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CASE REPORT

Chronic Intestinal Pseudoobstruction with Myopathy and Ophthalmoplegia

A Muscular Biochemical Study of a Mitochondrial Disorder

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KEY WORDS: chronic intestinal pseudoobstruction; ophthalmoplegia; mitochondrial myopathy; functional mitochondrial disorders.

Chronic intestinal pseudoobstruction (CIPO) is a clinical anatomic syndrome characterized by symptoms of occlusion without a mechanical obstructing lesion. Disturbance of intestinal motility is the physiopathological mechanism common to the different etiologies of CIPO. It can be idiopathic or secondary to a systemic disease affecting the intestinal nerve and/or muscle structures (1–5). The association of CIPO with ophthalmoplegia has been reported in visceral myopathies (1, 2, 6), some of which were familial (7–9). In a recently published case (10) of visceral myopathy with CIPO and ophthalmoplegia, morphological mitochondrial abnormalities were observed, which are responsible for the "ragged red fibers" in muscles. We describe an analogous case, in which precise morphological and functional analysis of muscle mitochondria showed deterioration differing from that described by Cervera et al (10). To our knowledge, this is the first observation of this type reported in the literature.

CASE REPORT

A 47-year-old white man was seen outside hospital because of asthenia in the four limbs, disturbances of intestinal transit, weight loss, and no effect of enteral nutrition for three months. He had no abdominal surgery. Since the age of 27 years, he has had ophthalmoplegia and intermittent watery diarrhea with five to six stools daily. Two years before admission, diarrhea was accompanied by repeated episodes of nausea and vomiting. Physical examination revealed major cachexia (weight 36 kg, normal weight 67 kg) and diffuse muscle wasting. Abdominal palpation was normal. Ophthalmoplegia with diplopia and symmetric bilateral ptosis, bilateral hearing loss, decreased visual acuity, and a nasal voice were noted. There was a decrease of the arm extension test (75 sec, normal > 150). Deep tendon reflexes of the upper limbs and Achilles tendon reflex were absent. Mental functions were preserved.

The patient's sister had the same ocular and digestive symptoms, but she has had no neurologic or gastrointestinal explorations.

Gastrointestinal Tract Studies

X-ray and ultrasonograms of the abdomen were normal. The upper gastrointestinal tract and colon endoscopic examinations with duodenal and colonic biopsies showed no abnormality. In contrast, a small bowel x-ray examination disclosed moderate jejunal dilation and a decrease in mucosal folds of the ileum (Figure 1). The carmine red test was normal. Laboratory data revealed anemia (96 mg/dl) with microcytosis (70 fl, normal 90) and iron deficiency (serum iron 2.7 mmol/liter, normal > 19; serum ferritin 15 mg/liter, normal > 170). Plasma electrolytes and glucose, serum levels of thyroid hormones, vitamins A, B_{12}, E, and folates were normal. Serum tests for autoantibodies were negative. Bacteriological and parasitological examination of stools showed no pathogenic bacteries. In contrast, the hydrogen respiratory test (D-glucose 50 g) disclosed an intestinal bacterial overgrowth. The respiratory test was negative after six weeks of antibiotic treatment (doxycycline, 200 mg/day) but had

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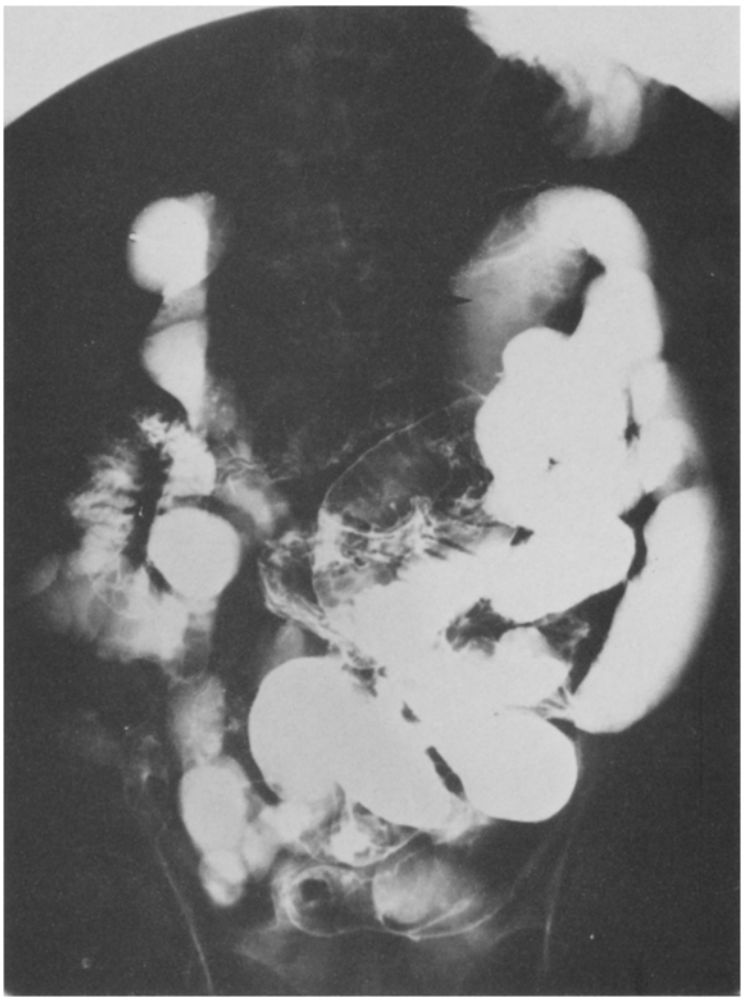

