Mitochondrial DNA A3243G mutation involved in familial diabetes, chronic intestinal pseudo-obstruction and recurrent pancreatitis


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Abstract

Aims. – To report on a family with five members who carry the A3243G mutation in mitochondrial tRNA for leucine 1 (MTTL1) and present with diabetes, chronic intestinal pseudo-obstruction (CIPO) and recurrent pancreatitis, and to screen for this mutation in a cohort of 36 unrelated patients with recurrent pancreatitis.

Methods. – The mutation was quantified in several tissue samples from patients. Respiratory chain activity was studied in muscle biopsies and fibroblast cultures. In addition, the thymidine phosphorylase gene (TP) involved in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and three genes involved in chronic pancreatitis – PRSS1, SPINK1 and CFTR – were sequenced in affected patients. Finally, the MTT1 gene was examined in 36 unrelated patients who had recurrent pancreatitis, but no mutations in the PRSS1 and SPINK1 genes.

Results. – Heteroplasmy for the mtDNA A3243G mutation was found in all tissue samples from these patients, but no mutations were found in the genes coding for thymidine phosphorylase, PRSS1, SPINK1 and CFTR. Also, none of the 36 unrelated patients with recurrent pancreatitis were carrying any MTT1 mutations.

Conclusion. – The mtDNA A3243G mutation associated with the gastrointestinal manifestations observed in the affected family should be regarded as a possible cause of CIPO and unexplained recurrent pancreatitis. However, the mutation is probably only weakly involved in cases of isolated recurrent pancreatitis.

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Résumé

Mutation A3243G de l’ADN mitochondrial dans une forme familiale de diabète, pseudo-obstruction intestinale chronique et pancréatite récidivante.

Objectifs. – Rapporter une famille de cinq sujets porteurs de la mutation A3243G de l’ARNt mitochondrial de la leucine 1 (MTTL1) et présentant un diabète, une pseudo-obstruction intestinale chronique (POIC) et des épisodes récidivants de pancréatite; 2) tester la présence de cette mutation dans un groupe de 36 sujets non apparentés de pancréatite récidivante.

Méthodes. – La mutation de l’ADN mitochondrial a été quantifiée sur différents tissus provenant des patients. L’activité de la chaîne respiratoire a été étudiée sur des biopsies musculaires et sur des cultures de fibroblastes. Le gène codant la thymidine phosphorylase (TP) impliqué dans l’encephalomyopathie neurogastrointestinal mitochondriale (MNGIE) et trois gènes responsables de pancréatites chroniques (PRSS1, SPINK1 et CFTR) ont été séquencés chez les patients. Enfin, le gène MTT1 a été examiné chez 36 sujets souffrant de pancréatite récidivante et non porteurs de mutations des gènes PRSS1 et SPINK1.

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Enzymatic defects of the mitochondrial respiratory chain usually affect a number of organs, resulting in heterogeneous and apparently unrelated clinical manifestations. The tissues and organs that require the greatest amount of energy—such as the muscles, heart, brain, optic nerves, liver and gastrointestinal (GI) tract—are generally the most vulnerable to defects of oxidative phosphorylation.

Digestive symptoms are reported in 15% of patients with mitochondrialopathy [1], but this rate is probably an underestimation as the involvement of the GI tract in mitochondrial disorders is rarely a prominent feature. Chronic intestinal pseudo-obstruction (CIPO) is among the more severe digestive disorders resulting from mitochondrial dysfunction. In particular, CIPO has been associated with the A3243G mutation in mitochondrial DNA (mtDNA) and is a cardinal feature of mitochondrial neurogastrointestinal encephalopathy (MNGIE) [1]. In contrast, mitochondrial dysfunction in the pancreas frequently results in diabetes mellitus and exocrine insufficiency, and only rarely leads to acute or chronic pancreatitis [2].

The family described in the present report carry the A3243G mutation in the mitochondrial tRNA for the leucine 1 (MTTL1) gene in at least five of its members, who present with either diabetes and CIPO or recurrent pancreatitis, or both.

1. Materials and methods

1.1. Case reports

The pedigree of the family is shown in Fig. 1.

