

Large Mitochondrial DNA Deletion in an Infant with Addison Disease

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Abstract *Background:* Mitochondrial diseases are a group of disorders caused by mutations in nuclear DNA or mitochondrial DNA, usually involving multiple organ systems. Primary adrenal insufficiency due to mitochondrial disease is extremely infrequent and has been reported in association with mitochondrial DNA deletion syndromes such as Kearns–Sayre syndrome.

Aim: To report a 3-year-old boy with Addison disease, congenital glaucoma, chronic pancreatitis, and mitochondrial myopathy due to large mitochondrial DNA deletion.

Method: Molecular analysis of mitochondrial DNA samples obtained from peripheral blood, oral mucosa, and muscle tissue.

Results: A novel large mitochondrial DNA deletion of 7,372 bp was identified involving almost all genes on the big arch of mtDNA.

Conclusions: This case reaffirms the association of adrenal insufficiency and mitochondrial DNA deletions and presents new evidence that glaucoma is another manifestation of mitochondrial diseases. Due to the genetic and clinical heterogeneity of mitochondrial disorders, molecular analysis is crucial to confirm diagnosis and to allow accurate genetic counseling.

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Introduction

Primary adrenal insufficiency, commonly known as Addison disease, is uncommon in the Western population and is estimated to affect 90–140 per 1 million people (Arlt and Allolou 2003). The spectrum of adrenal disorders differs in childhood and adult patients. Primary adrenal insufficiency in childhood and adolescence is due to abnormalities of gland development, gland responsiveness, and either to defects of steroid biosynthesis or target organ response.

Primary adrenal dysfunction associated with mitochondrial disease has been reported extremely infrequently (Boles et al. 1998) and is generally associated with mitochondrial DNA deletion syndromes such as Kearns–Sayre Syndrome (KSS).

Clinical features typical of mitochondrial diseases include ptosis, progressive external ophthalmoplegia (PEO), proximal myopathy and exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, and diabetes mellitus. KSS has also been associated with a variety of endocrine disorders such as short stature (38%), gonadal dysfunction (20%), and diabetes mellitus (13%) (Harvey and Barnett 1992).

We report the case of a boy with Addison disease associated with congenital glaucoma, chronic pancreatitis, and mitochondrial myopathy due to a large mtDNA deletion.

Methods

Clinical Case

We present a 3-year-old male, who was the second child of healthy and nonconsanguineous white parents, born after

uneventful pregnancy and delivery. He was a 3.2 kg and 49 cm term newborn without a family history of hereditary diseases, and who showed normal growth and development.

At 2 years of age, he was admitted to the hospital for abdominal pain, vomiting, and lethargy of unknown etiology; during that episode, he also had hyponatremia, elevated blood lactate of 6.6 mmol (normal < 2.2 mmol), and normal abdominal ultrasound.

Three months later, he presented with acute bilateral photophobia. The ophthalmologic examination revealed megalocornea and increased intraocular pressure. Congenital glaucoma was diagnosed and the patient underwent trabeculectomy.

At the age of 2^{10/12} years, endocrinological examination showed relative short stature (−1.7 SD), increased pigmentation of skin and mucosa, and normal development of genitals for age without pubarche. These findings and the history of hyponatremia suggested adrenal insufficiency, which was confirmed by normal cortisol (7.6 µg/dL) but markedly elevated serum ACTH (911 pg/mL) levels. After the intravenous administration of 250 µg ACTH, the cortisol level was 7.9 µg/dL, 17OH-progesterone was 0.6 ng/dL, and testosterone was < 10 ng/dL. Initially, plasma renin activity (PRA) was in the normal range. Very long chain fatty acid and phytanic acid were normal, antibodies to the adrenal cortex were not detected and brain MRI was normal.

Hydrocortisone replacement therapy was introduced (10 mg/m²/day) leading to normalization of ACTH and skin pigmentation. During follow-up, PRA increased to 12 ng/ml/h (normal 1.6–5.0 ng/ml/h), so Florinef™ was added at a dose of 0.1 mg/day.

Abdominal ultrasound showed a calcified pancreas and parenchymal changes consistent with chronic pancreatitis, although the patient had no history of recurrent abdominal pain, anorexia, diarrhea, or cyclic vomiting, nor was there any history of abdominal trauma or family pancreatic disease. The abdomen was soft, and there was no hepatosplenomegaly. Liver function, amylase, lipase, and blood cell count were normal; antinuclear and anti-DNA antibodies were negative. Extensive absorption work-up was negative. The magnetic resonance cholangiopancreatography (MRCP) revealed diffuse pancreatic calcification and decreased pancreatic size with abnormal limits and fatty infiltration. In addition, the main pancreatic duct was diffusely dilated with multiple ductal filling defects, probably due to intraductal pancreatic stones. Based on this evidence, the patient was diagnosed with chronic pancreatitis, and he was started with pancreatic enzyme supplementation (Creon®).

The multisystem involvement, characterized by congenital glaucoma, chronic pancreatitis, and Addison disease, suggested a mitochondrial disease. Echocardiogram, electrocardiogram, and neurologic examination were normal,

but muscle biopsy showed typical ragged-red fibers, confirming the diagnosis of mitochondrial disease.