Fig 1. Small bowel x-ray: jejunal dilatation (→).

no effect on vomiting and diarrhea. The patient was again admitted to the hospital for a subocclusive episode. Abdominal x-ray showed small bowel levels (Figure 2). This episode improved in 48 hr, but vomiting recurred, complicated by an acute aspiration pneumonia which necessitated parenteral nutritive support. The clinical picture, combining pseudoocclusive symptoms and symptoms of peripheral myopathy, suggested CIPO related to myopathy. During esophageal manometry, in the striated portion of the esophagus, the deglutition-induced waves were not propagated; the mean amplitude (10 mm Hg; normal 18–80) and duration of the contractions (1.7 sec; normal 2.1–4.6) were decreased. However, these two parameters were normal in the smooth part of the esophagus, but 95% of the waves recorded were not peristaltic. The basal pressure of the lower esophageal sphincter was normal. Anorectal manometry showed low basal pressures of the smooth and striated sphincter muscles, an inhibitory rectoanal reflex of low amplitude, as well as weak voluntary contraction of the external sphincter. These abnormalities indicated a disturbance of smooth and striated sphincter muscles. Gastrointestinal motility was explored by manometry (catheter perfusion method, with two orifices in the antrum, two in the duodenum, and one 5 cm distal to the angle of Treitz) after 6 hr of fasting (11). The graph showed abnormalities classically described in CIPO syndrome (12, 13), ie, absence of phase III of the migrating motor complex throughout the recording, and contractile activity consisting of clusters without propagation; some of these clusters were organized as minute-rhythm. Ingestion of a test meal (Polydiet, 600 kcal) induced repeated vomiting after several minutes, and the recording had to be terminated. CIPO secondary to diabetes, scleroderma, or amyloidosis was easily excluded by a glucose tolerance test, capillaroscopy (14, 15), and a deep rectal biopsy with Congo red staining.

Neurological and Muscular Studies

Plasma creatine kinase and hydroxybutyrate levels and urinary organic acid chromatograms were normal. Plasma levels of pyruvates (66 μmol/liter, normal < 62), acetoacetate (62 μmol/liter, normal < 50), lactic acid (2.8 mmol/

