Progress Report

Unexplained gastrointestinal symptoms: Think mitochondrial disease

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ABSTRACT

Defects in mitochondrial function are increasingly recognised as central to the pathogenesis of many diseases, both inherited and acquired. Many of these mitochondrial defects arise from abnormalities in mitochondrial DNA and can result in multisystem disease, with gastrointestinal involvement common. Moreover, mitochondrial disease may present with a range of non-specific symptoms, and thus can be easily misdiagnosed, or even considered to be non-organic.

We describe the clinical, histopathological and genetic findings of six patients from three families with gastrointestinal manifestations of mitochondrial disease. In two of the patients, anorexia nervosa was considered as an initial diagnosis. These cases illustrate the challenges of both diagnosing and managing mitochondrial disease and highlight two important but poorly understood aspects, the clinical and the genetic.

The pathophysiology of gastrointestinal involvement in mitochondrial disease is discussed and emerging treatments are described. Finally, we provide a checklist of investigations for the gastroenterologist when mitochondrial disease is suspected.

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1. Introduction

Defects in mitochondrial function are increasingly recognised as central to the pathogenesis of many diseases, both inherited and acquired [1]. Mitochondria are dynamic organelles present in every nucleated cell and play an essential role in cellular energy production. Consequently mitochondrial defects can result in dysfunction of almost any organ, particularly those with high energy demand.

Many of these mitochondrial defects result from abnormalities in mitochondrial DNA (mtDNA). While mtDNA disease may result from sporadic mutations, when transmission does occur it is classically through the maternal line, as either point mutations or complex mtDNA rearrangements [2–4]. However, as mtDNA relies upon the cell nucleus for replication and maintenance, nuclear gene defects can result in secondary mtDNA abnormalities. This is seen in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), which is inherited as an autosomal recessive disorder

[5]. In addition, there are many other genes involved in mitochondrial biogenesis and dynamics; an example is the OPA1 gene which plays a key role in mitochondrial dynamics, with mutations leading to optic atrophy syndromes [6]. Fig. 1 provides an overview of the common abnormalities in the mitochondrial genome, and the associated diseases. The diverse group of disorders that result from mitochondrial disease are described in greater detail in the following articles [7,8].

Mitochondrial disease is more common than previously thought, with an estimated prevalence of 1 in 500 [9]. Greater awareness of mitochondrial disease and improvements in analytical technique have led to improved detection of simple mtDNA defects, such as the single deletion commonly seen in Chronic Progressive External Ophthalmoplegia [10]. However, more complex mtDNA rearrangements which classically result in multisystem disease are still greatly under-diagnosed. This is partly because multisystem mitochondrial disease may present to many medical specialties without being diagnosed.

The prevalence of medically unexplained symptoms (MUS) in outpatients is common and ranges between 25% and 75% [11]. Comorbid psychiatric disorders are frequent in such patients, who present major challenges to conventional medical management, are commonly frequent attenders and may have misattributed

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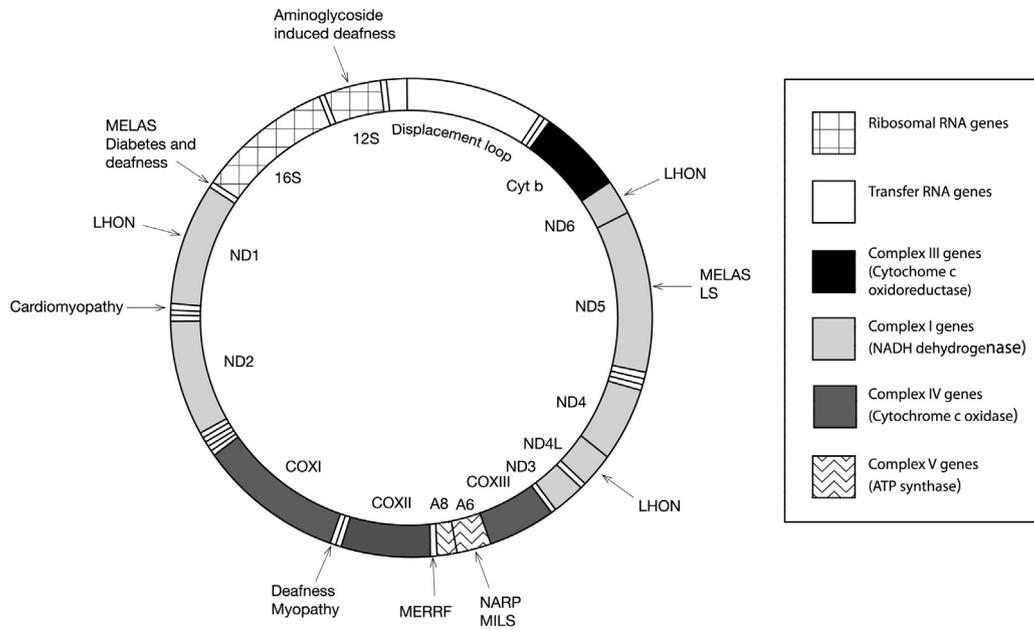E-mail address: simon.travis@ndm.ox.ac.uk (S.P. Travis).

