0031-3998/10/6802-0151
PEDIATRIC RESEARCH

Vol. 68, No. 2, 2010
Printed in U.S.A.

Hearing Loss in a Patient With the Myopathic Form of Mitochondrial DNA Depletion Syndrome and a Novel Mutation in the *TK2* Gene

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ABSTRACT: Mitochondrial DNA (mtDNA) depletion syndrome (MDS) is a devastating disorder of infancy caused by a significant reduction of the number of copies of mitochondrial DNA in one or more tissues. We report a Spanish patient with the myopathic form of MDS, harboring two mutations in the thymidine kinase 2 gene (*TK2*): a previously reported deletion (p.K244del) and a novel nucleotide duplication in the exon 2, generating a frameshift and premature stop codon. Sensorineural hearing loss was a predominant symptom in the patient and a novel feature of MDS due to *TK2* mutations. The patient survived up to the age of 8.5 y, which confirms that survival above the age of 5 y is not infrequent in patients with MDS due to *TK2* deficiency. **(*Pediatr Res* 68: 151–154, 2010)**

Mitochondrial DNA (mtDNA) depletion syndrome (MDS) is a severe disorder that encompasses a group of heterogeneous clinical presentations. The hallmark of this syndrome is a significant reduction of the number of copies of mtDNA in one or more tissues. The most severely affected organs are postmitotic, and the syndrome can be found as purely myopathic, encephalomyopathic, hepatocerebral (1), or more rarely multisystemic (2). Mutations in the gene encoding the mitochondrial kinase of pyrimidine nucleosides, thymidine kinase 2 (*TK2*, EC: 2.7.1.21) was the first genetic cause found for the myopathic form of MDS (3). So far, mutations in this gene account for ~20% of purely myopathic MDS (1).

Although there is a discrete variability in the clinical presentation of the patients with *TK2* mutations, many of them present several common features. In most cases, the age of onset is in the first or second year of life, manifesting as difficulty in standing up, hypotonia, and muscle weakness, which progressively worsens and finally compromises the respiratory function. Spinal muscular atrophy-like presentation and subacute myopathy with longer survival have been also reported (1,4).

Signs of mitochondrial dysfunction are usually found through biochemical and/or histochemical analysis of skeletal muscle, including the presence of ragged red fibers. mtDNA depletion in muscle is the hallmark of the disease, although the reversion of this molecular feature has been reported in one case (5). Additional features include moderate increase of serum creatine kinase and CNS involvement in isolated cases (6,7). In most patients, the disease is fatal before the sixth year of life, but cases of longer survival, even up to the teens, are not infrequent (5,8,9). Here, we report a Spanish patient, compound heterozygote for a mutation already documented and a novel mutation in the *TK2* gene, with sensorineural auditive involvement, not observed to date in patients with *TK2* mutations. This novel feature indicates that, as previously reported (6,7), *TK2* deficiency may involve organs or systems other than skeletal muscle.

CASE REPORT

This study was approved by the Institutional Review Board of the University Hospital Sant Joan de Deu. Written informed consent to do the study was obtained from the parents of the patient. A 6-y-old male, born to nonconsanguineous parents after normal pregnancy and delivery, had a normal newborn period and early development until the age of 2 y. He could stand up and walk without support at 13 mo. At the age of 2.5 y, he fell down frequently showing difficulties in walking and standing up. Over the next years, he developed progressive muscle weakness and wasting, mainly affecting proximal muscles of head and neck, shoulder, and pelvic girdle. Deep tendon reflexes were reduced, ocular movements were normal, and no ptosis was noticed. Cerebellar and cognitive functions were preserved, and fine motor abilities were normal. He

Received December 17, 2009; accepted April 8, 2010.
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Supported by grants from the Spanish Fondo de Investigación Sanitaria (PI03/0242; PI06/0735; PI04/0009; PI07/0045; PI04/1351) and the Diputación General de Aragón (Grupos Consolidados B33).