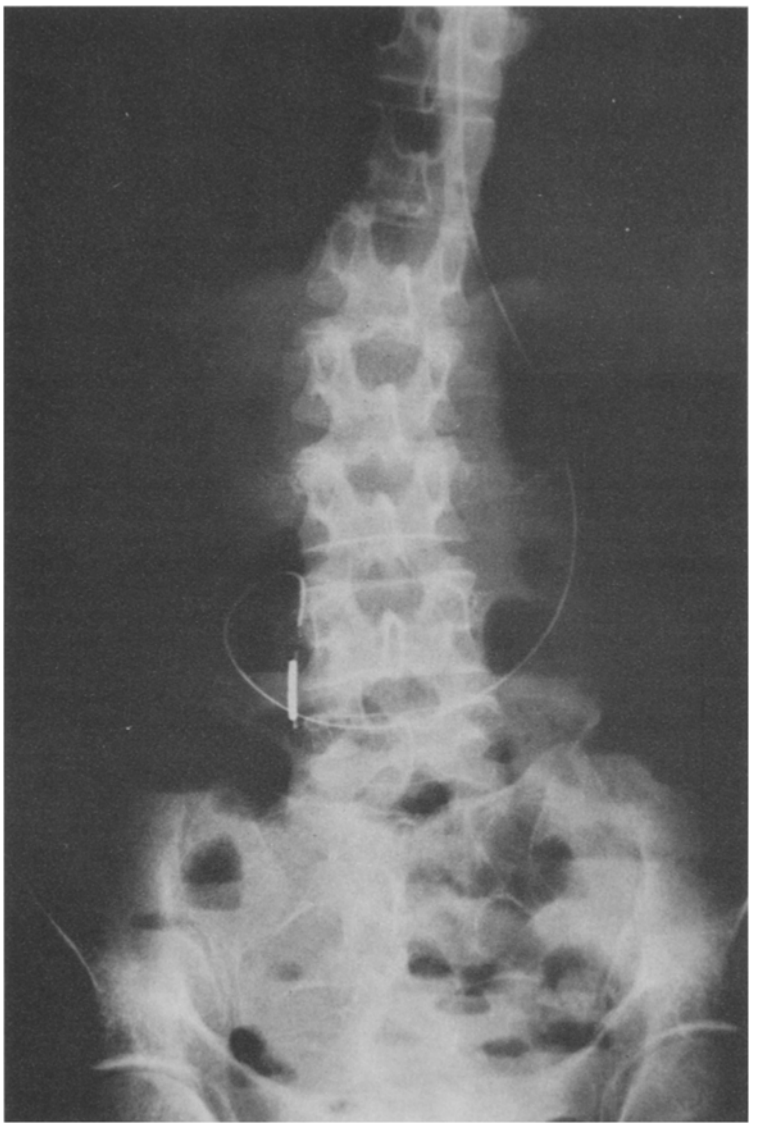

Fig 2. Abdominal x-ray: small bowel levels.

liter, normal < 1.8), and the lactate/pyruvate ratio (42.4, normal < 40) were high. During the tourniquet test, lactic acid level increased abnormally (4, 4.3, 3.7, 2.9 mmol/liter). Total plasma showed a carnitine deficit (11 μmol/liter, normal 38–63), both in free (6.4 μmol/liter, normal 30–50) and esterified forms (4.6 μmol/liter). The ratio of esterified to total carnitine was 42% (normal < 40%). However, correction of the total plasma carnitine level by parenteral supplement (carnitine, 3 g/day for six weeks) did not result in a clinical improvement. These abnormal laboratory data indicated a disturbance of muscular energy metabolism. Electromyography (EMG) of different muscle regions revealed signs of peripheral neuropathy, ie, degeneration of motor conduction (motor conduction velocity of the median nerves 31.9 m/sec, cubital 33.5 m/sec) and increased distal latencies (median nerve 6.8 m/sec, cubital 4.4 m/sec). There was no sensory potential in the nerves tested. The edrophonium test with EMG was negative, and antibodies to the acetylcholine receptor were absent, which excluded myasthenia. The EMG abnormalities were investigated by anatomopathological explorations of a musculocutaneous nerve sample. Light microscopy showed a decrease in the number of myelinated processes, particularly the large ones. This was confirmed by the increase in the myelination coefficient to 18 (normal 13.5). Moreover, electron microscopy (EM) revealed axon degeneration along with regeneration. Schwann cells contained mitochondria of atypical form (Figure 3). The clinical ocular and auditory abnormalities were investigated by different methods. Ophthalmological examination with a slit lamp revealed retinitis pigmentosa. Electroretinography showed a decrease in the amplitude of red light stimulation, indicating a macular disorder. The audiogram evidenced bilateral symmetric

