Clinical Case Reports

CASE REPORT

Large-scale mitochondrial DNA deletion underlying familial multiple system atrophy of the cerebellar subtype

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Key Clinical Message

A family with mitochondrial inheritance of multiple system atrophy of the cerebellar subtype. MRI brain shows significant cerebellar atrophy with mild pontine atrophy and the classical hot cross bun sign in Pons. The muscle biopsy was indicative of mitochondrial myopathy. Mitochondrial DNA analysis revealed a low-level large mtDNA deletion, m.3264_1607del12806 bp.

Keywords

Introduction

Mitochondria have a crucial role in cellular bioenergetics and apoptosis, and thus are important to support cell function and in determination of cell death pathways. Inherited mitochondrial diseases can be caused by mutations of mitochondrial DNA or of nuclear genes that encode mitochondrial proteins [1, 2].

Cerebellar ataxia is a frequently reported symptom in patients with mtDNA defects [3–12]. It is often progressive in these patients and is a major cause of disability [13]. Mutations in the nuclear-encoded Mitochondrial DNA polymerase gamma (*POLG1*) have been also described in patients with diverse clinical presentations that include cerebellar ataxia.

Multiple system atrophy of the cerebellar subtype (MSA-C) often presents with adult-onset progressive ataxia [14]. The motor features of MSA-C include predominant cerebellar dysfunction that manifests as gait ataxia, limb ataxia, ataxic dysarthria, autonomic dysfunction, and cerebellar disturbances of eye movements. The genitourinary dysfunction [14] and the MRI brain imaging [15] can differentiate MSA -C from sporadic ataxia.

For the first time, we describe a family that presented with maternal inheritance (Fig. 1) of adult-onset progressive cerebellar ataxia and signs of multiple system atrophy in association with large mitochondrial deletion.
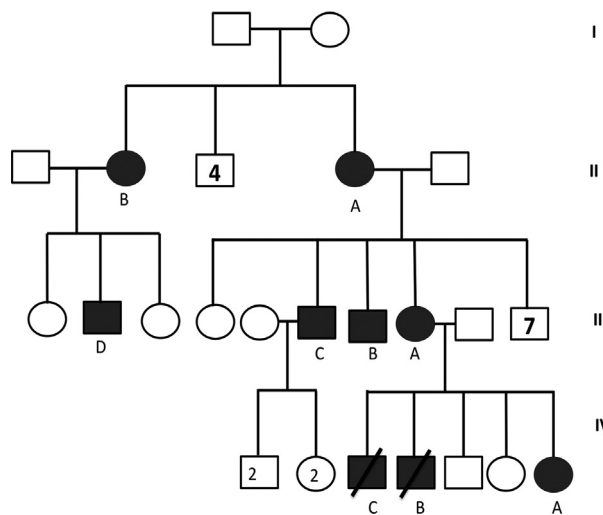

Figure 1. Family pedigree.

Material and Methods

Patients and subjects

We studied an affected family with progressive ataxia. Informed consent, blood samples were obtained. Clinical and radiological evaluations were performed for two affected family members. Muscle biopsy and molecular studies were done for one affected member. The investigations were arranged under protocols and approved by ethics committee of the hospital.

Muscle histopathology

A muscle biopsy was obtained from the right deltoid muscle of the affected patient (IV-A) and submitted for pathological assessment. The biopsy was processed according to standard practice where a portion is freshly frozen for the routine and special enzyme histochemistry studies and other portions reserved for ancillary studies including biochemical, electron microscopic, and molecular studies.

Molecular studies

Mitochondrial DNA analysis was done on the peripheral blood and frozen muscle tissue of the index patient at the Baylor medical genetics laboratories. Both point mutations and large mitochondrial DNA rearrangements involving deletions are analyzed by long range PCR amplification followed by massively parallel sequencing. Thirty-six mitochondrial point mutations were included m.1494T>C, m.1555A>G, m.1606G>A, m.3243A>G, m.3251A>G, m.3252A>G, m.3271T>C, m.3460G>A, m.4269A>G, m4274T>C, m.4300A>G, m.7445A>C, m.8344A>G, m.8356T>C, m.1555A>G, m.1606G>m.8993T>G, m.9176T>C, m.9176T>G, m.9185T>C, m.10010T>C, m.10158T>G, m.10191T>C, m.10197G>A, m.11777C>A, m.11778G>A, m.12147G>A, m.12258C>A, m.12315G>A, m.12320A>G, m.13513G>A, m.13514A>G, m.14459G>A, m.14484T>C, m.14674T>C, and m.14709T>C.

