Mitochondrial enteropathy: the primary pathology may not be within the gastrointestinal tract

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Abstract

Background—Mitochondrial DNA (mtDNA) defects are an important cause of disease. Although gastrointestinal symptoms are common in these patients, their pathogenesis remains uncertain.

Aim—To investigate the role of the mtDNA defect in the production of gastrointestinal dysfunction.

Patient—A 20 year old woman who presented at 15 years of age with recurrent vomiting and pseudo-obstruction, who did not respond to conservative management and ultimately had subtotal gastrectomy and Roux-en-y reconstruction. She subsequently presented with status epilepticus and was found to have a mitochondrial respiratory chain disorder due to a pathogenic mtDNA point mutation (A3243G).

Methods—Resected bowel was studied using light and electron microscopy and mtDNA analysed from both mucosal and muscular layers using polymerase chain reaction generated RFLP analysis.

Results—Histological and electron microscopic studies revealed no morphological abnormalities in the resected stomach, and molecular genetic analysis failed to identify the genetic defect in either the mucosal or muscle layers.

Conclusion—This study suggests that in some individuals with gastrointestinal symptoms associated with established mitochondrial DNA disease, the primary pathology of the mitochondrial enteropathy lies outside the gastrointestinal tract.

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Keywords: mitochondrial encephalomyopathy; cyclical vomiting; pseudo-obstruction

Although traditionally the domain of the neurologist, disorders caused by mitochondrial DNA (mtDNA) defects are now being recognised by physicians in almost every speciality. For example, mtDNA mutations cause 0.5–1% of all cases of diabetes mellitus, and up to 0.5% of strokes in the younger age group. Gastrointestinal features are common in patients with mitochondrial DNA disease. More than 10% of such patients complain of dysphagia; recurrent vomiting and anorexia are well recognised, and gastrointestinal pseudo-obstruction has been reported (for example, see Heil and colleagues). Several mtDNA defects have been described in association with gastrointestinal symptoms but the most frequently reported are the A3243G mutation in transfer RNA (tRNA)Leu(UUR), which is most commonly associated with the multisystem disorder MELAS (mitochondrial encephalomyopathy, lactic acidois, and stroke-like episodes) and an autosomally inherited predisposition to multiple mtDNA deletions.

Patients with mitochondrial DNA disease most often harbour a mixture of mutant and wild-type mtDNA (heteroplasmy). At the cellular level, the concentration of mutant mtDNA must exceed a critical threshold (usually 80–85%) before a mitochondrial respiratory chain deficiency is expressed. The percentage level of mutant mtDNA not only varies between individuals with mtDNA disease but also within individuals. This genetic variability contributes to the phenotypic variability seen among patients who harbour the same mitochondrial genetic defect.

Case report

A 20 year old Caucasian woman presented at age 15 to a surgical service with recurrent vomiting and dehydration. Barium studies demonstrated a poorly functioning stomach and dilated duodenum and she was diagnosed as having a pseudo-obstruction. She was treated conservatively which included a prolonged period of total parenteral nutrition during which time she developed glucose intolerance and required insulin treatment. With failure of conservative management she underwent gastrojejunostomy and insertion of a jejunal feeding tube. Small bowel biopsy at this stage revealed no pathological features. She relapsed three months later with vomiting and hypokalaemia that failed to respond to antietemics and potassium supplements, underwent subtotal gastrectomy and Roux-en-y reconstruction, and remained stable from a gastrointestinal viewpoint thereafter for several years. Histological examination of the resected bowel failed to identify the genetic defect.

Abbreviations used in this paper: EEG, electroencephalography; MELAS, mitochondrial encephalomyopathy with lactic acidois and stroke-like episodes; mtDNA, mitochondrial DNA; tRNA, transfer RNA; PCR, polymerase chain reaction; PBS, phosphate buffered saline.
bowl was again normal. Following operation she developed muscle weakness and tendoachilles contracture and was treated with physiotherapy.

Two years later she presented in status epilepticus after drinking a very small amount of alcohol. She was profoundly acidic (pH 6.9) with a blood lactate concentration of 22.4 mmol/l (normal <1.7 mmol/l), required paralysis, ventilation, and correction of her acidosis for seizure control, but was extubated 24 hours later. Following extubation, she developed a progressive rhabdomyolysis (creatine kinase >49 000) without renal dysfunction. At this stage mitochondrial disease was considered. Examination showed that she was small, thin, and generally wasted. She had sensorineural deafness but no retinal pigmentation or disorder of eye movement. Muscle strength was reduced symmetrically in both proximal and distal muscles and reflexes depressed, but preserved. Electroencephalography (EEG) showed changes compatible with encephalopathy, electromyography a minor myopathic disturbance, and fasting studies revealed elevated cerebrospinal fluid lactate (3.6 mmol/l; normal <1.7 mmol/l). Her mother had non-insulin dependant diabetes, as did her maternal grandmother. Her mother also had exercise induced muscle cramps and her sister an unexplained sensorineural deafness. The patient underwent diagnostic muscle biopsy.