Molecular Studies

DNA was extracted from peripheral blood lymphocytes, oral mucosa cells, and muscle tissue by a commercial method (QIAGEN). Mitochondrial DNA deletion analysis was performed by amplifying the entire mitochondrial genome (16,569 bp) in a single reaction, as well as seven smaller fragments in separate reactions, thus encompassing the big arc of the mitochondrial genome. For whole-genome analysis, the D1A and D1B primers were used (Kleinle et al. 1997). For the smaller fragments, we used the following primers: mtF5317 (5317–5333), mtF7392 (7392–7410), mtF8196 (8196–8215), mtF11632 (11632–11651), mtR13832 (13832–13812), mtR15613 (15613–15594), and mtR16134 (16134–16115), as described by Ferlin et al. (1997). The primers were used according to the following combinations: mtF5317/mtR13832 (fragment 1), mtF5317/mtR16134 (fragment 2), mtF7392/mtR15613 (fragment 3), mtF8196/mtR13832 (fragment 4), mtF8196/mtR16134 (fragment 5), mtF8196/mtR15613 (fragment 6), and mtF11632/mtR16134 (fragment 7). All fragments were amplified by long PCR under the same conditions, using a long PCR enzyme mix (Fermentas Life Sciences) in a final volume of 25 µL, and the cycling program used by Kleinle et al. (1997). Five µl of the products were separated on a 0.8% agarose gel and visualized under UV. The deletion breakpoints were studied by sequencing the fragments encompassing the deletion using the amplification primers.

Results

Molecular Studies

Long PCR analysis of the whole mitochondrial genome and of mtDNA segments with the first and second primer pairs showed shorter amplification fragments than those obtained with the wild-type sample. The deletion in peripheral blood, oral mucosa, and muscle had a size of ~7 kb as estimated by gel analysis (Fig. 1). After sequencing fragment 1, the exact deletion size was determined to encompass 7,372 bp, including 16 genes and flanked by direct repeats. Deletion size, breakpoints, the repeat sequence, and the genes abrogated by the deletion are shown in Fig. 2.

Discussion

Mitochondrial disorders are often considered rare conditions seen only in children with severe neurological

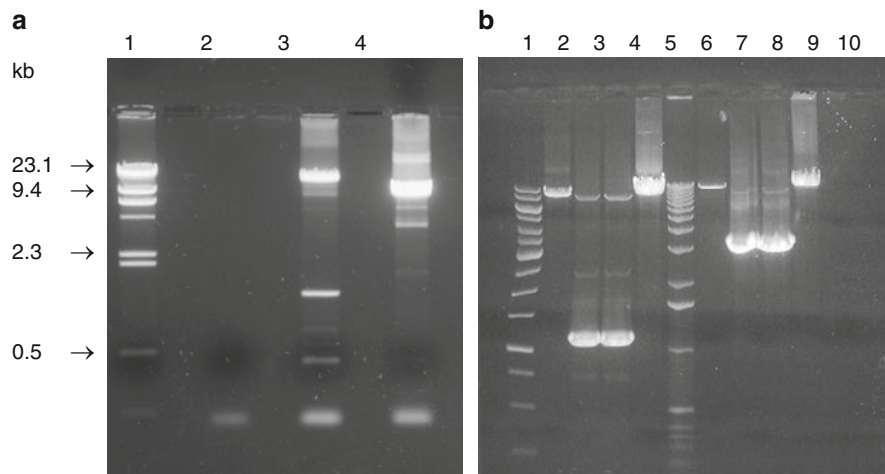


Fig. 1 (a): PCR products after whole mitochondrial genome amplification. Lane 1: molecular weight standard (23.1, 9.4, 6.6, 4.4, 2.3, 2.0, 0.5 kb), Lane 2: negative control, Lane 3: wild-type sample with a major fragment (16 kb), Lane 4: patient sample with a smaller fragment (9 kb). **(b)** PCR products after amplification of fragments 1

(lanes 2–5) and 2 (lanes 7–10). Lane 1 and 6: molecular weight standard (10, 8, 6, 5, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.75, 0.5, 0.25 kb), Lanes 2, 5, 7 and 10: wild-type sample. Lanes 3, 4, 8 and 9: patient samples (blood and mucosal cells, respectively). All other amplicons (fragments 3, 4, 5, 6 and 7) from the patient had normal-sized bands

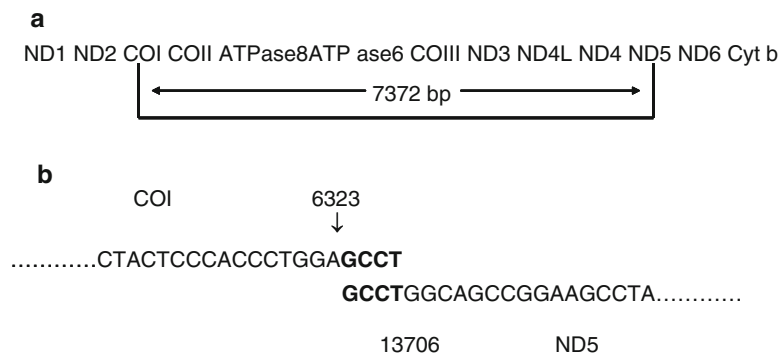


Fig. 2 (a): schematic of the deletion size and genes involved. **(b):** Nucleotide sequence and mitochondrial DNA position flanking the repeat region (repeat sequence in *bold*)

impairment and multisystem failure. Here, we describe a 3-year-old boy with Addison disease, congenital glaucoma, chronic pancreatitis, and mitochondrial myopathy, but without central nervous system involvement who harbored a single large mtDNA deletion.

