–	NA	NA	Tulinius et al,[18] 2005
M	24	16 y; RF	+	Ptosis at age 12 y	+	+	Pons et al,[9] 1996, and the present study
F	24	6 y; RF	+	–	NA	NA	Present study
M	7	19 mo; RF	+	Facial diplegia	+	+	Present study
F	24	Alive at 9 y	+	Facial diplegia	–	–	Present study

Abbreviations: CN, cranial nerve findings; GT, gastrostomy tube; NA, not available; PEO, progressive external ophthalmoplegia; RF, respiratory failure; *TK2*, thymidine kinase 2 gene; –, negative; +, positive.

Table 3. Laboratory and Molecular Features of 20 Patients Described in the Present Study and in the Literature

Sex	Age at Onset, mo	Age at Death and Cause	Weakness and Hypotonia	CK, U/L	Lactate	EMG	RRF/ RBF	COX-Fibers	*TK2* Mutation	Source
M	2	10 mo; RF	+	NA	+	NA	+	+	R183G and R254X	Carrozzo et al,[16] 2003
F	5	15 mo; RF	+	NA	–	NA	+	+	142insG	Carrozzo et al,[16] 2003
M	12	40 mo; RF	+	386	+	NA	+	+	T77M	Mancuso et al,[3] 2003
M	14	36 mo; RF	+	905	–	NA	+	+	T77M	Mancuso et al,[3] 2003
F	16	23 mo; RF	+	Normal	NA	NA	+	+	T77M	Mancuso et al,[3] 2003
M	12	40 mo; RF	+	1238	NA	NA	+	+	H90N and T77M	Mancuso et al,[10] 2002
F	16	Alive at 4 y	+	950	+	NA	+	+	H90N and T77M	Mancuso et al,[10] 2002
F	1	24 mo	+	NA	NA	Myopathic	+	+	I22M	Mancuso et al,[10] 2002
M	15	Alive at 2 y	+	Mild increase	+	Denervation	+	+	I22M	Mancuso et al,[10] 2002
F	8	Alive at 3 y	+	3436	NA	Myopathic	–	+	Ile181Asn	Saada et al,[2] 2001, and Nevo et al,[17] 2002
F	8	19 mo; RF	+	1875	+	Myopathic	–	+	Ile181Asn	Saada et al,[2] 2001, and Nevo et al,[17] 2002
M	6	Alive at 3 y	+	4010	+	Myopathic	–	+	Ile181Asn	Saada et al,[2] 2001, and Nevo et al,[17] 2002
F	Birth	4 y	+	908	+	Myopathic + SA	–	–	His90Asn	Saada et al,[2] 2001, and Nevo et al,[17] 2002
M	28	Alive at 12 y	+	Mild increase	NA	Myopathic	+	NA	T77M and R161K	Wang et al,[11] 2005
F	11	24 mo; RF	+	×8	NA	Normal	+	+	T64M and R183W	Tulinius et al,[18] 2005
F	12	28 mo; RF	+	×2	+	Myopathic	+	+	T64M and R183W	Tulinius et al,[18] 2005
M	24	16 y; RF	+	577	+	(1) Neurogenic (2) Myopathic	+	+	I212N and AT ins 337	Pons et al,[9] 1996, and the present study
F	24	6 y; RF	+	790	–	Myopathic	+	+	R120R and H121N	Present study
M	7	19 mo; RF	+	1724	–	NA	+	+	T108M and Q125X	Present study
F	24	Alive at 9 y	+	823	+	Neurogenic	+	+	N93S and AT ins 337	Present study

Abbreviations: CK, creatine kinase; COX, cytochrome-*c* oxidase; EMG, electromyographic; NA, not available; RBF, ragged blue fibers; RF, respiratory failure; RRF, ragged red fibers; SA, spontaneous activity; *TK2*, thymidine kinase 2 gene; –, negative; +, positive.

tochondrial-encoded proteins and significantly lower activity of *TK2* in muscle compared with the liver and brain are the main determinants of the selective muscle involvement in this disease.[19]

The marked increase in serum CK values seen in most patients is an unusual finding in mitochondrial myopathies and a useful diagnostic clue. Other features of mitochondrial dysfunction seen in most patients include lactic acidosis, ragged red– (with the Gomori trichrome stain) or ragged blue– (with the succinate dehydrogenase stain) and cytochrome-*c* oxidase–negative fibers on muscle biopsy specimens, and a myopathic

pattern on EMG. However, all of these investigation results have been normal in some patients, and the results will depend in part on the timing of these tests. A high index of suspicion is warranted in any child with proximal weakness and hypotonia of unclear etiology. The diagnosis is confirmed by means of Southern blot analysis for mtDNA quantitation and subsequent *TK2* mutation screening.

Mitochondrial myopathy due to mtDNA depletion syndrome is a rare condition, and *TK2* mutations do not account for all cases. Early studies[3,17] described severe infantile myopathy with motor regression and early death. However, the clinical spectrum has now expanded to include spinal muscular atrophy type 3–like presentation,[3,9] rigid spine syndrome (patient 4), and a milder myopathic phenotype without motor regression (patients 2 and 3) and with longer survival (patients 1 and 2). These observations highlight the expanding phenotypic spectrum of this disease, which should be included in the differential diagnosis of all unexplained myopathies of infancy and childhood.

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