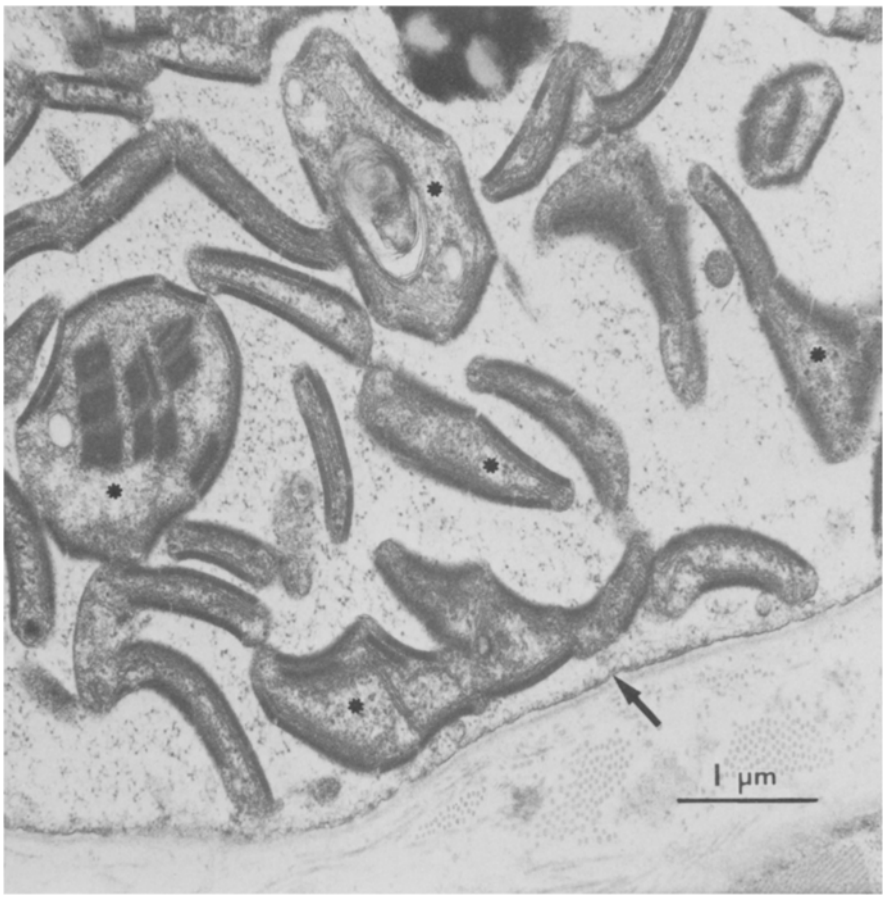

Fig 3. Mitochondria (*) of atypical form within a Schwann cell (→).

sensorineural hearing loss. Results of lumbar puncture, cerebral computer tomography, and magnetic resonance were normal (16, 17). Lastly, the cardiac conduction disorder was investigated by electrocardiography, which showed a sinus rhythm of 80 beats/min and a P-R interval of 0.14 sec, but an RSR′ on the precordial V_2 lead indicated a right bundle branch block. The echocardiogram was normal. Intravenous urography and urodynamic evaluation excluded evident disorders of the urinary tract smooth muscles. Muscle biopsies were carried out to determine the etiology of the abnormalities.

Muscular Biochemical Study

Superficial biopsy of the deltoid muscle and deep biopsy of the quadriceps muscle (1 g) were carried out under local anesthesia. A portion of the deltoid muscle biopsy specimen was frozen in isopentane, cooled by liquid nitrogen to −180° C for histochemical analysis. The second portion was fixed in 2.5% glutaraldehyde for EM study. The quadriceps muscle biopsy specimen was divided into three portions: the first for histochemical analysis, the second for biochemical analysis of the mitochondria, and the third for analysis of mitochondrial deoxyribonucleic acid (mtDNA) by molecular biological methods.

Histochemistry and EM. The deltoid muscle biopsy specimen was cut into sections 10 μm thick and subjected to various stains, ie, the conventional stains (hematoxylin-eosin, Gomori trichrome, Sudan red, Sudan black, and peroxide of Schiff) and those revealing oxidases (nicotinamide dinucleotide, reduced dehydrogenase, succinyl dehydrogenase, and a glycerophosphate) and adenosine triphosphatase (18). The specimen fixed for EM was treated according to the methods of Dubowitz and Brooke (18).

Biochemical Studies. The frozen muscle specimen was homogenized [210 mM mannitol, 70 mM sucrose, 50 mM Tris, 10 mM potassium ethylenediaminetetraacetic acid (EDTA), pH 7.4]. Mitochondria were then isolated and succinate cytochrome *c* reductase (SRC) and cytochrome *c* oxidase (COX) activities were measured by spectrophotometry according to the method of Morgan-Hughes et al (19, 20).

For the carnitine assay, the frozen muscle specimen was homogenized (10% ice-cold 120 mM HEPES, 0.1 mM EDTA buffer, pH 7.4). The concentrations of free and total carnitine were determined by the radiochemical method (21).

Polarographic Study of Mitochondria. Oxygen consumption was measured with a Clark-type oxygen electrode (19) in a total volume of 0.5 ml respiratory buffer (75

mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM Trizma-phosphate, 50 μM K2 + EDTA, 10 mM Tris HCl, pH 7.4) at 25° C using a Gilson oxygraph. Various substrates donating reducing equivalents to different sites of the respiratory chain system were investigated, ie, nicotinamide adenine dinucleotide, oxidized form-linked substrates, pyruvate (5 mM), glutamate (10 mM), and palmityl carnitine (80 mM), each added with malate (2.5 mM) and succinate (10 mM). The state 3 to state 4 transitions for oxidation of substrates were induced with small additions (125 nM) of adenosine diphosphate. Oxygen consumption, expressed in nanogram atoms per minute per milligrams protein and the values of the respiratory control ratio or state 3–state 4 were determined.

Southern blot analysis of mtDNA (22). For preparation of mtDNA, DNA was extracted from 50 mg of skeletal muscle by overnight digestion with 100 μg/ml proteinase K (40 ml Tris HCl, pH 8.0, 0.4 M NaCl, 10 mM EDTA, 0.5% SDS at 37° C). Nucleic acids were recovered by phenol extraction and ethanol precipitation and were treated for 30 min with 10 μg/ml ribonuclease A at 37° C before extraction with phenol–chloroform and precipitation with ethanol.