Fig. 1. Diagram of the human mitochondrial genome and sites of mutations leading to important clinical phenotypes. Adapted from "Seminars in medicine of the Beth Israel Hospital, Boston. Mitochondrial DNA and disease" by Johns [7]. The 16,569 np mtDNA map demonstrates the genes for the subunits of NADH dehydrogenase (ND), cytochrome c oxidase (COX), cytochrome b (cyt b), ribosomal RNA (12S and 16S) and ATPase (A6 and A8). LHON, Leber's hereditary optic neuropathy; MELAS, syndrome of mitochondrial encephalomyopathy, lactic acidosis and stroke like episodes; LS, Leigh's syndrome; NARP, neuropathy, ataxia and retinitis pigmentosa; MILS, maternally inherited Leigh's syndrome; MERRF, myoclonic epilepsy with ragged-red fibres.

psychiatric conditions. MUS can, however, only be diagnosed when organic disease has been excluded [11]. Gastrointestinal involvement is common in patients with mtDNA disease, affecting up to 15%, yet symptoms are frequently overlooked as they may be non-specific such as abdominal pain, chronic constipation or vomiting [12]. Other manifestations include severe gut dysmotility and profound weight loss, which may be among the principal presentations of mitochondrial disease, as in MNGIE [13]. Importantly, mitochondrial disease can be easily mistaken for anorexia nervosa. Indeed the misdiagnosis of organic disease as 'anorexia nervosa' is well recognised in the literature [14–17].

In addition to the often non-specific presentation of mitochondrial disease, the genetics present further challenges to diagnosis. Firstly, even when sought, underlying complex mtDNA rearrangements are often missed by routine analytical techniques or are indistinguishable from simple, single deletions, consistent with low overall rates of detection [5,18,19]. Accurately defining the genetics has important implications both for the transmission and clinical presentation of mitochondrial disease. Complex mtDNA rearrangements including duplications are frequently maternally inherited and multi-systemic, whereas simple deletions are generally sporadic and myopathic. Secondly, there is marked variability in clinical presentation of mtDNA disorders, even within the same family and with apparently similar genotypes. Two unique features of mitochondrial genetics play an important role in determining phenotypic differences: these are heteroplasmy (the existence of two or more mitochondrial genotypes within the same cell, the proportion of which may vary both between and within individuals) and the threshold effect (the level of mutant mtDNA load necessary for clinical expression), but there is still much to explain [1]. A better understanding of complex mtDNA mutations is essential to provide further insight; it is likely that incomplete mapping of complex mtDNA rearrangements may account for some of the unexplained phenotypic variation seen with mtDNA disorders.

2. Case series

2.1. Family A

Three siblings from a consanguineous family (mother and father were first cousins), who initially presented with medically unexplained gastrointestinal and neurological symptoms are described, in whom the diagnosis of a mitochondrial disease due to MNGIE was subsequently established. The genetic pedigree is shown in Fig. 2. A psychiatric disorder (anorexia nervosa) was considered in two of the family members.
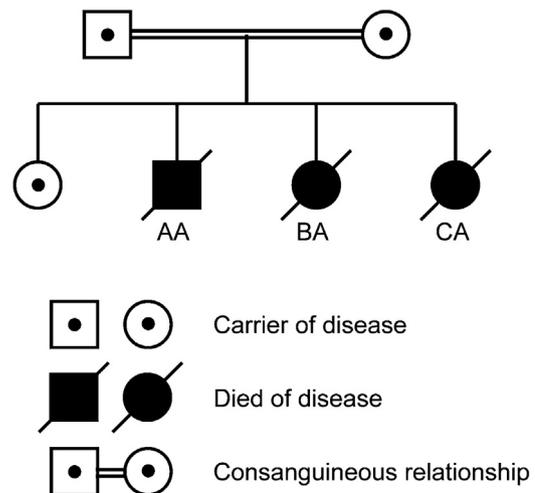

Fig. 2. The genetic pedigree of family A.

2.2. Patient AA

The eldest son had intermittent vomiting from age two and diarrhoea from age four. In 1981 aged 12 years, he was admitted to hospital with vomiting and suspected bulimia nervosa. However, stenosis of the duodeno-jejunal junction leading to obstruction was subsequently found at exploratory surgery, and a gastro-jejunostomy performed. He continued to vomit and an antrectomy was performed later that year. At 13 he was readmitted with poor weight gain and a peptic ulcer was diagnosed. Symptoms persisted and at a third laparotomy, gastro-duodenal dilation was found, so the gastro-jejunostomy was disconnected, the upper jejunum resected and an end-to-end jejuno-jejunal anastomosis performed with a duodenoplasty. A further laparotomy for vomiting at age 14 after treatment with cisapride and a period of parenteral nutrition, resulted in a Roux-en-Y gastrojejunostomy. Intestinal neurohistochemistry assessing adrenergic and cholinergic innervation was normal.