Patient II.1, a man born in 1948, had sensorineural deafness, non-insulin-dependent (type 2) diabetes mellitus and mild cognitive impairment. A weight loss of approximately 20 kg had occurred over the last five years of his life. At age 53, he underwent an operation for haemorrhagic duodenal ulcer. At age 56, he was admitted to the neurology department with acute ischaemic stroke. Three months later, he suffered acute intestinal obstruction requiring two subsequent exploratory laparotomies. As no mechanical causes of obstruction were found, CIPO was diagnosed. Thereafter, a mitochondrial disorder was confirmed by the finding of ragged red fibres (RRF; Fig. 2A), and complex I and III deficiency on muscle biopsy, and by the identification of the mtDNA A3243G mutation. In addition, magnetic resonance imaging (MRI) of the brain showed abnormalities in the periventricular white matter. At age 57, he required long-term total parenteral nutrition with prokinetic treatment using octreotide and sequential courses of antibiotic treatment. Surgical ileoeccecal resection together with gallbladder resection and construction of a gastric stoma were also carried out. Subsequently, total resection of the small intestine was necessary, but recovery of oral nutrition proved to be impossible. No pancreatic involvement was seen in this patient. He died at age 58, probably due to acute ischaemic stroke. An autopsy was not performed.

Patient II.2, a man born in 1949, also had sensorineural deafness and insulin-dependent (type 1) diabetes that began at age 20. From age 22, he developed chronic calcific pancreatitis that required cephalic pancreatectomy. No classical aetiology for the pancreatitis—including biliary tract disease, excessive alcohol abuse, drug toxicity, dyslipidaemia or hypercalcaemia—could be established. At age 53, he developed a chronic post-traumatic subdural haematoma and epilepsy. Two episodes of acute intestinal obstruction, requiring exploratory laparotomy, occurred at ages 52 and 53 but, again, no mechanical causes were found. He died at age 54, probably due to acute ischaemic stroke.

Patient II.3, a woman born in 1951, had recurrent headaches from the age of 20. At age 53, she suffered an acute episode of necrotizing pancreatitis requiring surgery. The diagnosis of pancreatitis was based on abdominal pain and her increased lipase serum (1052 IU/L; normal < 200 IU/L). Computed tomography (CT) was normal, and pancreatitis was classified as

![Fig. 1. Pedigree of the family carrying the mitochondrial DNA A3243G mutation: squares represent men and circles represent women.](image-url)
Fig. 2. Muscle biopsies from patients II.1 (A, B) and II.3 (C, D): routine haematoxylin-eosin staining reveals numerous ragged red fibres (A, C), while COX–SDH staining shows COX-negative fibers (B, D). The mesenteric artery from patient II.3 (E) shows the original arterial lumen (arrow) and dissection of the arterial wall with extension of blood into the adventitia (arrowhead). × 40 (A–D) and × 1.6 (E).

grade A on the Balthazar score [3]. No classical cause of the pancreatitis was found. Thereafter, the patient experienced recurrent abdominal pain suggestive of mesenteric intestinal ischaemia. Abdominal CT revealed a stricture in the superior mesenteric artery, including the duodenopancreatic artery, suggesting an ischaemic cause of the pancreatitis that required surgery.

Patient II.4, a man born in 1953, had a history of recurring episodes of acute intestinal obstruction. At age 52, he had an ischaemic stroke of no identifiable cause despite exhaustive investigations. Myoclonic epilepsy occurred after this stroke episode.

Patient III.7, a man born in 1976, had suffered from recurrent pancreatitis since the age 13 (abdominal pain, increases in amylase and lipase serum levels). All classical causes of pancreatitis were ruled out. At age 16, abdominal CT – performed because of a new episode of pancreatitis – showed enlargement of the pancreas and infiltration of the peripancreatic tissues. The pancreatitis was classified as grade C on the Balthazar score. At that time, retrograde cholangiopancreatography
failed to reveal any abnormality of the biliary tract. He underwent cholecystectomy, but no gallstones were found. Pancreatic sphincterotomy, performed in 1993, proved to be ineffective. The last episode of pancreatitis occurred in September 2006. Two further laparotomies were carried out at ages 20 and 24 – one for abdominal trauma, the other for acute intestinal obstruction – but no mechanical causes were identified.

Patient II.5, for this woman, few clinical details are available. It is, however, known that she developed insulin-dependent (type 1) diabetes from age 20 and had a history of recurrent miscarriage. She died at age 30, probably due to stroke.

1.2. Pathological analysis

Muscle biopsies were carried out for patients II.1 and II.3: 5 μm sections of muscle tissue were stained with haematoxylin-eosin (HE), Gomori and succinic dehydrogenase. A specimen of the mesenteric artery, obtained from patient II.3, was also processed for HE staining.

1.3. Biochemical analysis

Citrate synthase, complex I (NADH–quinone reductase), complex II (quinone–dichloro indophenol reductase succinate dehydrogenase), complex II+III (succinate–cytochrome c reductase) and complex IV (cytochrome c oxidase) activity in muscle homogenates from patient II.1 were measured, as described elsewhere [4].