Abbreviations: COX, cytochrome *c* oxidase; **MDS,** mitochondrial DNA depletion syndrome; **mtDNA,** mitochondrial DNA; **TK2,** thymidine kinase 2 (EC: 2.7.1.21)

made no further progress in gross motor development. At the age of 5 y, he was able to walk indoors with a waddling gate, but he was unable to stand up alone. At the age of 6 y, he lost head control, followed by involvement of axial muscles with respiratory failure that led to nocturnal ventilator dependency. Language development started at the expected age, but from the age of 3 y, pronunciation difficulties were observed and his comprehension level was barely acceptable, with no history of aminoglycoside treatment. At the age of 4 y, brainstem auditory evoked potentials showed no response at 100 dB. A standard audiometric study showed an auditory threshold of 45 dB (Fig. 1). At the age of 5 y, bilateral audiphones were applied, and at 6 y, the audiometric study showed a hearing threshold above 65 dB, but comprehension was reached at 95 dB. When wearing the audiphones, the auditory threshold was reached at 30 dB and comprehension at 60 dB.

Treatment with carnitine and triacetyluridine was established with poor clinical improvement. Laboratory analysis showed increased serum creatine kinase (maximum levels, 540 UI/L; reference, <190) and mild hyperlactacidemia (lactate, 2.30 mM; reference, 0.66–1.88).

Complementary studies. Motor and sensory nerve conductions were normal, and needle EMG showed a myogenic pattern. MRI of the brain, cardiac evaluation, visual evoked potentials, and renal and hepatic function were normal.

Biochemical and morphologic analysis of skeletal muscle showed increased variability in muscle fiber diameter with increased connective tissue and several regenerating and vacuolated fibers. Modified Gomori trichrome stain revealed abundant ragged red fibers and increased connective tissue. Histochemical stain for cytochrome *c* oxidase (COX) showed a mosaic pattern with COX negative fibers. Oil red stain revealed moderate increase of lipid droplets (Fig. 2). Mitochondrial respiratory chain enzyme activities were determined in muscle homogenate according to described spectrophotometric methods (10), showing a severe decrease of complexes I, III, and IV in addition to near normal activity of complex II (Table 1).

Molecular studies. DNA from muscle was analyzed through real-time quantitative PCR, revealing depletion of more than 90% of mtDNA. Because of evidence of a myopathic form of

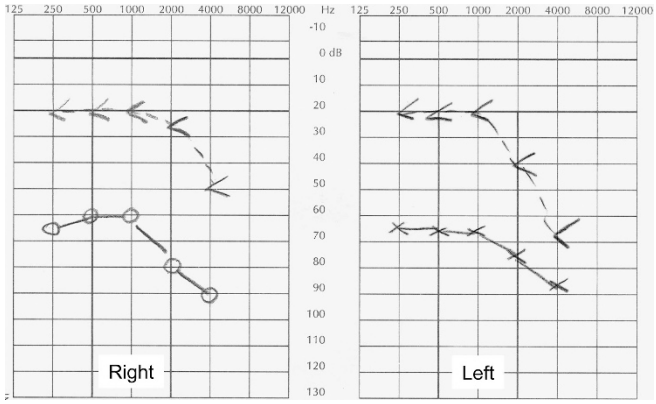

Figure 1. Audiogram showing a moderate down-sloping hearing loss. *X* axis, frequency in Hertz; *Y* axis, hearing threshold level in decibel; *circles* and *crosses*, air conduction; *arrow-heads*, bone conduction.

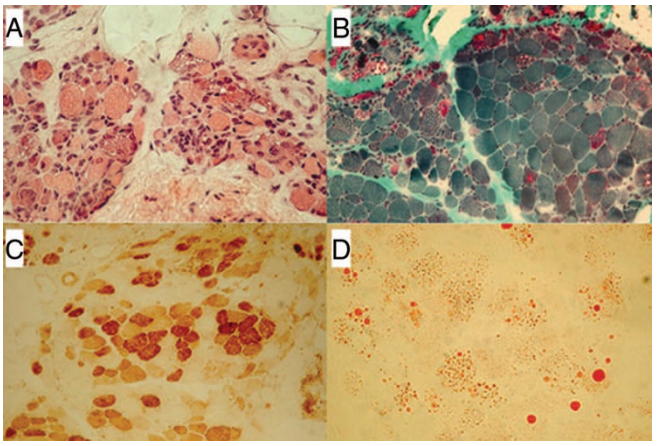

Figure 2. Histochemical study of patient's skeletal muscle. *A*, Hematoxylin & eosin stain showing an increased variability in muscle fiber diameter with increased connective and fat tissue. Several regenerating fibers and some vacuolated fibers were also seen. *B*, Modified Gomori trichrome stain revealing abundant ragged red fibers and lipid droplets. *C*, Histochemical stain for COX showing a mosaic pattern with COX-negative fibers. *D*, Oil red stain revealing increase in lipid droplets.