Results

Clinical report

This is a 33-year-old female (IV-A) who was referred because of history of progressive tremor, unsteadiness, and difficulty in walking with significant family history of similar illness (Fig. 1). The age onset of the disease was at 26 years old. The patient was a product of uneventful pregnancy and normal spontaneous vaginal delivery with normal developmental milestone.

Initially, her speech had become slurred with significant horizontal nystagmus. Then, she had difficulty climbing stairs and with time, she started to have difficulty carrying objects with frequent falls. Later, her coordination and tremor worsened, and she started to be unable to walk and consequently, she developed urinary incontinence, swallowing difficulty, and frequent choking episodes, but without cognitive impairment. Muscle power was normal with present reflexes. T2-weighted and flair sequence MRI brain images showed significant cerebellar atrophy with mild pontine and medulla oblongata atrophy (Fig. 2A,B). Flair MRI brain images show faint hot cross bun sign in Pons (Fig. 3A), with significant pontine and cerebellar atrophy (Fig. 3B). The Gradient echo MRI sequence shows hot cross bun sign in Pons (Fig. 4). Her pyruvate level was elevated.
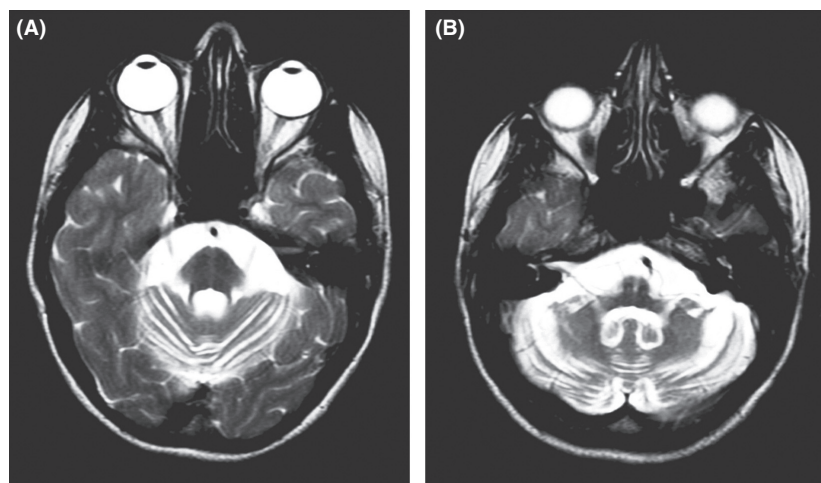

Figure 2. T2-weighted MRI brain images show significant cerebellar atrophy with mild Pons (A) and Medulla oblongata atrophy (B).
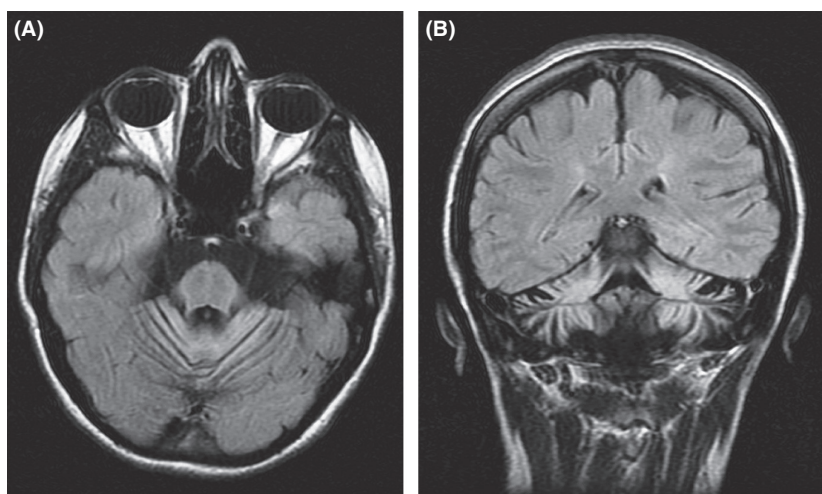
Figure 3. Flair MRI brain images show faint hot cross bun sign in Pons (A) with significant Pontine and Cerebellar atrophy (B).
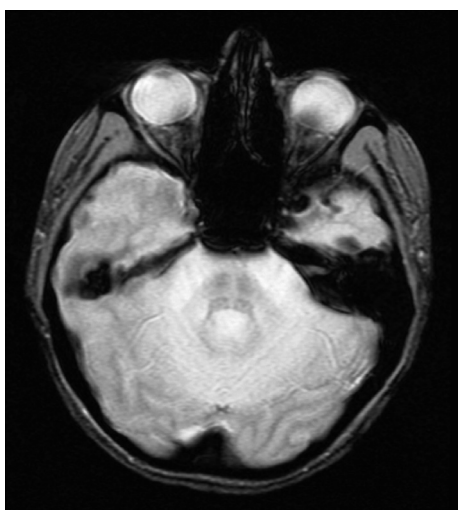

Figure 4. Gradient echo MRI sequence shows hot cross bun sign in Pons.