For Southern blot analysis, DNA (1 μg) was subjected to restriction endonuclease digestion with BamHI, separated by electrophoresis on an 0.8% agarose gel, denatured for 1 hr (1 M NaOH/1.5 M NaCl), neutralized (1 M Tris HCl, 1.5 M NaCl, pH 8.0), transferred to a nitrocellulose filter and heated for 2 hr at 80° C. The dried filter was washed for 2 min in 6 × SSC (15 mM trisodium citrate, 150 mM NaCl, pH 7.0) and prehybridized for 2 hr at 68° C (6 × SSC, 0.5% SDS, 1 mg/ml Ficoll, 1 mg/liter polyvinylpyrrolidone, 1 mg/l bovine serum albumin, and 100 μg/liter denatured salmon sperm DNA and ^{32}P nick-translated denatured mtDNA probe. The filter was washed at room temperature for 5 min in 2 × SSC, 0.1% SDS, for 15 min in 2 × SSC, 0.5% SDS, and for 2 hr at 68° C in 0.1 × SSC, 0.5% SDS, and was then subjected to autoradiography for 12–24 hr at −70° C.

RESULTS

Histochemistry and EM

Muscle fiber diameters ranged from 38 to 56 μm. With Gomori trichrome stain, most fibers were found to be vacuolated (Figure 4A); no ragged red fibers could be seen (23, 24). Lipid droplets were often present in type II muscle fibers (Figure 4B). Abnormal lipid-filled vacuoles were observed with Sudan red stain (Figure 4C). With oxidase stains, all type I and type II muscle fibers had a normal appearance. Mitochondria showed many morphological abnormalities under EM: they were involuted, with a crystalline appearance and a dense core (Figure 5). There were no nuclear inclusions (25).

Biochemical Studies

Respiratory Chain Enzymes. The absolute values of COX and SRC activities were low (Table 1), and compared to citrate synthetase activity they were 30–50% below normal (Table 2).

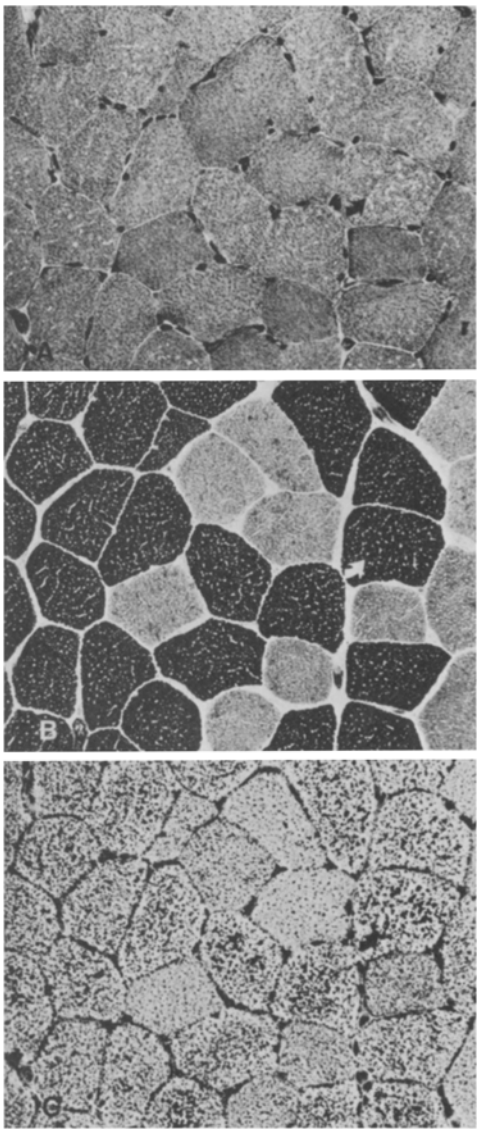

Fig 4. Frozen sections of quadriceps muscle. (A) Gomori trichrome stain: vacuoles (→) can be seen in most muscle fibers. (B) ATPase, pH 9.4: most muscle fibers with vacuoles (→) are type II. (C) Sudan red stain: vacuoles (→) contain large amounts of lipids.

Muscle Carnitine. Free carnitine was low (8.4 μmol/liter, normal 9.8) and the proportion of esterified carnitine was clearly excessive (5.6 μmol/liter).

Polarographic Study of Mitochondria. No abnormality was found in this study. The respiratory chain appeared normal (Table 3).

Southern Blot Analysis of mtDNA. No abnormality was found, specifically no deletion of mtDNA.