He was treated extensively at a tertiary children's hospital in London. On his final hospital admission aged 15, he was 159 cm tall and weighed 28.5 kg (BMI 11.2 kg/m^2). Lower limb weakness and foot drop with a broad-based gait was noted. Large joint position sense was poor, with marked sensory loss in feet and ankles. By the age of 16, he was unable to stand without support and had peripheral sensory loss in upper and lower limbs. Electromyography showed a demyelinating polyneuropathy, with chronic inflammation on a sural nerve biopsy. Brain CT scan, cerebrospinal spinal fluid and visual evoked responses were normal. Correction of selenium and chromium deficiency had no effect. Chromosomal, hormonal and toxicology screens were normal or negative. He had an episode of haemolytic anaemia (haemoglobin falling to 3.7 g/dL). No single diagnosis could be reached. He was intermittently fed intravenously while his physical debility progressed, with treatment withdrawn 'in the patient's best interests, with no hope of a cure'. He died at home at the age of 17. The death certificate records bronchopneumonia, chronic malnourishment, severe gastro-intestinal motility disorder and peripheral neuropathy. A post-mortem showed that aspiration pneumonia was due to mechanical intestinal obstruction from adhesions, with *Clostridium difficile* colitis and emaciation as contributing factors.

The differential diagnosis in the notes serially records Munchausen's syndrome, anorexia nervosa and severe peripheral neuropathy of unknown cause. Comments are revealing: 'physical symptoms are far from being purely psychosomatic, but constitute significant organic pathology, albeit with eternally puzzling aetiology'. Diagnostic and therapeutic manoeuvres had proved 'singularly useless'. The 'family psychopathology' was said to be 'untreatable'. The patient was said to be 'depressed and have a love of operations', with an 'obsessive interest in medical conversation'.

2.3. Patient BA

In 2004, AA's sister presented at the age of 18 with neurological symptoms. She had a two month history of dropping objects, weak grip strength and an inability to bend her forefinger to her thumb. She also had balance problems and had fallen; at presentation to Accident and Emergency aged 17 with a sprained ankle, a bilateral foot drop was noted. She subsequently described that her thighs started burning after short walks, and she injured herself without realising it when shaving her legs. She was underweight, but not underdeveloped.

At the age of 18, she was investigated by her local neurology service. She had wasting of the thenar eminence and reduced muscle tone in all four limbs. She was areflexic with down going plantars, and had absent peripheral proprioception and vibration sense and impaired sensation to pinprick or light touch. Nerve conduction studies demonstrated a severe sensory and motor peripheral neuropathy and a possible diagnosis of chronic inflammatory demyelinating polyneuropathy was suggested. She was consequently treated with intravenous immunoglobulin, and also received physiotherapy, ankle and wrist splints.

She was seen again later the same year, needing support to walk. Nerve conduction studies were repeated and confirmed significant deterioration. She was prescribed further immunoglobulin and prednisolone. She deteriorated and was transferred to the regional neurology service in Oxford, where it was noted that she lacked the sensory features commonly associated with demyelinating polyneuropathy. A trial of plasma exchange was unsuccessful.

Urinary thymidine (for thymidine phosphorylase deficiency) was negative. This false negative result was likely due to bacterial degradation of urinary thymidine during transit and could have been avoided either by adding preservative or by analysing blood simultaneously. However, a muscle biopsy from the left deltoid showed cytochrome oxidase negative fibres consistent with a mitochondrial myopathy. On DNA analysis, she was found to carry multiple mtDNA deletions. At this stage she was vomiting frequently, with abdominal pain and constipation. She had lost close to 40% of her body weight, and weighed 34 kg (165 cm tall, BMI of 12.5 kg/m^2).

The following year, it was decided to concentrate on symptomatic management and nutrition. A percutaneous gastrojejunostomy (PEG-J) was inserted. Although she was said to eat reasonably well, she had nocturnal vomiting, intermittent diarrhoea and was wheelchair bound. She died in hospital at the age of 19.

Final genetic analysis revealed that BA had MNGIE.

2.4. Patient CA

In January 2006, just months after the death of her sister, the final sibling CA presented aged 22 with weight loss, mild impairment of balance, numbness and weakness of her toes. She weighed 37.6 kg. She had been thin since birth. At age 14 she had been admitted to a children's ward for 'anorexia watch'. The stress of the fatal illness of her siblings and 'difficult family dynamics' were considered to be contributory factors. Numbness in her feet first manifested as difficulty in driving, when she had difficulty in working the pedals. Her thumbs had started to 'lock' and she was easily tired. Gastrointestinal symptoms of bloating and abdominal pain had led to a decreased appetite. She was easily satiated, frequently nauseated and had increased bowel frequency. Domperidone provided some relief.

In view of her sister's condition she was admitted for an elective muscle biopsy and clinical geneticist opinion. She had features of external ophthalmoplegia, peripheral neuropathy and limb weakness. Although her initial urinary thymidine was borderline, repeat urine and blood levels were elevated. Muscle biopsy showed features consistent with a mitochondrial myopathy.

Multiple mitochondrial deletions were detected on DNA analysis. A diagnosis of MNGIE due to a defect in the thymidine phosphorylase gene (homozygous for c.1431dupT, with a frame shift leading to loss of authentic stop codon) was made and functional thymidine phosphorylase activity was almost absent. It is now accepted that the central pathogenic mechanism of MNGIE is caused by defects in intergenomic communication, due to mutations of the nuclear gene encoding thymidine phosphorylase controlling the replication and expression of the mitochondrial genome [20]. Thymidine phosphorylase reversibly phosphorylates the nucleosides thymidine and deoxyuridine, levels of which are usually undetectable in healthy individuals. CA's urine deoxyuridine and thymidine concentrations were 0.199 and 0.107 mmol/l

respectively. In addition, plasma concentrations of deoxyuridine and thymidine were found to be 20 μmol/l and 6 μmol/l.