Her mother (III-A) was affected with similar illness of progressive ataxia of gait and limbs. Her onset was at the age of 25 years. Subsequently, she became wheelchair bound and developed urinary incontinence, severe swallowing difficulty, and anarthria speech, but she lived with normal cognitive function. She died at the age of 50 years . MRI brain showed atrophy and signal changes within lower brainstem, the cerebellum, and spinal cord. She had normal alpha fetoprotein level and normal immunoglobulin level.

The maternal uncle (III-C) of the index patient had similar illness with progressive incoordination of hands and legs at the age of 27 years , which eventually progressed to loss of balance requiring a walker then a wheelchair, and then he developed dysphagia, and choking episodes which led to death after 15 years since the onset. Her grandmother (II-A) and her grandmother's sister (II-B) were affected with progressive ataxia. Also, another maternal uncle (III-B) is similarly affected. Our index case has two brothers who died at the age of 2 and 4 years (IV-B, C). They suffered from progressive brain atrophy.

Muscle histopathology

The hematoxylin- and eosin-stained fresh frozen sections (Fig. 5A) revealed mild variation in myofiber sizes with scattered small/atrophic fibers as well as occasional split fibers. There were no necrotic or regenerative fibers, inflammatory reaction or endomysial fibrosis. With the modified Gomori trichrome stain, many of the small fibers showed significant increase in subsarcolemmal mitochondria appearing as red granules in a partial circumferential fashion just short of being "ragged red" fibers (Fig. 5B). These granules represent mitochondria as illustrated by the activity of the specific mitochondrial enzymes, succinic dehydrogenase (SDH) and cytochrome c oxidase (COX). The former showed the so-called "ragged blue" fibers (Fig. 5C). There were no COX-negative fibers. Under electron microscopy, there is subsarcolemmal accumulation of mitochondria (Fig. 5D) in the affected fibers. Many of the mitochondria are enlarged, and some have abnormal shapes including elongation and branching, and some have concentric cristae.

Mitochondrial DNA analysis

MtDNA analysis of blood specimen did not reveal deletions or deleterious point mutations. However, mtDNA
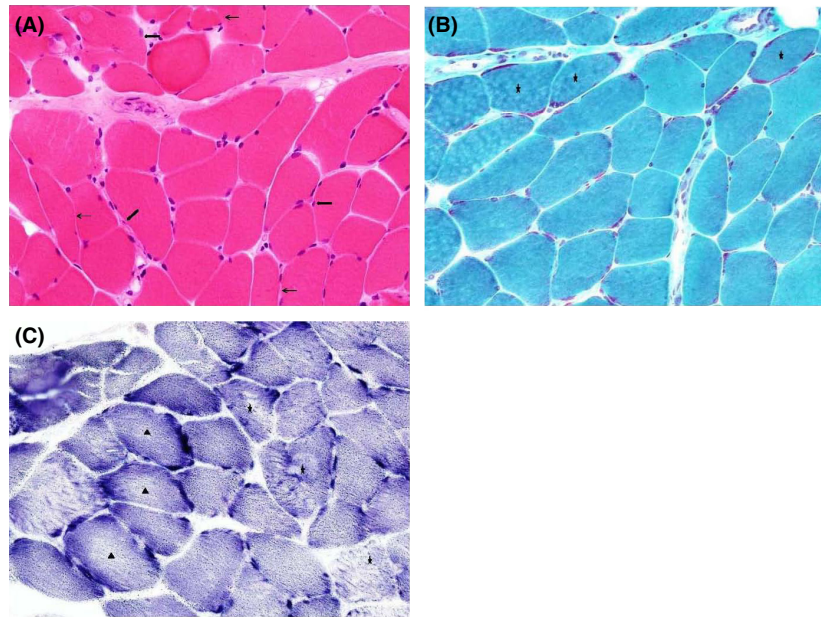
Figure 5. (A) A cross sectional view of muscle biopsy shows variation in myofiber sizes with mildly to moderately atrophic fibers (arrows) as well as split fibers (head arrow). (Hematoxylin and eosin, original magnification ×400). (B) Many of the smaller fibers have subsarcolemmal increase in red granules corresponding to mitochondria (stars).The endomysial connective tissue is intact. (Modified Gomori trichrome, original magnification ×400). (C) The subsarcolemmal red granules in the modified Gomori trichrome show increased SDH activity (ragged blue fibers) (stars), a specific mitochondrial enzyme. There are also scattered moth-eaten fibers (triangles) (Succinic dehydrogenase enzyme histochemistry, original magnification ×400).