Table 1. *Mitochondrial respiratory chain enzyme activities in skeletal muscle*

	(nmoles/min · mg prot)		mU/U citrate synthase	
Enzyme activities	Patient	Control range	Patient	Control range
Complex I + III	3	12–56	18	107–560
Complex II	4	4–10	24	33–69
Complex II + III	0.2	7–24	1	60–149
Complex III	17	55–259	103	498–1760
Cytochrome *c* oxidase	20	59–170	121	503–1300
Citrate synthase	165	71–200	—	—

MDS, the coding region and intronic boundaries of the *TK2* gene were sequenced. Two heterozygous mutations were found: 1) a previously reported deletion (c.730_732delAAG) abolishing lysine at codon 244 (p.K244 del) (5); and 2) a novel duplication of an A in a polyA row in the exon 2 (c.276dupA) (Fig. 3), generating an out-of-frame reading and a premature stop codon (p.S93IfsX99). This mutation generates a similar frameshift and the same premature stop codon as the previously reported p.E90GfsX102 mutation (7). The numeration of nucleotides refers to GeneBank accession # NM_004614.3. We sequenced the exon 2 of the *TK2* gene in DNAs from 54 healthy controls (108 alleles) and did not find the presence of the novel c.276dupA mutation. The family rejected more genetic studies, and so the allelic distribution of the mutations could not be further investigated.

DISCUSSION

The first gene associated to the myopathic form of the MDS was *TK2*, which encodes the enzyme that catalyzes the first step of the mitochondrial salvage of pyrimidine deoxynucleosides (3). Several cases of patients with myopathic MDS due to *TK2* mutations have been reported since then (1,4–7,11–13). In recent years, mutations in three other genes have been reported to cause MDS with encephalomyopathy (*SUCLA2*, encoding the beta subunit of ADP-forming Succinyl-CoA synthase) (14), fatal infantile lactic acidosis (*SUCLG1*, encoding the alpha subunit of

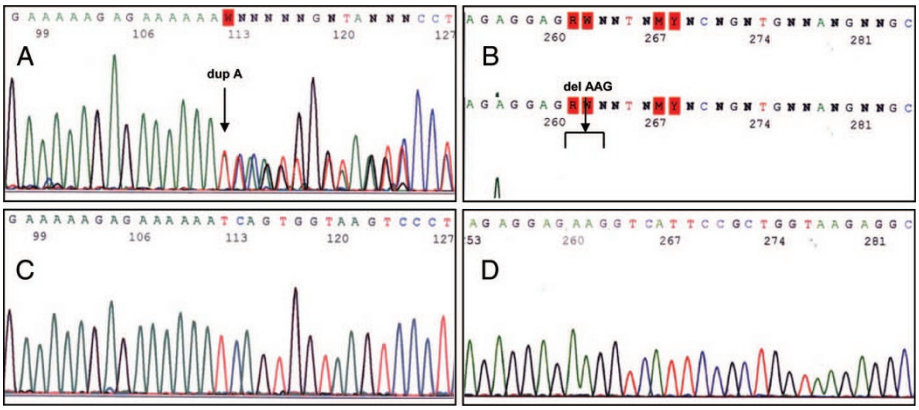

Figure 3. Electropherograms showing the *TK2* heterozygous mutations found in the patient (*arrows*). *A*, Novel mutation (c.276dupA) in exon 2. *B*, Previously reported mutation (c.730_732delAAG) in exon 8. *C* and *D*, Wild-type sequences obtained from a healthy control.

the same enzyme) (15), or a more multisystemic presentation including myopathy (*RRM2B*, encoding the p53-controled R2 subunit of ribonucleotide reductase) (2,16). Other genes, most of them related to deoxynucleotide metabolism, have been also linked to the encephalomyopathic or hepatocerebral forms of MDS (1,13). Although the genetic cause of many cases of MDS still remains unknown, the recent findings on the genetic and biochemical causes of this syndrome anticipates future improvements in the knowledge of the pathomechanisms involved in MDS.