Because thymidine phosphorylase is not expressed in skeletal muscle, it was thought that enzyme replacement by bone marrow transplant might be beneficial. Allogeneic stem cell transplantation had previously corrected biochemical derangements in MNGIE [21].

No bone marrow match, however, could readily be found for CA. Thymidine load was reduced by cutting dietary pyrimidine and purine intake. It was suggested that high dose folate administration might increase the activity of thymidylate synthetase, an enzyme which might contribute to raised deoxyruridine levels in urine [22].

Ubiquinone (CoEnzyme Q10) 300 mg daily was prescribed. Later that year, she had gained 0.8 kg in weight, but was walking with a stick and had worsening pes cavus. She was enrolled into a compassionate use study of carrier erythrocyte-entrapped thymidine phosphorylase enzyme replacement therapy. This therapeutic approach was first used in 2006 where a single administration of erythrocyte encapsulated thymidine phosphorylase was given to a seriously ill patient [23]. Although initial cycles evoked a dramatic response in CA, later cycles provided only transient relief lasting 5 days.

She stopped her low purine diet after 18 months despite initial weight gain as the benefit was not sustained. Metoclopramide was more successful than domperidone, amitriptyline, or bisacodyl at relieving her nausea.

Attempts were made at nasogastric feeding but were limited by a sensation of dysphagia. Barium swallow revealed no oesophageal pathology but a delayed film showed a grossly dilated stomach with markedly delayed emptying. The globus type symptoms eventually improved with the addition of amitriptyline. Her nutritional status, however, became critical.

CA was admitted aged 23 (weight 32.7 kg) for intravenous feeding via a peripherally inserted central catheter (PICC), after treatment to prevent re-feeding syndrome following chronic malnutrition, secondary to gastroparesis and gastrointestinal dysmotility. It was considered that bowel symptoms were due to atrophy of smooth muscle and loss of mitochondrial activity in autonomic nerves. Small bowel bacterial overgrowth was considered a contributing factor, so ciprofloxacin was given on the first week of every month. Omeprazole was used for gastro-oesophageal reflux related to gastroparesis, confirmed by isotope gastric emptying studies. Symptomatic control of abdominal pain was achieved with fentanyl patches and octreotide. Despite these interventions, CA died aged 24.

2.5. Family B

A second family, a mother and son, who presented in adulthood with progressive ptosis, external ophthalmoplegia, fatigable limb weakness and diabetes are described. Interestingly, gastrointestinal involvement was a dominant feature in the son but not the mother. A diagnosis of mitochondrial disease due to complex mtDNA rearrangements was established.

2.6. Patient AB

A 22 year old man presented in 1999 with a history of gastrointestinal disturbance, lower limb myopathy, ptosis, external ophthalmoplegia and heart block. Although motor milestones during childhood had been acquired normally, the patient had never been as physically active as his two brothers, because he was easily fatigued. Apart from the mother, there was no family history of illness, unexplained death, disease or consanguinity. He had been seen by a paediatrician at five years of age, but no diagnosis had been made. In early teenage years he developed a bilateral

ptosis, more marked in the evenings. Gastrointestinal symptoms became prominent, with intermittent diarrhoea and constipation, as well as a sensation of incomplete faecal evacuation. He had lower abdominal, groin and anal pain, with bloating, although appetite and body weight were normal. He also had infrequent episodes of severe generalised colicky abdominal pain lasting several days at a time and necessitating absence from school, although no investigation had been undertaken. Heavy physical exertion was avoided until the age of 17, when a job involving manual labour produced exercise-related muscle pain in the lower back and legs, relieved by rest. From 18 years, the patient reported episodes of pre-syncope. Heart block was discovered on a resting electrocardiogram, leading to cardiac pacemaker insertion at the age of 21. Diagnosis of a probable mitochondrial disease in the mother led to referral of the son to the mitochondrial genetics group for further investigation.

On examination he had bilateral ptosis, with external ophthalmoplegia more marked in the horizontal plane. He had peripheral retinal pigmentation, with preserved visual acuity. Upper limb strength was normal, but muscle bulk in the lower limbs was poorly developed, with weakness and fatigability. Abdominal examination was unremarkable.

Investigation of his gastrointestinal symptoms with esophagogastroduodenoscopy (EGD), colonoscopy and small bowel barium enema revealed only mild esophagitis and haemorrhoids. A diagnosis of constipation-predominant intestinal dysmotility, commonly found in mitochondrial disease, was made on clinical grounds. Treatment with osmotic laxative, peppermint oil and dietary advice to increase soluble fibre led to symptomatic improvement. His groin pain appeared to be neurogenic in origin, although responded poorly to simple analgesics, amytriptyline, or carbamazepine.

Muscle biopsy of the right quadriceps was performed. Histochemical staining was abnormal with scattered myofibers demonstrating excessive succinate dehydrogenase (SDH) activity and several deficient in cytochrome c oxidase (COX). There were also structurally abnormal mitochondria on electron microscopy. The findings were compatible with a mitochondrial myopathy.

