CASE REPORT

Familial visceral myopathy associated with a mitochondrial myopathy

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Abstract

A 27 year old man with intestinal pseudo-obstruction who developed parenteral nutrition induced hyperlipidaemia and who also had ophthalmoplegia and an undifferentiated myopathy is described. Histological examination of biopsy specimens and molecular analysis show that this patient had both familial visceral myopathy and a mitochondrial myopathy, suggesting that a mitochondrial DNA mutation is the molecular lesion in familial visceral myopathy.

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The familial visceral myopathy syndromes are a clinically heterogeneous group of rare disorders of unknown molecular origin. Segregation patterns consistent with both autosomal dominant and autosomal recessive inheritance have been described in affected kindreds.¹ The hallmark of the syndromes is the histological intestinal abnormality characterised by smooth muscle cell vacuolar degeneration, loss, and fibrosis, especially of the outer longitudinal muscle layer.²

The mitochondrial myopathies encompass a distinct group of well described neurological syndromes (Kearns-Sayre, myoclonic epilepsy lactic acidosis and stroke-like syndromes (MELAS), myoclonic epilepsy and ragged red fibres (MERRF), and Leber's hereditary optic neuropathy) characterised histologically by the presence of coarse subsarcolemmal red staining aggregates in skeletal muscle, stained according to the modified Gomori trichrome method.³ Expression of these syndromes frequently reflects impaired mitochondrial function consequent to mitochondrial DNA (mtDNA) changes,⁴ most commonly deletions within the region spanning nucleotide bases 8000-9500.⁵ Because mitochondria are acquired from the ova, mitochondrial myopathies are generally maternally transmitted.⁶

Individuals affected by either familial visceral myopathy⁷ or a mitochondrial myopathy^{8,9} manifest varying degrees of intestinal pseudo-obstruction, polyneuropathy, leukoencephalopathy, lactic acidosis, and ophthalmoplegia. The nearly identical clinical features have prompted previous suggestions of an association between these two disorders.¹⁰⁻¹² We describe a 27 year old man with an undifferentiated myopathy. Histological examination of biopsy specimens and molecular analysis show that this patient had

both familial visceral myopathy and a mitochondrial myopathy, suggesting that a mtDNA lesion is associated with familial visceral myopathy.

Methods

PROBAND

A 27 year old, non-smoking, heterosexual Yugoslavian man presented with perforated duodenal diverticula. His past medical history included 10 years of diarrhoea alternating with constipation, intermittent abdominal cramps, inability to maintain weight, and a recent onset of muscle weakness with paraesthesia in all limbs. He also complained of difficulty with concentration. He was using no medications. His younger brother had nearly identical complaints and two sisters had died in infancy from unknown causes (Fig 1). Physical examination showed that the patient had a profound loss of body fat and muscle mass. His height, weight, and body mass index were 178 cm, 48 kg, and 15.1 kg/m², respectively. Abdominal examination showed an acute abdomen without organomegaly; stools were positive for occult blood. Neurological examination showed deficits in attention and concentration. There was moderate ophthalmoparesis on upward gaze and mild bilateral weakness in flexion and extension of muscle groups in the legs. Proprioception, vibration sense, and light touch were impaired in the legs. Cerebellar function and gait were normal.

The patient had a partial duodenal resection and a primary reanastomosis. He received total parenteral nutrition (TPN) postoperatively.

PATHOLOGY STUDIES

The duodenal tissue obtained at surgery was fixed and stained with haematoxylin and eosin. A gracilis muscle biopsy was examined with paraffin histology, a Gomori trichrome stain, and by electron microscopy.

MOLECULAR STUDIES

Total cellular DNA was extracted from 500 mg of frozen skeletal muscle using an established procedure.¹³ Mitochondrial DNA spanning the region of 8530-9110 nucleotides was amplified using the Taq polymerase chain reaction (PCR). Total cellular DNA (10 µg) was incubated in a Tempcycler (Coy) with Taq polymerase

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Figure 1: Pedigree structure showing first degree relatives. Closed symbols represent affected subjects, hatched symbols clinically suspect subjects, and open symbols unaffected subjects. The proband is depicted by the arrow.

(BRL) and the oligonucleotide primers 5'-ACGAAAATCTGTTTCGCTTCA-3' (spanning mtDNA nucleotides 8530–8550) and 5'-ATTGTGAAGATGATAAGTGT-3' (spanning mtDNA nucleotides 9090–9110).¹⁴ The target domain was amplified in 30 three-step cycles: denaturing at 94°C for 90 seconds, annealing at 55°C for 90 seconds, and extension at 72°C for 240 seconds. The amplified product was then used as a probe for Southern blot analysis and as a template for nucleotide sequence analysis. Total cellular DNA was digested with endonuclease BamHI, EcoRI, or HindIII. Southern analysis was performed as described¹³ using the PCR amplified mtDNA product labelled with the PhotoGene Nucleic Acid Detection System (BRL) as a probe. The PCR amplified mtDNA product from the proband and a control were sequenced directly using the PCR oligonucleotide primers in dideoxy sequencing.