METHODS
Bowel samples for histology and electron microscopy were processed routinely. Muscle histochemistry and biochemistry were performed as previously described.

Genomic DNA was extracted using a standard proteinase K/SDS protocol from skeletal muscle and blood. The stomach was the only gastrointestinal tissue available for analysis. The resected stomach had been fixed in formaldehyde but was not wax embedded. The mucosal and smooth muscle layers were dissected under a dissecting microscope (Zeiss, Axioplan 2) before being frozen in liquid nitrogen. Solid blocks of tissue from mucosal and smooth muscle layers were, on three separate occasions, ground into a powder in an autoclaved mortar and pestle. This powder was suspended in phosphate buffered saline (PBS, pH 7.4) and the suspension was washed three times in PBS, before being digested with proteinase K in the presence of 10% SDS solution (24 hours while being gently agitated at 37°C). The samples were briefly centrifuged at 13 500 rpm and the supernatant removed and purified by three rounds of phenol/chloroform-isooamylalcohol extractions. DNA was precipi-

Figure 1  (A) Histology of the resected stomach. Cross section of the stomach showing normal histology. (B) Succinate dehydrogenase (SDH) activity in skeletal muscle. Skeletal muscle in cross section. SDH is a mitochondrial enzyme and this section shows the classical accumulation of mitochondria around the periphery of the fibre (classically called ragged red with Gomori Trichrome staining) which suggests a mitochondrial disorder. (C) Last hot cycle PCR of mtDNA. Autoradiograph of a 12% polyacrylamide gel showing the results of the Hae III digest. The 135 bp radiolabelled PCR uncut product is shown in lane 6. When this is digested it produces two bands (98 bp and 37 bp) in the wild-type (lane 5, normal control). The A3243G MELAS mutation introduces an additional Hae III site in the 98 bp fragment, resulting in 73 bp and 25 bp fragments. Patient tissues are shown in lanes 1–4: lane 1, skeletal muscle; lane 2, stomach mucosal layer; lane 3, stomach muscle layer; lane 4, white blood cell. The two extra bands arising from the A3243G mutation are easily seen in her skeletal muscle and blood, but not in either the muscle or mucosal layers of her stomach.
Mitochondrial enteropathy was incorporated in the "last hot cycle" of the PCR reaction before digestion with 5 U of Hae III (Roche Diagnostics, UK). For the wild-type template the 135 bp fragment cuts into 98 and 37 bp fragments. The A3243G mutation creates an additional restriction site, and the 98 bp fragment is cut into 73 and 25 bp fragments. The digestion products were then electrophoresed on a 12% non-denaturing polyacrylamide gel (150 V for 4–5 hours). The intensity of each band was quantified using a phosphorimage screen and Imagequant software (Molecular Dynamics), allowing calculation of the relative proportion of mutant and wild-type mtDNA. Direct sequencing of mtDNA was carried out as described previously. In brief, 500 bp segments of mtDNA were amplified using M13 tailed primers, dye-primer sequencing chemistry and an ABI 377 automated sequencer. Sequence data were analysed with Factura software (Perkin-Elmer, UK).

RESULTS

Histological examination of the resected stomach showed no abnormality of the mucosa, submucosa, or muscularis propria. The neural plexus and ganglion cells also appeared normal (fig 1A). Histochemical analysis of fresh muscle revealed that 10% of her muscle fibres had the classical "ragged red" appearance (fig 1B) but none was deficient in cytochrome c oxidase, a mitochondrial respiratory chain enzyme. Mitochondrial respiratory chain studies revealed a deficiency of complex IV (50% of normal), with normal activity of complexes I, II and III. Molecular genetic analysis confirmed the presence of the A3243G (so-called MELAS) mutation, with 25% mutant mtDNA in her blood and 52% in skeletal muscle. The A3243G MELAS mutation was not detected in either the mucosal or muscle layer of her stomach (fig 1C). We sequenced the highly polymorphic mitochondrial DNA d-loop (1000 bases) from the stomach muscle layer DNA and the skeletal muscle DNA. There was a complete sequence match between the two d-loops. We identified the following changes with reference to the revised Cambridge reference sequence12: G16129A, T16224C, T16311C, T16519C, A73G, T146C, T195C, A263G, C303 insertion, C311 insertion, AC524 insertion. This sequence was observed only twice in a forensic database of 1962 Caucasian hypervariable region sequences (HV1: nt 16024–16365 and HV2: nt 73–340). Bootstrap analysis ("FreqBoot" TJ Parsons, JA Irwin. Armed Forces DNA Identification Laboratory) revealed that the probability of any two unrelated individuals having the same HVRS as our patient had an upper 95% confidence limit of 0.3%. Likelihood ratio calculations indicate that the matching sequence was 300 times more likely if the samples were from the same individual rather than if they were from unrelated Caucasian individuals chosen at random. In addition, we also sequenced the leucine UUR tRNA gene in the DNA extracted from the stomach and found that it was identical to the Cambridge reference sequence for the human mitochondrial leucine tRNA gene.

