Amyotrophic Lateral Sclerosis With Ragged-Red Fibers

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Background: Motor neuron diseases (amyotrophic lateral sclerosis [ALS] and spinal muscular atrophy [SMA]) have been rarely associated with mitochondrial respiratory chain defects.

Objectives: To describe a patient with typical ALS and the finding of ragged-red fibers in muscle biopsy specimens and to review the literature on respiratory chain defects in ALS and SMA.

Design: Case report and review of the literature.

Setting: Collaboration between tertiary care academic hospitals.

Patient: A 65-year-old man with typical ALS.

Main Outcome Measures: The patient had 10% ragged-red fibers and 3% cytochrome-c oxidase-negative fibers in muscle biopsy specimens but no biochemical defects of respiratory chain enzymes or alterations of mitochondrial DNA (mtDNA).

Results: Amyotrophic lateral sclerosis with ragged-red fibers has been reported in 5 families and is associated with mtDNA mutations in some subjects. Spinal muscular atrophy without mutations in the survival motor neuron gene (SMN; OMIM 600354) has been associated with mtDNA depletion or with mutations in the cytochrome-c oxidase assembly gene (SCO2; OMIM 604377).

Conclusion: Respiratory chain defects can mimic ALS or SMA and should be considered in the differential diagnosis.

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Mitochondrial dysfunction has an important role in the pathogenesis of familial amyotrophic lateral sclerosis (ALS), in which 20% of patients appear to have toxic gain-of-function mutations in copper-zinc superoxide dismutase 1 (SOD1), a cytosolic and mitochondrial protein. In patients with sporadic ALS, respiratory chain enzyme deficiency has been found in the spinal cord, and accumulation of abnormal mitochondria has been observed in anterior horn cells.1

Primary mitochondrial diseases (ie, those due to defects of the respiratory chain) have been associated with motor neuron disease, including ALS and spinal muscular atrophy (SMA). The neurologic manifestations of primary mitochondrial diseases are diverse and include sensorimotor peripheral polyneuropathy.2 However, purely lower motor neuron disorders are seen infrequently. We describe a patient having what seems to be typical ALS but in whom muscle biopsy specimens showed ragged-red fibers (RRFs), a sign of abnormal mitochondrial proliferation.

METHODS

PATIENT

The patient was a businessman who worked in the agricultural industry of Italy until age 58 years. After retiring, he was an enthusiastic golfer and a ballroom dancer. At age 60, he noticed twitching of his thigh muscles and stiffness of the legs. After 64, he had calf cramps, and his legs had become weak by age 65. He also had cramps in the arms and used a cane to walk. He had to use his arms for support on rising from chairs. He experienced a fall and had difficulty rising. He had no cognitive problems, weight loss, paresthesias, dysarthria, dysphagia, dyspnea, or bladder or bowel symptoms.

On examination in Italy at age 63 years, findings included profuse fasciculations in all 4 limbs, proximal arm weakness, and distal more
than proximal leg weakness, worse on the right. Sensation was normal. Tendon reflexes were brisk, with bilateral Babinski signs. Cognition and cranial nerve functions were normal. Laboratory data included the following abnormal levels: creatine kinase (466 U/L; normal, <190 U/L), aspartate aminotransferase (78 U/L; reference range, 10-45 U/L), alanine aminotransferase (71 U/L; reference range, 10-50 U/L), \( \gamma \)-glutamyltransferase (218 U/L; reference range, 3-65 U/L), and alkaline phosphatase (148 U/L; reference range, 50-120) (to convert these levels to microkatal per liter, multiply by 0.10167). His anti-myelin-associated glycoprotein (MAG) IgM titer was 1:4700 (reference range, <1:1000), but his anti-MAG IgG and anti-GMI titers were normal. His antinuclear antibody titer was greater than 1:20. The following findings were normal or negative: anti–hepatitis B surface antigen, C3-C4, anti-Sm, anti-Jo1, anti-Scl70, anti-SSA, anti-SSB, and cryoglobulin levels. His cerebrospinal fluid protein level was 8.1 g/dL (reference range, 6.0-8.0 g/dL) (to convert to grams per liter, multiply by 10.0), and his \( \gamma \)-globulin level was 21% (normal range, 11%-18.6%), with no oligoclonal bands.

Results of nerve conduction studies of motor nerves in the legs showed decreased amplitudes, and electromyography demonstrated moderate denervation in the legs but not in the glutei, suggesting motor neuropathy in the legs. Study findings in the arms were normal. The following results were normal or negative: spirometry, electrocardiogram, neuropsychological testing, and brain single-photon emission computed tomography perfusion with technetium Tc 99m.

One year later, the findings were similar. Nerve conduction studies and the electrocardiogram revealed slight slowing of sural sensory nerves and denervation in the 4 limbs. Transcranial motor-evoked potentials showed central conduction defects in the legs. Cerebrospinal fluid glucose and protein levels were normal. Tendon reflexes were brisk, with bilateral Babinski signs. Sensation was normal. Tendon reflexes were overactive throughout, with clonus at the ankles, bilateral Hoffmann signs, a right Babinski reflex, and bilateral tensor fascia lata responses.

HISTOCHEMISTRY AND BIOCHEMISTRY

Histochemical study of muscle biopsy specimens using 8-μm-thick frozen sections was performed as previously described. Biochemical analysis was performed in a 10% muscle homogenate using published procedures.

MOLECULAR ANALYSIS

Total DNA was extracted by standard protocol (PUREGENE; Gentra Systems, Inc, Minneapolis, Minnesota) following the manufacturer's instructions. Direct sequencing of the 22 transfer RNA (tRNA) genes of mitochondrial DNA (mtDNA) was performed (ABI Prism 310 Genetic Analyzer using the Big Dye Terminator Cycle Sequencing Reaction Kit; Perkin-Elmer Applied Biosystems, Foster City, California).