Results

CLINICAL FEATURES

The patient's abnormalities are given in the Table. The most important were intestinal pseudo-obstruction, polyneuropathy, leuko-

encephalopathy, and many metabolic abnormalities that included a chronic lactic acidosis, total parenteral nutrition induced hyperlipidaemia, and a fasting respiratory quotient exceeding 1.0. The serum concentration of carnitine and the carnitine palmitoyl transferase activity were normal. A normal glucose response to fasting was observed (data not shown). Investigations of the proband's brother were similar, with the exceptions of the absence of total parenteral nutrition induced metabolic disturbances and the presence of type II diabetes mellitus.

PATHOLOGY

The surgical duodenal specimen from the proband showed mild villous blunting and a slightly increased cellularity of the lamina propria. There was smooth muscle cell loss with fibrosis, especially within the outer longitudinal muscle layer (Fig 2A). The myenteric plexus was unremarkable, containing numerous and normal ganglion cells. Electron microscopy examination confirmed myocyte loss and the presence of collagen fibrosis (Fig 2B). In clinical terms, these histological findings established a diagnosis of familial visceral myopathy.

The gracilis muscle biopsy showed scattered degenerating and regenerating fibres with no inflammation, fibrosis, or neurogenic changes. The Gomori trichrome stained sections showed subsarcolemmal aggregates of red granular material corresponding to abnormal mitochondria (not shown). Electron microscopy showed aggregates of mitochondria with intramitochondrial inclusions consisting of closely apposed parallel membranes resembling 'zippers' or 'parking lots' (Fig 3). These skeletal muscle fibre changes were considered pathognomonic of a mitochondrial myopathy.

MOLECULAR ANALYSES

Southern blot analysis of BamHI, EcoRI, and HindIII digested muscle DNA from our patient, hybridised with a labelled mtDNA probe,

Clinical abnormalities found in a patient with familial visceral myopathy and a mitochondrial myopathy

Laboratory test	Finding	Interpretation
Barium studies:		
Upper gastrointestinal	Normal oesophagus, mild dilatation of stomach, multiple diverticula within small bowel with stasis of contrast material	Intestinal pseudo-obstruction
Lower gastrointestinal	Few colonic diverticula with dilatation	
Nerve conduction studies	Marked delay/absence of nerve conduction in upper and lower limbs	Chronic demyelinating neuropathy
Magnetic resonance imaging of cranium	Diffuse white matter lesions	Leukoencephalopathy
Serum lactate	>3.5 mmol/l	Chronic lactic acidosis
Fasting RQ	>1	Incompetent fat/lipid metabolism
Total parenteral nutrition feeding	Milky serum, cholesterol >18 mmol/l, triglycerides >15 mmol/l, no weight gain after 3 months	Total parenteral nutrition induced hyperlipidaemia

Figure 2: (A) Light microscopy of duodenum showing myocyte loss and fibrous replacement (arrow) within the outer longitudinal muscle layer. (B) Electron microscopy of same specimen showing myocyte loss and fibrous replacement with collagen (upper half of figure).

Figure 3: Electron microscopy of gracilis muscle biopsy tissue showing an aggregate of mitochondria containing 'parking lot' inclusions.

showed fragments of predicted size when compared with a normal control (Fig 4). No heterogeneity of mtDNA species (heteroplasmy) was found.

The nucleotide sequence of the mtDNA domain 8530–9110 nucleotides (a region that encodes subunit 6 of ATPase) from the proband was identical to the wild type sequence, except for a single base substitution of a thymidine for cytosine at position 8634 (Fig 5). This substitution was silent at the protein level: a tyrosine residue was translated both from the patient and the wild type mtDNA.

Discussion

We present a patient with the concomitant diagnoses of familial visceral myopathy and a mitochondrial myopathy. The diagnosis of familial visceral myopathy was based on characteristic clinical features and histological abnormalities within the small bowel. The diagnosis of a mitochondrial myopathy was based on the pathognomonic findings within the mitochondria of skeletal muscle cells when examined by Gomori trichrome and electron microscopy. The findings in this patient suggest that familial visceral myopathy and mitochondrial myopathies may have a similar molecular origin and could represent two points on the spectrum of a single disease.