1.4. Molecular genetic analysis

After obtaining the patients’ informed consent, standard procedures were used to extract mtDNA from samples of blood, urine, skeletal muscle, GI tract and skin fibroblasts taken from the five affected family members. Detection of the A3243G mutation was performed by PCR amplification of the mtDNA (nucleotide position 3108-3433), followed by sequencing. Quantification of the A3243G mutation was performed twice for each sample by PCR amplification (nucleotide position 3108-3433), restriction enzyme digestion (Apa1), electrophoretic separation and densitometric quantification.

The thymidine phosphorylase (TP) gene responsible for the MNGIE syndrome was studied by direct sequencing. In addition, exons 1, 2 and 3 of the PRSS1 gene, and exons 1 and 3 of the SPINK1 gene – both of which are involved in hereditary pancreatitis – and all exons of the CFTR gene were analyzed by denaturing high-performance liquid chromatography (DHPLC).

Finally, we looked for the A3243G mutation in 36 other patients affected with either hereditary or recurrent pancreatitis of no known biliary or alcoholic cause, in whom mutations in the PRSS1, SPINK1 and CFTR genes had been previously ruled out. Of these patients, 11 were affected with a familial form of chronic pancreatitis and had a pedigree compatible with a maternal mode of inheritance. The remaining 25 patients were sporadic cases of recurrent acute pancreatitis, 15 of whom were aged less than 20 years.

2. Results

2.1. Pathological findings

Muscle biopsies from patient II.1 showed 10% RRF (Fig. 2A) and 6% in patient II.3 (Fig. 2C), and 30% cytochrome c oxidase (COX)-negative fibres in patient II.1 (Fig. 3B) and 15% in patient II.3 (Fig. 3D). Analysis of the mesenteric artery from patient II.3 revealed the presence of typical blood-vessel dissection (Fig. 2E), with a second channel around the ostia. There was no visible alteration in the arterial wall evocative of arterial dysplasia.

2.2. Biochemical analysis

Respiratory chain activity studied on muscle tissue from patient II.1 showed reduced activity of complex I (1.3 nmol/min/g protein; normal values: 16–52 nmol/min/g) and complex III (82 nmol/min/g; normal values: 125–418 nmol/min/g).

2.3. Molecular genetic analysis

Heteroplasmy for the A3243G mutation in mtDNA was found in all samples from the affected patients. The proportion of mutated mtDNA varied from 10 to 75%, depending on the tissue analyzed (Fig. 3). In keeping with a recent report [5], the lowest mutational load was found in muscle mtDNA (10%) whereas the highest was in skeletal muscles (up to 75%). Intermediate values were found in urine and skin fibroblasts (25–60%). Interestingly, a relatively low mutational load (20–30%) was found in GI samples from two of the affected patients.

In addition, in the affected patients, no mutations were found in the TP, PRSS1, SPINK1 and CFTR genes. Finally, the A3243G mutation was not present in any of the 36 unrelated patients, who had a history of recurrent pancreatitis, but had no known biliary, alcoholic or genetic cause.

3. Discussion

The A3243G transition in the MTTL1 mitochondrial gene is the most common pathogenetic mutation found in mtDNA. This mutation is associated with a broad spectrum of clinical manifestations, including mitochondrial encephalomyopathy, lactic acidosis, the stroke-like episodic syndrome (MELAS) and the maternally inherited diabetes and deafness syndrome (MIDD). MELAS, a multisystem disorder, typically appears during childhood and is characterized by:

- stroke-like episodes before age 40;
- encephalopathy with seizures or dementia;
- mitochondrial myopathy, revealed by lactic acidosis or RRF on muscle biopsy [5,6].

MIDD, also a disease usually arising in adulthood, is defined as the association of early-onset non-insulin-dependent (type 2) diabetes and sensorineural deafness [6]. However, as reported here, the clinical symptoms due to the A3243G mutation
GI manifestations have been reported with varying frequency in patients bearing the A3243G mutation and affected with either MELAS [7–13] or MIDD [14–20]. Narbonne et al. [20] found that MIDD patients had significantly more bouts of constipation, diarrhoea, nausea, vomiting and abdominal pain compared with a control group of type 1 diabetic patients (88% versus 28%, respectively). CIPO has been reported in a number of patients harbouring the A3243G mutation [8–13,17,18,20] and, in most of these cases, the digestive complications were severe, often leading to death. To our knowledge, however, to date there has been only one report of familial cases of CIPO associated with the A3243G mutation [9].