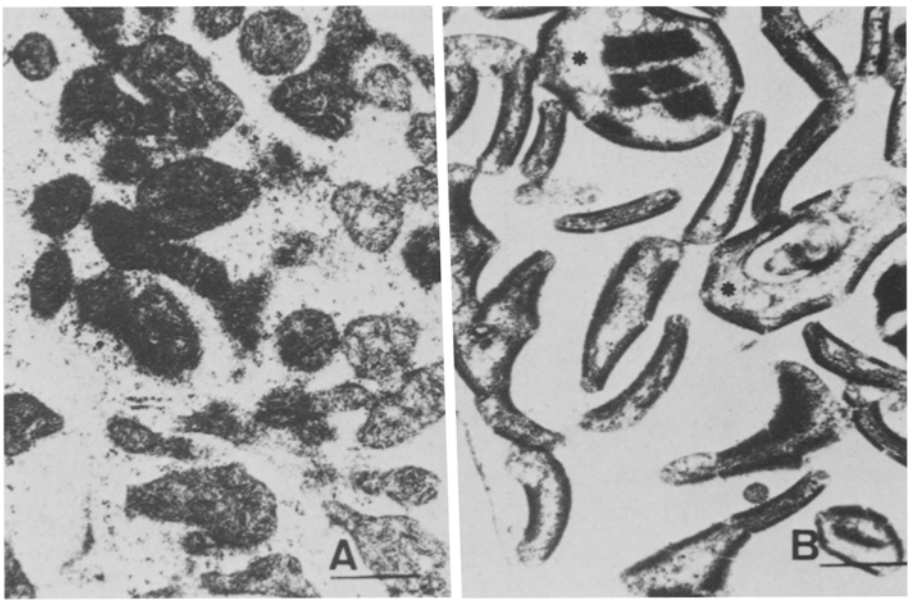

Fig 5. Mitochondria. (A) Normal mitochondria. (B) Morphological abnormalities of mitochondria (*) in a muscle fiber (→). They are involuted and have a crystalline appearance with a dense core.

DISCUSSION

Mitochondrial myopathies are a group of diseases in which morphological and histochemical abnormalities are mainly found in mitochondria. In the first case studies published (17), all patients had progressive ophthalmoplegia variably associated with high cerebrospinal fluid (CSF) protein levels, cardiac conduction disorders, retinitis pigmentosa, hearing loss, growth disturbances, electroencephalogram abnormalities, or ataxia. Lipid overloading was visible in striated muscles as was an accumulation of mitochondria with abnormal forms. The abnormalities, visualized by Gomori trichrome stain, corresponded to "ragged red fibers." The disorder was called "oculocraniosomatic neuromuscular disease" with "ragged red fibers" (17). Different types of mitochondrial myopathies have since been reported, such as Kearns-Sayre syndrome (22, 26), MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes) (23), and more recently MNGIE syndrome (myoneurogastrointestinal encephalopathy) (16).

CIPO syndrome is observed during different systemic disorders (1, 2), particularly myopathy. Anuras (1) has reported 11 families with CIPO and visceral myopathy, classified into three types according to the mode of transmission and the digestive and extradigestive symptoms. Type 2 corresponds to CIPO with ophthalmoplegia (oculogastrointestinal muscular dystrophy, OGIMD) (7, 8). It is characterized by autosomal recessive inheritance and usually appears in childhood. Cervera et al (10) re-

TABLE 1. MITOCHONDRIAL ENZYMES IN ISOLATED MUSCLE MITOCHONDRIA*

	COX (complex IV)	*SCR (complexes II+III)*	*CSase*
Controls	2582 ± 626 (N = 14)	534 ± 206 (N = 14)	2153 ± 402 (N = 13)
Patient	1447	198	2000

*Activities are expressed in nanomoles per minute per milligram protein. All assays were performed at two different protein concentrations and values are the mean of the two determinations. Results in the control group are expressed as means ± SD. COX = cytochrome *c* oxidase; SCR = succinate cytochrome *c* reductase; CSase = citrate synthase; N = number of determinations.

TABLE 2. MITOCHONDRIAL ACTIVITIES OF CYTOCHROME *C* OXIDASE (COX) AND SUCCINATE CYTOCHROME *C* REDUCTASE (SCR)*

	COX/CSase		*SCR/CSase*	
	S1	*C4*	*S1*	*C4*
Controls	1.9 ± 0.4 (N = 9)	1.2 ± 0.3 (N = 10)	0.24 ± 0.1 (N = 10)	0.24 ± 0.1 (N = 10)
Patient	1.1	0.7	0.06	0.1

*Results are expressed per unit of citrate synthase (CSase) in the supernatant (S1) of the first centrifugation (1000*g*) and in the mitochondrial pellet (C4). Results in the control group are expressed as means ± SD.