Familial visceral myopathy and the mitochondrial myopathies have been distinguished from each other on the basis of muscle type affected. Familial visceral myopathy has been associated with smooth muscle cell abnormalities, whereas the mitochondrial myopathies have been characterised by skeletal muscle cell abnormalities. None of the reported cases of familial visceral myopathy, however, have undergone a Gomori trichrome stained muscle biopsy.^{1 2 7 15–22} Likewise, none of the reported cases of mitochondrial myopathies, specifically those in which patients were predominantly affected with a chronic intestinal pseudo-obstruction, have reported the histological findings of the small bowel wall.⁹ It is thus possible that the failure to detect coexistent abnormalities in these conditions is the result of a lack of specific histological examination.

There are similarities in the expression of the clinical phenotypes of familial visceral myopathy and of the mitochondrial myopathies. For example, intestinal diverticulosis is a characteristic accompaniment of familial visceral myopathy and has also been described in the mitochondrial myopathies.⁹ Furthermore, extra-intestinal neurological manifestations in familial visceral myopathy include ophthalmoplegia, megacystis, dementia, and seizure disorders,^{2 19 20 22} features that are also characteristic of mitochondrial disorders. In addition, a patient with familial visceral myopathy had lactic acidosis, total parenteral nutrition induced hyperlipidaemia, right bundle branch block, polyneuropathy, and leukoencephalopathy,⁷ features that are characteristic of mitochondrial disorders. Our patient had many of these clinical features.

A factor that could theoretically distinguish

LEGEND:

probe = mtDNA(8530-9110)
 lane 1 = FVM subject BamHI
 lane 2 = normal EcoRI
 lane 3 = FVM subject EcoRI
 lane 4 = normal HindIII
 lane 5 = FVM subject HindIII

Figure 4: Southern analysis of muscle mitochondrial (mt) DNA from the proband and control subject. The band sizes are shown. Lane 1 represents mtDNA from the proband after digestion with BamHI. Lanes 2 and 3 compare mtDNA from a normal subject with mtDNA from the proband, respectively, after digestion with EcoRI. Lanes 4 and 5 compare mtDNA from the normal subject with mtDNA from the proband, respectively, after HindIII enzymatic digestion. Fragments of expected size were observed in each case: digestion with BamHI produced a 16.5 kb fragment corresponding to the linearised, wild type mtDNA. Digestion with EcoRI and HindIII produced the expected fragments of 7.3 and 5.5 kb, respectively.

between familial visceral myopathy and the mitochondrial myopathies is their mode of inheritance. Familial visceral myopathy is inherited as an autosomal trait,¹ whereas the mitochondrial myopathies classically display a maternal inheritance.²³ Our patient had an affected male sibling and two female siblings who had both died in infancy, suggesting autosomal dominant inheritance with variable penetrance. This would be compatible with familial visceral myopathy but not with the classical mitochondrial myopathies. However, three pedigrees with mitochondrial myopathies have been reported in which defective paternal genes affecting nuclear control over mtDNA replication resulted in large scale mtDNA deletions.²⁴ Another study has shown paternal transmission of mtDNA from males in backcrossed mice.²⁵ Thus, the mode of transmission does not necessarily distinguish familial visceral myopathy from a mitochondrial myopathy.

The genetic defect in familial visceral myopathy is unknown. In contrast, 40% of patients with mitochondrial myopathies have large scale deletions in muscle mtDNA usually including the region between nucleotides 8000 and 9500. In addition, one form of MERRF and one form of Leber's hereditary optic neuropathy are caused by point mutations within the mitochondrial genome.^{26,27} Our patient had no gross structural changes of the muscle mtDNA genome. Furthermore, detailed sequence analysis of our patient's muscle mtDNA between nucleotides 8530 and 9110, a region that is often affected in mitochondrial myopathies, showed only a silent DNA polymorphism. The molecular defect in our patient remains to be determined. It is still possible that a single lesion affecting the mtDNA genome is responsible. The finding of

Figure 5: Direct dideoxy sequencing of muscle mitochondrial (mt) DNA of the proband and a control subject. The reading frame is 5' to 3' from bottom to top and the mtDNA sequences are compared for a normal control and the proband. A single base substitution of thymine (T) for cytosine (C) at position 8634 was found. The codons each specify tyrosine.

position	8629-31	8632-34	8635-37
codon	Lys	Tyr	Leu
normal	AAA	TAT	CTC
patient	AAA	TAC	CTC
		Tyr	

concomitant familial visceral myopathy and mitochondrial myopathy in this family suggest that familial visceral myopathy may be a mitochondrial myopathy.

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