Paralytic ileus is also a cardinal feature of MNGIE, an autosomal-recessive disorder caused by mutations in the TP gene, resulting in alteration of mtDNA [1]. The diagnosis of MNGIE can be suspected clinically in the presence of severe GI dysmotility, cachexia, ptosis, external ophthalmoplegia, sensorimotor neuropathy and asymptomatic leukoencephalopathy, as revealed by brain MRI, and confirmed by the finding of mutations in the TP gene. However, in the patients reported here, their clinical presentations differed from those of classical MNGIE, the autosomal-recessive mode of inheritance appeared to be unlikely and the search for mutations in the TP gene proved negative. CIPO has been reported in other mitochondrial disorders [1] involving various mtDNA point mutations and mutations in POLG1, the gene encoding mitochondrial DNA polymerase gamma [21]. However, neither mtDNA depletion nor multiple mtDNA deletions, possibly indicative of POLG1 involvement, were found in the patients described here.

Diabetes mellitus and exocrine insufficiency are the most common pancreatic features of mitochondriopathy. In contrast so far, there have been only six reports of patients with acute or chronic pancreatitis associated with mtDNA mutations. Four of these cases involved the A3243G mutation [7,22–24], one patient had Kearns-Sayre syndrome [2] and another patient had myoclonic epilepsy associated with ragged red fibres (MERRF) [25]. Nevertheless, pancreatitis may be underdiagnosed in patients severely afflicted by mitochondrial disorders. On the other hand, we could find no A3243G mutations in any of the other 36 patients with hereditary or recurrent acute pancreatitis in which mutations of the PRSSI1, SPINK1 or CFTR genes had been excluded.

Although the number of patients included in our study was small, the results suggest that the A3243G mutation is probably weakly involved in cases of recurrent pancreatitis that have no associated signs of mitochondriopathy. Moreover, given the low heteroplasmy rate of 10% found in the blood cells of the pancreatitis patients in this report, an analysis based solely on leukocyte mtDNA would be insufficient to definitively discard mitochondrial involvement in cases of isolated recurrent pancreatitis.

The pathophysiology of CIPO and recurrent pancreatitis due to the A3243G mutation and, more generally, of mtDNA mutations remains uncertain. Several hypotheses have been proposed regarding CIPO in the MELAS and MIDD syndromes. Firstly, heteroplasmy – the proportion of mutant mtDNA – has been observed in intestinal tissue samples from at least nine affected patients, including two of the patients reported on here (Fig. 3) [10,12,14,15,17,19,20]. This suggests that intestinal dysmotility may be related to an energy metabolism defect, resulting in GI...
dysmotility or myenteric plexus neuropathy. However, in one reported case of CIPO [11], no mutated mtDNA was found in any of the digestive tract samples, suggesting that GI dysmotility might originate from the autonomic nervous system. Secondly, vascular involvement may also be implicated in GI symptoms, as it has been demonstrated that MELAS patients have an endothelial dysfunction, resulting in a decreased vasodilatory capacity in the small arteries [26]. In fact, Hess et al. [14] reported on a case of ischaemic colitis associated with the A3243G mutation, and we observed dissection of the mesenteric artery in patient II.3 in our present report. Finally, energy defects in GI smooth muscle and vascular involvement are not mutually exclusive. Indeed, in two post-mortem studies of women with CIPO due to the A3243G mutation [8,16], extensive accumulations of large, abnormal mitochondria were found not only in the smooth muscle cells and ganglion cells of the GI tract, but also in the intestinal vascular media: the endothelium also revealed scattered necrotic zones [16].

Acute pancreatitis may also be explained by a similar mitochondrial energy defect or vascular dysfunction. Although there is no direct evidence of vascular involvement in the pancreas of patients with the A3243G mutation, it is known that the organ is susceptible to ischaemic injury, and that perturbations of the systemic and pancreatic microvascularization play a significant role in the pathogenesis of pancreatitis [27]. In addition, mitochondrial diseases, including MELAS, are associated with an excess production of reactive oxygen species (ROS), which mostly occurs in mitochondria as byproducts of oxidative phosphorylation, and has been described as an important factor in the pathogenesis and progression of pancreatitis [28].

The familial case of CIPO and recurrent pancreatitis reported here, implicating the mtDNA A3243G mutation, suggests that it would be useful to look for mtDNA mutations – in particular, the A3243G mutation – in cases of unexplained CIPO or recurrent pancreatitis, especially when the disorders are associated with signs or a family history evocative of a mitochondrial disorder.

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References