Discussion

Our patient presented with symptoms of gastric outflow obstruction and radiological investigation showed gastric dysmotility. After failing to respond to conservative treatment, including long term parenteral nutrition, she underwent gastric resection. Two years later she presented in status epilepticus with a very high blood lactate, which suggested the presence of a mitochondrial disorder. This was confirmed by investigation, including muscle biopsy, and the finding of a known pathological mtDNA mutation at position 3243 in tRNA16. Examination at this stage showed many of the features recognised previously in association with this mtDNA mutation, namely, small stature, deafness, glucose intolerance, myopathy, and lactacidosis.

Gastrointestinal symptoms are common in patients with mitochondrial disease. In many, nausea and vomiting are presumed secondary to lactic acidosis, which is known to induce gastric stasis. In others mitochondrial dysfunction within the gastrointestinal tract is the presumed explanation. Patients with mitochondrial neurogastrointestinal encephalopathy17 or, the probably synonymous condition, polyneuropathy, ophthalmoplegia leukoencephalopathy, and intestinal pseudo-obstruction,18 present with gastrointestinal symptoms, particularly obstruction, and in some this is due to an autosomal gene defect that induces multiple mtDNA deletions. Ischaemic colitis19 and chronic diarrhoea20 have been described in association with the A3243G (MELAS) mutation and in one case investigation showed the presence of mutation within the bowel mucosa.21 Prominent bowel symptoms have also been described with other point mutations.19 The pathophysiology of the gastrointestinal symptoms associated with mitochondrial disease remain, however, uncertain. Subtle histological abnormalities in isolated cases have pointed towards a gut myopathy or a myenteric plexus neuropathy.5

Our patient presented with gastrointestinal symptoms but a diagnosis was only made when she manifested more frequently encountered features of mitochondrial disease. In order to understand the pathophysiology of her original symptoms, we investigated her resected bowel. The stomach and small intestine appeared histologically normal. We separated the mucosal and smooth muscle layers of the stomach to look for the A3243G mutation and were surprised to find it absent, even using the sensitive technique of last cycle hot PCR. In case we were mistakenly looking at tissue from another patient, we sequenced the highly polymorphic D-loop (1000 base pairs) from both the stomach and skeletal muscle and found a complete sequence match. The probability of identifying this sequence in the HVRS of two unrelated individuals has an upper 95% confi-
Incidence limit of 0.3%, making this possibility highly unlikely. We also sequenced the whole tRNA\(^{\text{Leu(UUR)}}\) gene in the stomach and found that it was identical to the standard (Cambridge) sequence making it highly unlikely that we were looking at an artefact of nuclear pseudogene amplification. Thus our patient who presented with gastric dysmotility did not appear to have the A3243G mutation at significant levels in her bowel.

How, therefore, can we explain her gastrointestinal symptoms and radiological findings? Mutations in mtDNA are usually heteroplasmic (mutant and wild-type mtDNA present in the same individual and even within the same cell) and it appears that the level of mutant must exceed a threshold before phenotype is manifest. Recent studies have shown that mtDNA mutations may increase and indeed decrease with time. Could this have occurred in the patient’s gut? We feel this is unlikely as the stomach was removed at a time when she was symptomatic and investigation confirmed dysmotility. It is possible that significant levels of mutant mtDNA were present in other regions of the small intestine, and pathology within these regions was responsible for the pseudo-obstruction. While these tissues were, unfortunately, not available for further analysis, the stomach, duodenum, and jejunum share a common embryological origin and should therefore have a similar mitochondrial composition. Nausea and vomiting are prominent features of other mitochondrial disorders, particularly Leigh’s disease in which the major site of pathology is thought to be within the brain stem. Moreover, migraine aura and classical migraine are associated with MELAS. Again the site of dysfunction thought to produce these features is central with outflow through the autonomic nervous system, particularly the vagus nerve. We suggest, therefore, that this patient suffered gastrointestinal symptoms that were due to autonomic nervous system involvement. Where in the central nervous system this dysfunction arises is unclear. Her epilepsy and persisting EEG abnormality point, however, to involvement within the brain even though imaging studies have thus far been unremarkable.

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