Mitochondrial DNA analysis revealed a complex type mitochondrial DNA single rearrangement in muscle DNA. Southern blotting of a SnaBI digest showed two bands of 11.7 and 28.3 kb, in addition to the normal 16 kb band (Fig. 3). Regional probes showed that these reflected an mtDNA deletion and a corresponding duplication (that is, a molecule corresponding to a deleted molecule in series with a normal molecule) (Fig. 4). The junction fragment for both deletion and duplication was hence the same [24]. Sequence analysis showed that the deletion constituted 4863 bp and the corresponding duplication 11,706 bp (mapping from 9522 to 14,385 with 8 bp almost exactly flanking direct repeats). He subsequently developed insulin dependent diabetes mellitus and hypogonadism in his early thirties. Progressive sensorineural deafness, impairment of visual acuity and dystonic tremor of both hands developed from his mid thirties. Gastrointestinal symptoms persist, but have not deteriorated. His weight and appetite remain stable.

2.7. Patient BB

As she had ptosis, the patient's mother (then aged 46 years) was investigated concurrently. From childhood she had been reluctant to run or take vigorous exercise, with marked general fatigue by early evening. This had allowed her to empathise with the physical limitations in her son. Hypothyroidism was subsequently diagnosed, but thyroxine only improved, rather than eliminated her fatigue. She had no gastrointestinal or cardiac symptoms. At the age of 42 she had been referred for a neurological opinion with
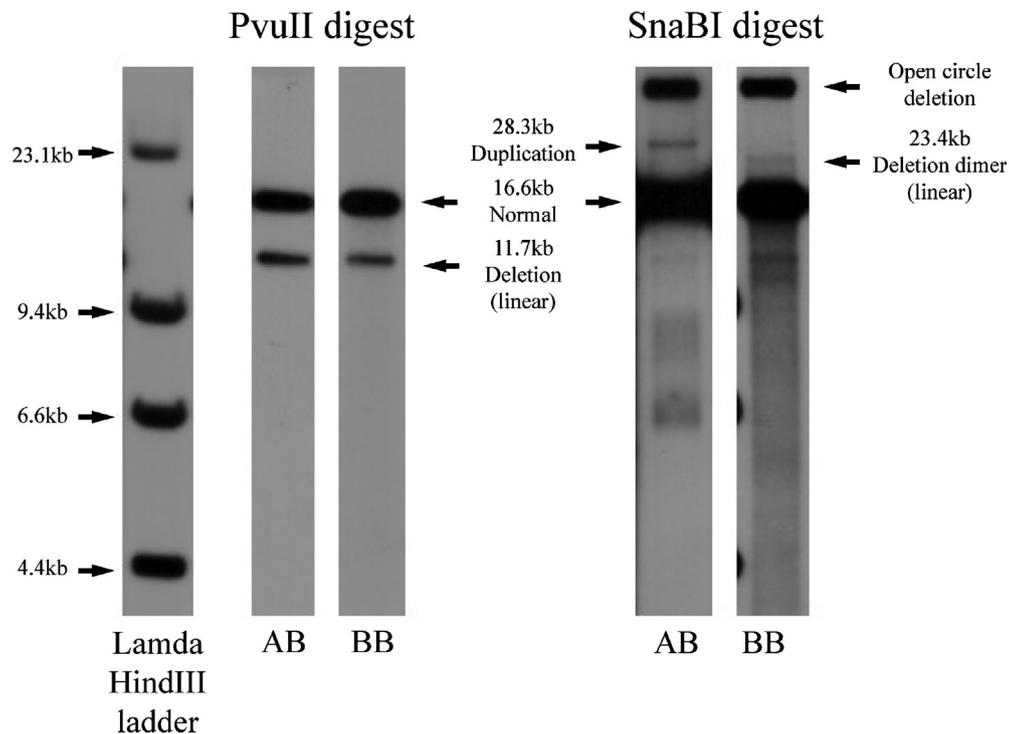
PvuII digest   SnaBI digest

Fig. 3. Southern analysis. *Pvu*II digest confirmed the presence of an approximately 5 kb (sized as 4.9 kb) rearrangement in muscle DNA from the son (AB) and mother (BB); the proportion of rearrangement in the son was approximately 30%, and in the mother approximately 10%. *Sna*BI digest showed that the major rearranged species in son and mother is single deletion; a low level of deletion dimer was detected in the mother; a low level of duplication and very low level of deletion dimer was detected in the son.

fatigue and diplopia; she was treated for myasthenia gravis. When there was no improvement in symptoms, an electromyogram was performed, revealing excessive polyphasic motor units, suggestive of a myopathy rather than a neuromuscular transmission defect. A muscle biopsy demonstrated features consistent with a mitochondrial myopathy, which in turn had lead to appropriate investigation of her son.

On examination she had mild bilateral ptosis, with external ophthalmoplegia in all planes. There was minor peripheral retinal pigmentation, but visual acuity was normal. Her upper limb muscle bulk was normal, but with discernible weakness and fati-

gability. She had decreased muscle bulk in the lower limbs, with demonstrable weakness and fatigability.