TABLE 3. POLAROGRAPHIC STUDIES WITH PURIFIED MITOCHONDRIA AT 25° C*

	ADP		
	Controls	*Patient*	*RCR*
Malate + glutamate (complexes I+III+IV)	114 ± 39	130	5.1 ± 1.6
Malate + pyruvate (complexes I+III+IV)	115 ± 42	86	4.9 ± 2.1
Malate + palmityl carnitine (complexes I+III+IV)	109 ± 31	80	3.6 ± 1.3
Succinate (complexes II+III+IV)	109 ± 32	99	2.2 ± 0.7

*Activities are expressed in nanogram atoms per minute per milligram protein. Results in the control group are expressed as means ± SD. ADP = adenosine diphosphate; RCR = respiratory control ratio.

cently reported a case of CIPO with ophthalmoplegia in which the presence of ragged red fibers indicated mitochondrial myopathy. Morphological mitochondrial abnormalities also occur in MNGIE syndrome (16), along with metabolic disturbances characterized by decreased COX activity. This syndrome is also transmitted as autosomal recessive. It can be compared to Kearns-Sayre syndrome (23) in which the same enzyme deficit is observed. However, the symptoms can be reduced by substitutive treatment with coenzyme Q10 (26).

Our observation differs in several respects from descriptions of other digestive diseases associated with ophthalmoplegia: (1) unlike OGIMD syndrome (9), our patient showed hearing loss, retinitis pigmentosa, and disturbance of cardiac conduction; (2) in contrast with MNGIE syndrome (16), CIPO was present, CSF was normal, and encephalopathy and ragged red fibers were absent; (3) unlike the only case of CIPO with mitochondrial myopathy reported in the literature (10), CSF was normal, cardiac conduction was disturbed, deep tendon reflexes were weak, ragged red fibers were absent. Despite the absence of ragged red fibers in our patient, muscle lipidosis and morphological abnormalities of the mitochondria (Figure 5) indicate a mitochondrial cause for the disease. A carnitine deficit is often found in mitochondrial myopathy (22), corresponding to an increase in metabolites from fatty acid oxidation and oxidative phosphorylation. Functional deterioration of the mitochondria was evidenced in our patient by the decrease in the activities (COX and SRC) of the complexes in the respiratory chain. This may indicate abnormality of either the internal membrane or the synthesis of proteins coded by the mitochondrial genome. Muscle mtDNA deletion has been described in Kearns-Sayre syndrome (22), but the absence of clear deletion does not exclude the possibility of a mitochondrial cause for the disease: mtDNA is of maternal origin (27), and mitotic divisions are responsible for heterogeneous distribution not only in given tissues, but also in cells of the same tissue. Heteroplasmy and variable mtDNA distribution are known to produce disorders in different organs and different clinical signs of myopathy within the same family (27, 28). Thus, the absence of evident mtDNA deletion in the skeletal muscle of our patient does not necessarily exclude localized deletion or mutation of mtDNA in digestive muscle.

To summarize, the patient was diagnosed as having CIPO with myopathy and ophthalmoplegia caused by a mitochondrial disorder. The familial character of the disorder was confirmed by the clinical observation of a bilateral ptosis in the patient's sister. In the absence of a known treatment for the disorder, the patient was given total nutritional assistance by parenteral feeding at home for 18 months.

SUMMARY

The association of chronic intestinal pseudoobstruction with ophthalmoplegia has been reported previously in visceral myopathies. We report a case of this association in which muscle mitochondria had a crystalline appearance, a dense core, and decreased cytochrome *c* oxidase and succinate cytochrome *c* reductase activities. The absence of evident mitochondrial DNA deletion in the skeletal muscle of this patient does not exclude the possibility of localized deletion or mutation of mitochondrial DNA in digestive muscle.

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REFERENCES

1. Anuras S: Intestinal pseudo-obstruction syndrome. Annu Rev Med 39:1–15, 1988
2. Krishnamurthy S, Schuffler MD: Pathology of neuromuscular disorders of the small intestine and colon. Gastroenterology 93:610–639, 1987