We report the third case of a Spanish patient harboring mutations in *TK2*. Genetically, our patient shares one mutation with the first Spanish patient reported, (p.K244del) (5). It is noteworthy that the novel mutation in the exon 2 reported here (duplication of an additional A within the wild-type 271–276 poly-A row) is very close to a previously reported duplication (c.268dupG; p.E90GfsX102) (7). Both mutations predict a similar frameshift resulting in the same premature stop codon. Exon 2 is a short sequence (32 nucleotides), rich in poly-A segments, and the presence of two different duplications, a tetra nucleotide deletion, plus 2 additional point mutations and a splice mutation in the adjacent donor site of I2 (9,12,13) could indicate that the exon 2 region is a possible hot-spot for mutations in the gene.

The clinical presentation of the patient, as well as the histologic and biochemical pictures, coincide with the main features observed in other patients with *TK2* mutations (7). Our case presents progressive sensorineural hearing loss, not previously reported in patients with mutations in *TK2*, although it was observed in other patients with MDS (14). Interestingly, sensorineural hearing loss was documented in some patients carrying mutations in mtDNA. In most cases, mitochondrial syndromic hearing loss was associated to mutations in several tRNAs encoded by mtDNA or to the common deletion. One of the pathogenic changes most frequently found in mtDNA, the A3243G mutation in the $tRNA^{Leu(UUR)}$ has been associated in some cases to diabetes plus deafness, although the same mutation was also reported in isolated deafness (17). Nonsyndromic hearing loss is a landmark in patients with the A1555G mutation in the 12S rRNA gene (18,19). In most patients harboring this mutation, hearing loss occurs after aminoglycoside treatment, but cases of deafness caused by A1555G without aminoglycoside exposure are not infrequent (19). Hearing impairment associated to severe encephalomyopathy has been only reported in one family with MDS caused by *SUCLA2* mutations (14). To the best of our knowledge, no other MDS patients with deafness have been reported elsewhere, although we cannot exclude that, in some cases, hearing dysfunction in newborns may have been masked by severe encepahlomyopathy and/or multiorganic presentation. The case presented here broadens the clinical features of MDS with *TK2* mutations. The patient did not show symptoms of noticeable hearing dysfunction before the age of 3 y, suggesting a progressive hearing loss to moderate deafness starting around this age. This progressive pattern is common in deafness due to mitochondrial dysfunction (18).

Together with the presence of hearing loss, it is also noteworthy that the patient survived until the age of 8.5 y. More than 20 cases of MDS caused by *TK2* mutations have been reported to date (1,4–7,11–13). Approximately, half of them died before 5 y, mainly due to complications derived from the respiratory insufficiency. Ages of patients reported to be alive at the time of the report range from 5 y to more than 15 y, among those that survived after 5 y. In the case reported here, the product of the allele harboring the frameshift mutation is likely to be completely nonfunctional, and the other mutation is the same observed in the previously reported Spanish patient (5) who, interestingly, is still alive at 22 y (personal observation).

Mutations in the *TK2* gene are the most frequently reported cause of myopathic cases of MDS to date (13), probably because this was the first gene associated to this form of MDS (3), but still the reduced number of patients harboring different mutations precludes attempts to correlate genotype and phenotype. There is a discrete variability in the clinical presentations of *TK2* deficient patients, including the relatively wide range of the ages of survival discussed above. Few reports provide biochemical assessment of *TK2* enzyme activity in tissues from patients; therefore, it is difficult to determine whether differences in these residual activities among patients account for different phenotypes (*i.e.* life span or CNS involvement). Alternatively, other genetic factors and/or environmental influence could account for this variability, rather than the specific mutation present in the patient. It was noted that none of the patients reported so far is homozygous for mutations leading to complete loss of function (1) (*e.g.* nonsense mutations), which might indicate that totally nonfunctional *TK2* could be incompatible with life. In fact, when *TK2*

activity was determined in samples from patients, residual activity was always reported, in some cases reaching 30–40% of normal (3,4,6,8) and only in one case the activity was found to be negligible (<1%) (5). The residual activity may account for lack of involvement of other tissues, as proposed by Saada *et al.* (20), based on the differences in basal *TK2* activities in several tissues.

In summary, our report reveals that sensorineural hearing loss can be found in patients with MDS caused by *TK2* mutations. In addition, the extended survival of the patient reported here provides further evidence that the life span of these patients has a rather wide variability.

Acknowledgments. We thank S. Moliner for her skilful technical work, and Michael Terry for his excellent English language assistance.

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