Mitochondrial DNA analysis of her muscle DNA revealed the same mtDNA 4863 bp deletion as her son, but no duplication (Figs. 3 and 4). In addition to the 11,706 bp deleted molecules, she had a 23.4 kb band corresponding to deletion dimers (two deleted molecules joined in series). As expected, the junction fragment was identical for both rearranged molecules (Fig. 3).

A calcium channel antagonist (amlodipine), commenced by a cardiologist for suspected exertional angina, led to marked improvement in activity levels, fatigability and her range of eye movements. Magnetic resonance spectroscopy during a standard work-incremented exercise protocol (plantar flexion) was performed in order to assess the effect of amlodipine on mitochondrial function and recovery from exercise. Following a two week trial of amlodipine, there was significant improvement in oxidative ATP production in mitochondria measured by spectroscopy. The calcium antagonist, nifedipine, is able to partially block ATP sensitive potassium channels of the type found in skeletal muscle. As these channels probably influence muscle fatigue, we reasoned that amlodipine might improve fatigue by doing the same. Hence we initiated a trial of amlodipine in the son but this provided no symptomatic improvement.

The mother subsequently developed diabetes in her mid fifties, treated with oral hypoglycaemics. At 57 years old, she developed paroxysmal atrial fibrillation, which could not be prevented by beta blockers or flecainide. Most notably, she has never had the debilitating abdominal symptoms of pain, bloating and constipation experienced by her son.
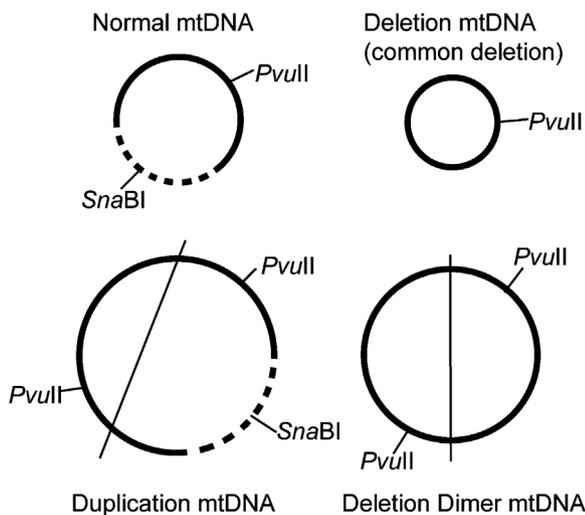
2.8. Family C

We finally describe a male patient, AC, with cognitive decline, neuropathy and optic atrophy, who later developed prominent gastrointestinal symptoms of dysphagia and vomiting. A diagnosis of

Fig. 4. Schematic diagram of mitochondrial DNA showing the location of *Sna*BI and *Pvu*II restriction enzyme recognition sites, and illustrating rearranged mtDNA molecules which commonly occur in patients with mtDNA rearrangements. The most commonly deleted region is indicated as a dashed line.

autosomal dominant optic atrophy (DOA) due to a well established pathogenic mutation in the *OPA1* gene was made. The Opa1 protein, encoded by *OPA1*, plays an important role in mitochondrial dynamics, and thus gene mutations can lead to mitochondrial disorders. He has no evidence of any mitochondrial DNA abnormality however. As patient AC has neurological features in addition to his optic neuropathy, his condition is termed 'DOA plus' (for "Dominant Optic Atropathy plus").

AC first manifested signs of disease aged between two and three years. It was noted that he often fell over, overshot when reaching for objects and failed to make eye contact with adults. He was diagnosed with optic atrophy, having had a right convergent squint corrected at the age of 14 months old. There was an early history of 'failure to thrive' and acid reflux.

He presented to local services in 2006, aged 9 with a two year history of motor problems, with weakness and unsteadiness. On brain MRI, no destructive brain lesions were seen, with the only abnormality small optic nerves and optic chiasm. Vision by this stage was limited to perception of light only. No mutations in the POLG gene, which encodes the catalytic subunit of the mtDNA polymerase were found, nor was there evidence of Leber Hereditary Optic Neuropathy. Urinary organic acids were normal.

By age 11, AC had left mainstream school with cognitive decline, dysarthria and sensory ataxic neuropathy. He required callipers to walk. Neuropathy was confirmed on EMG with 'sensory motor axonal neuropathy affecting sensory fibres more severely than motor'. A muscle biopsy was consistent with denervation and reinnervation but there was no evidence of mitochondrial disease.

By age 12 he was wheelchair bound. He had marked neuropathic pain and his co-ordination had deteriorated. He also had hearing difficulties, consistent with an auditory nerve neuropathy.

At age 13, the genetics for his 'DOA plus' syndrome were defined. One copy: c.2707_2711delTTAG originated from his paternal grandfather. This is a well characterised mutation that leads to impaired vision in 60% of individuals who carry it. The other copy in exon 6 of his *OPA1* gene was found in his mother: c.661G>A, a never before described mutation predicted to be that of p.Glu221Lys. The optic atrophy was caused by hypoplastic optic nerves, the mechanism for which was postulated to be due to increased 'mitophagy', a subtype of autophagy, literally to 'self-digest'. The 'plus' features included sensory neuropathy and cognitive decline.