3. Mayer EA, Schuffler MD, Rotter JI, Hanna P, Mogard M: Familial visceral neuropathy with autosomal dominant transmission. Gastroenterology 91:1528–1535, 1986
4. Schuffler MD, Pope CE: Studies of idiopathic intestinal pseudo-obstruction. II. Hereditary hollow visceral myopathy. Family studies. Gastroenterology 73:339–344, 1977
5. Schuffler MD, Zonak Z: Chronic idiopathic intestinal pseudo-obstruction caused by degenerative disorder of the myenteric plexus: The use of Smith's method to define the neuropathology. Gastroenterology 82:476–486, 1982
6. Cave DR, Compton CC: A 21-year-old man with progressive gastro-intestinal stasis, hepatomegaly, and a neurologic disorder. N Engl J Med 322:829–841, 1990
7. Anuras S, Mitros FA, Nowak TV, Ionasescu V, Gurll NJ, Christensen J, Green JB: A familial visceral myopathy with external ophthalmoplegia and autosomal recessive transmission. Gastroenterology 84:346–353, 1983
8. Ionasescu V, Thompson SH, Ionasescu R, Searby CH, Anuras S, Christensen J, Mitros F, Hart M, Bosch P: Inherited ophthalmoplegia with intestinal pseudoobstruction. J Neurol Sci 59:215–228, 1983
9. Ionasescu V, Thompson H, Aschenbrener C, Anuras S, Rick WS: Late-onset oculogastrointestinal muscular dystrophy. Am J Med Genet 18:781–788, 1984
10. Cervera R, Bruix J, Bayes A, Blesa R, Illa I, Coll J, Garcia-Puges AM: Chronic intestinal pseudoobstruction and ophthalmoplegia in a patient with mitochondrial myopathy. Gut 29:544–547, 1988
11. Hostein J, Bost R, Bonaz B: Motricité de l'intestin grêle. Gastroenterol Clin Biol 13:T6–T9, 1989
12. Summers RW, Anuras S, Green J: Jejunal manometry patterns in health, partial pseudo-obstruction and pseudo-obstruction. Gastroenterology 85:1290–1300, 1983
13. Camilleri M, Brown ML, Malagelada JR: Impaired transit of chyme in chronic intestinal pseudoostruction. Correction by cisapride. Gastroenterology 91:619–626, 1986
14. Hostein J, Bost R, Carpentier P, Franco A, Fournet J: Motricité oesophagienne au cours de la maladie de Raynaud, de la sclérodermie systémique et du syndrome de Raynaud présclérodermique. Gastroenterol Clin Biol 19:130–135, 1985
15. Hostein J, Fournet J: Gastrointestinal manifestations of collagen diseases. Dig Dis Sci 4:240–252, 1986
16. Bardosi A, Creutzfeldt W, DiMauro S, Felgenhauer K, Friede RL, Goebel HH, Kohlschutter A, Mayer G, Rahif G, Servidei S, Van Lessen G, Wetterling T: Myo-, neuro-, gastrointestinal encephalopathy (MNGIE syndrome) due to partial deficiency of cytochrome-*c*-oxidase. Acta Neuropathol 74:248–258, 1987
17. Olson W, Engel WK, Walsh GO, Einaugler R: Oculocraniosomatic neuromuscular disease with " ragged-red fibers." Arch Neurol 26:193–211, 1972
18. Dubowitz UK, Brooke MH: Muscle Biopsy: A Modern Approach. Philadelphia, WB Saunders, 1973
19. Morgan-Hughes JA, Darneviza P, Kahn SN, Landon DN, Sherratt RM, Land LM, Clark JB: A mitochondrial myopathy characterized in reducible cytochrome *b*. Brain 100:617–640, 1977
20. Morgan-Hughes JA, Hayes DJ, Clark JB, Landon DN, Swash M, Stark RJ, Rudge P: Mitochondrial encephalomyopathies: Biochemical studies in two cases revealing defects in the respiratory chain. Brain 105:553–582, 1982
21. Parvin R, Pande S: Microdetermination of (-)carnitine and carnitine acetyltransferase activity. Anal Biochem 79:190–201, 1977
22. Romero NB, Lestienne P, Marsac C, Paturneau-Jouas M, Nelson I, François D, Eymard B, Fardeau M: Immunocytological and histochemical correlation in Kearns-Sayre syndrome with mtDNA deletion and partial cytochrome *c* oxidase deficiency in skeletal muscle. J Neurol Sci 93:297–309, 1989
23. DiMauro S, Bonilla E, Zeviani M, Nakagawa M, De Vivo DC: Mitochondrial myopathies. Ann Neurol 15:521–538, 1985
24. Serratrice G, Pelissier JF: Myopathies oculaires. Etude nosologique de 49 cas. Nouv Presse Med 16:1969–1974, 1987
25. Tome FMS, Fardeau M: Nuclear inclusions in oculopharyngeal dystrophy. Acta Neuropathol 49:85–95, 1980
26. Bresolin N, Bet L, Binda A, Moggio M, Comi G, Nador F, Ferrante C, Carenzi A, Scarlato G: Clinical and biochemical correlations in mitochondrial myopathies treated with coenzyme Q_{10}. Neurology 38:892–899, 1988
27. Poulton J, Turnbull DM, Mehta AB, Wilson J, Gardiner RM: Restriction enzyme analysis of the mitochondrial genome in mitochondrial myopathy. J Med Genet 25:600–605, 1988
28. Wallace DC: Maternal genes: Mitochondrial diseases. Birth Defects 23:137–190, 1987