RESULTS

Muscle biopsy specimens revealed neurogenic changes, with 10% RRFs and 3% cytochrome-c oxidase (COX)–negative fibers (Figure). The activities of the respiratory chain complexes were normal in a muscle extract (data not shown). Southern blot analysis of mtDNA showed no large-scale rearrangement or depletion, whereas the more sensitive “long polymerase chain reaction” revealed faint abnormal bands indicative of multiple mtDNA deletions. This is probably a normal finding in a 65-year-old subject. Sequencing of the whole mtDNA showed no pathogenic mutations.

COMMENT

The clinical findings of the patient were compatible with ALS. The findings of RRFs and COX-negative fibers in muscle biopsy specimens suggested a mitochondrial motor neuron disease or 2 independent disorders. The mitochondrial proliferation revealed by the RRFs was not severe enough to be reflected in increased activity of citrate synthase, a marker of mitochondrial mass. Despite the presence of COX-negative fibers, the activities of all respiratory chain complexes were normal.

That primary mitochondrial dysfunction can cause motor neuron disease is an idea based on only a few cases. The first was in a family described by Dobkin and Verity in 1976. The propositus had dysarthria and dysphagia from childhood. His hands became weak at age 28 years, and he manifested almost purely a motor syndrome (but he had paresthesias and slowing of sural nerve conduction). A muscle biopsy specimen showed 3% RRFs, and electron microscopy demonstrated clusters of normal and giant mitochondria. His mother had a similar syndrome. Her autopsy results showed no loss of motor neurons in the brain, but anterior horn cells were depleted. The maternal grandmother of the propositus also had an autopsy performed because of weight loss and “anorexia nervosa,” but the spinal cord was not examined. This was the first reported family with SMA and mitochondrialopathy. The evidence of maternal inheritance was compatible with an mtDNA mutation, but mitochondrial genetics was to come of age only 10 years later.

The next report was in 1991, when Rowland et al mentioned a case in a series of patients with mitochondrial diseases. This was the same patient described in detail by Pons et al in 1996 as an example of the myopathic variant of mtDNA deletion syndrome due to mutations in the thymidine kinase 2 nuclear gene (TK2; OMIM 188250). Additional infants with TK2 mutations had the
SMA phenotype, confirming the importance of sequencing this gene in infants with SMA but without mutations in the SMN gene.10,11

In the meantime, Comi et al12 described a man who had developed spastic paraparesis at age 29 years. At age 32, an electromyogram showed widespread denervation. His cerebrospinal fluid protein level rose from 5.7 to 8.0 g/dL in 1 year, and his serum lactate level was borderline high. Succinate dehydrogenase stains showed a pattern of RRFs, and biochemical analysis demonstrated isolated decreased COX activity (43% of normal). Sequencing showed a 5–base pair microdeletion in the mtDNA-encoded subunit I of COX, the first described mtDNA mutation in a motor neuron disease. A description of 3 similar cases followed. Finsterer13 described a mother and 2 daughters with symptoms consistent with ALS, COX-negative fibers, and ultrastructurally abnormal mitochondria in muscle, without mutations in the SOD1 gene (OMIM 147450) but with as many as 3 different mtDNA mutations, one in the tRNAIle gene (OMIM 590045), a second in the cytochrome b gene (OMIM 516020), and a third in the adenosine triphosphatase 6 gene (OMIM 516060).

Fetoni et al14 described a man who was believed to have monomelic amyotrophy but had bilaterally overactive reflexes. The patient had diabetes mellitus, and other family members had hearing loss in a pattern of maternal inheritance. His serum lactate level was normal, but muscle biopsy specimens showed COX-negative fibers and RRFs, as well as a pattern of denervation. A mutation in the tRNASer(UCN) gene (OMIM 590080) of mtDNA was found in the patient and in a maternal niece.

Rubio-Gozalbo and colleagues15 described a child who had a severe lower motor neuron syndrome from birth and had died at age 5 months without an autopsy. The SMN gene was normal, excluding conventional SMA, but his lactate levels were high in serum and in cerebrospinal fluid. Muscle biopsy specimens showed no RRFs but demonstrated diffuse COX deficiency. The coexistence of cardiomyopathy in this child suggests that he may have had a deficiency of the nuclear-encoded COX assembly protein SCO2, as 2 other infants with SMA, and cardiomyopathy, and no mutations in the SMN gene.16,17

Borthwick et al18 described a 73-year-old man who had limb weakness for 6 months before he died of cardiac arrest while under study. He had diabetes mellitus and had undergone placement of a pacemaker after a myocardial infarction. Autopsy results showed normal motor cortex and corticospinal tracts. The number of anterior horn cells was undiminished, but many showed chromatolysis without inclusions, failing to confirm the clinical diagnosis of adult-onset progressive muscular atrophy. The findings of COX-deficient motor neurons suggested the possibility of a mitochondrial disorder. This was confirmed by mtDNA sequencing, which showed a heteroplasmic mutation in the tRNAIle gene. Although this was 1 of 3 mutant genes in the patient described by Finsterer,13 the mutation was different (J. Finsterer, MD, PhD, e-mail communication, August 2, 2007).

There seems to be little doubt now that disorders of the mitochondrial respiratory chain can cause lower motor neuron disorders compatible with SMA of children, progressive muscular atrophy of adults, or ALS. The abnormalities may be mutations in mtDNA or in nuclear genes controlling respiratory chain complexes (SCO2).
or mtDNA replication (TK2). The diversity of clinical syndromes is matched by inconsistent findings biochemically and genetically, without a clear genotype-phenotype correlation.

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