There was no family history of consanguinity. There was however a strong history of neurological and ophthalmological impairment. His paternal grandmother had what was thought to be 'congenital' right sided facial paralysis. Her maternal great grandmother was blind from 15, and this grandmother's brother and sister had suffered from blindness and mobility problems at a young age. A paternal uncle had epilepsy, a squint and minor learning difficulties.

At age 13, AC developed significant gastrointestinal symptoms, with dysphagia and vomiting. A PEG was sited the same year, but he struggled with abdominal pain on feeding, relieved with defecation. Aged 15 he was admitted with diarrhoea and had lost 10 kg in weight. Stool cultures were negative. EGD with duodenal biopsies was normal. Ileocolonoscopy demonstrated oedematous colonic mucosa with patchy erythema but no ulceration, and normal terminal ileum. Histopathology showed mild acute on chronic colonic inflammation, with cryptolytic granulomas in the transverse colon. MRI enteroclysis suggested marked wall thickening of the right colon consistent with an infiltrative process, but was not typical of inflammatory bowel disease. The diarrhoea did however initially respond to a course of steroids.

AC was readmitted 6 days post discharge with severe abdominal pain and further diarrhoea. Laparoscopy was performed but no clear diagnosis reached. Following a haemoglobin drop of 5 g/dl, a CT scan to assess for haemorrhage revealed gas within the colonic

Table 1
A summary of the clinical and genetic features of the six patients.

Tissue	Family A			Family B		Family C
	AA	BA	CA	AB	BB	AC
Gastrointestinal	Intestinal dysmotility Gastroparesis	Intestinal dysmotility Gastroparesis	Intestinal dysmotility Gastroparesis Ophthalmoplegia	Intestinal dysmotility		Pneumatosis coli Dysphagia
Muscle				Weakness/exercise intolerance Ptosis	Weakness/exercise intolerance Ptosis Ophthalmoplegia	
Central nervous system				Dystonic tremor Ataxia		Cognitive impairment Dysarthria Epilepsy Sensory ataxic neuropathy
Peripheral nervous system	Peripheral neuropathy	Peripheral neuropathy	Peripheral neuropathy			
Cardiac				Conduction block	Atrial fibrillation	
Eye				Peripheral retinal pigmentation Impaired visual acuity	Peripheral retinal pigmentation	Optic atrophy Impaired visual acuity
Ear				Sensorineural deafness		Sensorineural deafness
Endocrine				Diabetes mellitus Hypogonadism Hypothyroidism	Diabetes mellitus Hypothyroidism	
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Maternal		Autosomal dominant
Genetic abnormality	MNGIE	MNGIE	MNGIE	mtDNA deletion and duplication	mtDNA deletion	OPA1 gene mutation

wall, most marked in the right colon. A diagnosis of pneumatosis coli was made and he was treated with high flow oxygen. It is plausible that mitochondrial disease in the muscularis propria of the intestine may have rendered him susceptible to consequences from an infective or ischaemic insult. He recovered and was discharged, but his prognosis from the underlying disease remains extremely guarded.

Table 1 summarises the clinical and genetic features of the six patients described in this paper.

3. Discussion

Our case series highlights two important but poorly recognised aspects of mitochondrial disease: the clinical and the genetic. All patients had clinical evidence of multisystem disease that is characteristic of patients with more complex mitochondrial defects. This is an important clinical signal, because it means that such patients can present to any of many specialists, who will all (most probably) be unfamiliar with mitochondrial disease. This contrasts with the common, sporadic chronic progressive external ophthalmoplegia associated with single mtDNA deletions, where the main feature is an isolated myopathy. Once again, this is an important educational point for clinicians, since most will assume that myopathic symptoms predominate in patients with a mitochondrial disease and tend to ignore 'non-specific features' such as tiredness, easy fatigability, systemic symptoms, or diabetes. It is notable for example that patient AB's concerns were his gastrointestinal symptoms and neurogenic pain, which were potentially treatable symptoms, but which may not be given a high priority in a general muscle clinic. Like a coin, a medical condition always has two sides: what is familiar to one group of specialists may be obscure to another.

Gastrointestinal symptoms are a major feature of MNGIE, and upper gut disorders including gastroparesis are common in autosomal-inherited syndromes associated with multiple mtDNA deletions such as Leigh syndrome [12,25]. In MNGIE, chronic intestinal pseudo obstruction (another term for severe intestinal dysmotility) is a dominant symptom that can lead to intestinal failure requiring nutritional support [12]. It appears to be due to marked atrophy of the external layer of the muscularis propria in which there is prominent mitochondrial proliferation and loss of cytochrome oxidase activity. Genetic analysis shows a selective depletion of mtDNA confined to the external layer of the muscularis propria of the small intestine [26,27]. Although this is a plausible explanation of the gut failure that can characterise MNGIE, recent evidence suggests that loss of the intestinal interstitial cells of Cajal may also be important [28]. Cajal cells appear to have a high energy requirement, since mitochondria comprise 5–10% of their cytoplasmic volume, so their function is likely to be badly affected by mitochondrial disease. This is consistent with morphometric observations dating back decades that have found abnormal interstitial cells of Cajal in Hirschsprung disease, non-specific chronic intestinal pseudo-obstruction and idiopathic intractable constipation [29]. Interestingly, mtDNA has recently been implicated in some patients with irritable bowel syndrome, which suggests that there may indeed be a spectrum of mtDNA disorders reflected in a spectrum of intestinal motility disorders [30]. If this is confirmed, it would shed substantial light on the pathophysiological darkness of intestinal dysmotility.