Mitochondrial DNA deletion syndromes included three phenotypes: KSS (OMIM, # 530000), Pearson syndrome (OMIM, # 557000), and PEO. KSS is characterized by the triad of onset before age 20, PEO, pigmentary retinopathy, and at least one of the following features: heart block, ataxia, and cerebrospinal fluid (CSF) protein > 100 mg/dl. Frequent additional signs include short stature, microcephaly, sensorineural hearing loss, cardiomyopathy, renal tubular acidosis and Fanconi syndrome, muscle weakness, basal ganglia calcifications, diffuse signal abnormality of central white matter, dementia, and sensory or motor neuropathy.

Pearson syndrome is characterized by refractory sideroblastic anemia and vacuolization of marrow precursors, as well as exocrine pancreatic dysfunction with malabsorption and pancreatic fibrosis.

Abnormalities of the adrenal axis have been found in patients with KSS. Boles et al. (1998) published a case with mtDNA deletion and Addison disease as a prominent clinical feature. Sanaker et al. (2007) reported a young woman who developed Addison disease, hypothyroidism, and glucose intolerance associated with thyroid peroxidase antibodies and adrenal 21-hydroxylase antibodies. Other endocrinopathies such as early-onset diabetes mellitus (Rötig et al. 1993), growth hormone deficiency (Gücüyener et al. 1998), and hypoparathyroidism (Cassandrini et al. 2006) have also been report in patients with single mtDNA deletions.

Congenital glaucoma was described in a child with KSS and has been reported in two adult patients as a rare

coincidence (Simaan et al. 1999). Normally, the ciliary muscles supplies some force to the trabecular meshwork, increasing the absorption of the aqueous humor. Thus, a decrease in outflow might be secondary to the ciliary mitochondrial myopathy, leading to reduction in aqueous humor absorption, increased ocular pressure, and glaucoma.

Acute or chronic pancreatitis associated with mitochondrial diseases is rare in childhood (Kishnani et al. 1996a). The incidence of pancreatic calcification varies from 19.9% to 83.1% in adults with chronic pancreatitis (Stobbe et al. 1970) and the relationship between calcification and exocrine pancreatic function is not linear. Pancreatic calcifications are virtually pathognomonic of chronic pancreatitis and develop in up to 90% of adult patients with alcoholic chronic pancreatitis (Ammann et al. 1988), but no information is available for younger patients. We could not document pancreatic insufficiency in this patient, but it is likely that the calcifications characterize more severe forms of chronic pancreatitis even in the early phases of the disease (Scuro et al. 1990).

Pancreatic dysfunction has been described in Pearson's disease associated with diabetes mellitus. There have been only a few reports of mitochondrial myopathy associated with pancreatitis (Kato et al. 1990; Toyono et al. 2001). Tsao et al. (2000) reported an infant with chemical pancreatitis due to mtDNA depletion. In addition, pancreatitis and pancreatic calcifications have been reported in patients with the m.3243A > G mutation in mitochondrial DNA, the "MELAS mutation" (Kishnani et al. 1996b; Schleiffer et al. 2000). In our patient, the abdominal ultrasound and MRCP suggested the diagnosis of chronic pancreatitis, although he was asymptomatic and had no steatorrhea. Finsterer (2000) recommends that, when a mitochondrial disease is suspected, the history should always include queries on the presence or absence of pancreatitis (Finsterer 2007).

In this case, the clinical suspicion of a mitochondrial deletion was very strong. Using this methodological approach, we were able to describe a novel large mitochondrial DNA deletion involving almost all genes on the big arc, flanked by short direct repeats, as often described for mtDNA deletions (Kleinle et al. 1997; Yamashita et al. 2008). We demonstrated this large deletion of mitochondrial DNA in at least three different tissues of this patient. Given the clinical manifestations, we may assume that it is present in other cell types, but we could not prove it, as well as the heteroplasmic mutation level. The patient reported by Boles et al. (1998), who also presented with Addison disease, had a smaller deletion. On the other hand, there are cases with similar deletion sizes but without Addison disease, reinforcing the concept that genotype does not always or exactly correlate with phenotype (Yamashita et al. 2008). Due to the genetic and clinical heterogeneity of mitochondrial disorders, it is especially important to have access to

molecular analysis to confirm diagnoses and to achieve more precise genetic counseling (Wong 2007).

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