An intriguing aspect of Family B is why gastrointestinal symptoms were so prominent in the son and almost absent in the mother. The key genotypic difference between mother and son was the presence of the 11,706 bp duplication (mapping from 9522 to 14,385) in the latter. We suggest that the mother may have carried mtDNA duplications in her oocytes, but lost them progressively from her muscle, as in a previous report [31]. It is tempting

Table 2
Clinical clues and checklist of investigations for mitochondrial disease in a patient with intractable symptoms of irritable bowel syndrome or suspected visceral myopathy.

Careful re-clerking to include a systems enquiry, family history and full neurological examination
Consider mitochondrial disease if gastrointestinal symptoms are associated with additional organ involvement (for example):
• Nervous system: Developmental delay, seizures, ataxia, dystonia, hemiparesis, visual loss, peripheral neuropathy
• Muscle: Weakness, exercise intolerance, ptosis, ophthalmoplegia
• Eye: Optic atrophy, retinal pigmentation, cataracts
• Auditory: Sensorineural hearing loss
• Endocrine: Diabetes mellitus, thyroid and pituitary dysfunction
• Cardiac: Cardiomyopathy, conduction disease
• Other: Fanconi syndrome, hepatopathy (abnormal liver function, otherwise unexplained)
Investigations for mitochondrial disease (telephone the hospital Consultant Clinical Biochemist or appropriate colleague to discuss)
• Plasma glucose (may detect diabetes mellitus, increasing likelihood of mitochondrial disease)
• Electrocardiogram and echocardiogram (to explore potential cardiac involvement)
• Fasting plasma lactate and pyruvate level
• Plasma and urinary thymidine and deoxyuridine
• Urinary organic acids
• Plasma acylcarnitines
• If abnormal neurology or evidence of muscle weakness, discuss with Neurologists the role of
• Lumbar puncture with cerebrospinal fluid analysis for lactate and protein
• Electromyography
• Nerve conduction studies
• Magnetic resonance imaging of brain
• Muscle biopsy for histological and histochemical analysis

to speculate that the duplication might account for the visceral, intestinal symptoms in the son's phenotype, while the 4863 bp deletion common to both mother and son accounted for the effects on the peripheral muscles. It is only conjecture, but a possibility worth pursuing, since it is a common clinical observation that some patients with a mitochondrial disease have severe intestinal symptoms and others are relatively spared. Our findings illustrate the need for precise analysis of individual mtDNA, since duplications and higher order mtDNA rearrangements are often overlooked on routine testing.

Although mitochondrial disease cannot currently be cured, in recent years four exciting potential therapies have emerged. Firstly, the use of allogeneic hematopoietic stem cell tranplantation to restore thymidine phosphorylase activity in MNGIE shows promise. It has been effective in 5 of 11 patients treated to date, with the first transplanted patient showing markedly improved gastrointestinal function [32]. A prospective clinical trial is planned. Secondly, there is interest in the benefits of promoting new mitochondrial formation (biogenesis) and function. Particular attention has focused on the transcriptional coactivator, peroxisome proliferator-activated receptor (PPAR) y coactivator-1α protein (PGC-1α) which is a strong promoter of mitochondrial biogenesis. Recently bezafibrate, a drug treatment for hyperlipidaemia, has been shown to stimulate PGC-1α activity and improve mitochondrial respiratory chain function in cell lines of patients with mtDNA disease [33]. Thirdly, the use of gene therapy to prevent mtDNA transmission in affected families appears feasible, although there are clear ethical considerations with donor mtDNA from unaffected individuals required [34]. Finally, the efficacy of CoEnzyme Q10 (ubiquinone) in patients with CoEnzyme Q10 deficiency is noteworthy. This is an example of a mitochondrial disorder resulting from the deficiency of a key metabolite that can be treated with supplementation [35]. Unfortunately the benefits of CoEnzyme Q10 do not extend to other mitochondrial disorders which have quite separate pathophysiologies, underlining the challenges of developing treatments for this heterogeneous group of diseases [35].

Mitochondrial disease should be part of the differential diagnosis for anorexia nervosa and also for medically unexplained gastrointestinal symptoms. We conclude by proposing a checklist of investigations that may be helpful in establishing a diagnosis of mitochondrial disease (Table 2). The checklist cannot be comprehensive, but should increase awareness that apparently rare mitochondrial disorders can account for common symptoms. The key component is the alert physician, whether in primary or secondary care: when the clinical picture cannot readily be explained and does not quite fit (such as unaccountable weight loss in someone with irritable bowel syndrome), mitochondrial disease should be considered. The checklist includes rudimentary questions pertaining to family history and a full neurological examination that might provide the initial clues to an organic cause. At present a correct diagnosis will guide supportive management and allow genetic counselling, but as outlined above there is also reason to hope that effective treatments may soon be available. The true prevalence of mitochondrial disease may well be masked by failing to consider the diagnosis, which in turn limits the research that could potentially ameliorate outcomes for these patients.

Conflict of interest statement

None declared.

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