analysis of the skeletal muscle detected a low-level large mtDNA deletion, m.3264_1607del12806 bp. The result was confirmed by Sanger sequencing. The deleted segment (12806 bp) extends to involve all the mtDNA encoding the 13 essential polypeptides of the Oxidative phosphorylation (Fig. 6).

Discussion

We describe a family with adult-onset of progressive cerebellar ataxia and radiological signs of multiple system atrophy of the cerebellar subtype(MCA-C). The magnetic resonance imaging of the index case showed cerebellopontine atrophy and T2 hyperintensity within the Pons (hot cross bun sign). The family history is well matched with a maternal mode of inheritance.

The muscle biopsy was indicative of mitochondrial disease. There are frequent fibers with increased subsarcolemmal staining, with many poorly formed ragged red fibers. Mitochondrial DNA analysis of the skeletal muscle was diagnostic of a mitochondrial disease. A low-level large mtDNA deletion, m.3264_1607del12806 bp, was detected.

Multiple system atrophy (MSA) is a rare adult-onset synucleinopathy associated with dysautonomia and the variable presence of parkinsonism (MSA-P) and/or cerebellar ataxia (MSA-C). Magnetic resonance imaging

(MRI) of the MSA-C may show T2 hyperintensity within the Pons (hot cross bun sign), with volume loss in the Pons and cerebellum [16, 17].
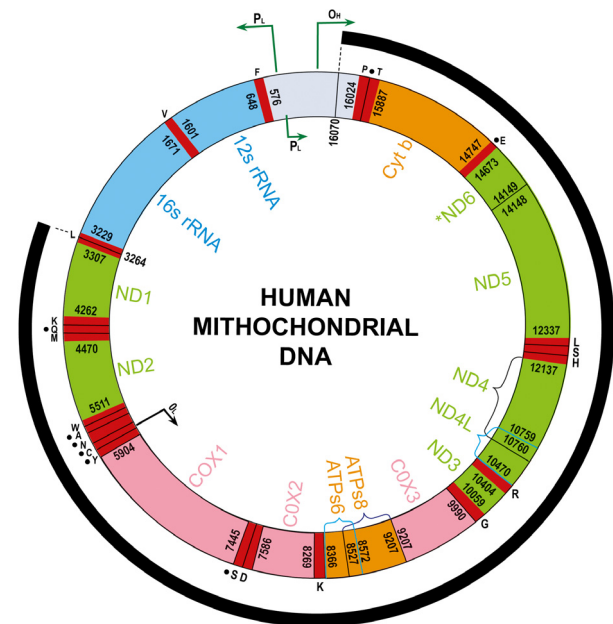

Figure 6. Large deleted segment (12806 bp) involves the mtDNA genes encoding the 13 essential polypeptides of the Oxidative phosphorylation.

A definite diagnosis of MSA-C is usually based on postmortem histological analysis of olivo-ponto-cerebellar tissue documenting glial and neuronal cytoplasmic inclusions with a-synuclein as a major component along with myelin loss [18], nevertheless the characteristic MRI brain changes and the bladder dysfunctions of our patient meet the criteria for the diagnosis of MSA-C.

Our family represents a genuine evidence of underlying genetics basis of MSA-C. To date, the association between MSA-C and primary mitochondrial disorder was not reported. However, mitochondrial mimicry of multiple system atrophy of the cerebellar subtype was described previously in one patient with POLG1 Gene heterozygous mutation [19.]

On the other hand, a homozygous mutation (M78V-V343A/M78V-V343A) and compound heterozygous mutations (R337X/V343A) in COQ2 were also identified in two multiplex families [20]. Furthermore, a common variant (V343A) and multiple rare variants in COQ2, all of which are functionally impaired, are associated with sporadic multiple system atrophy. The V343A variant was exclusively observed in the Japanese population [20].

Previous studies had shown an evidence of mitochondrial respiratory-chain dysfunction or oxidative injury in patients with multiple system atrophy [21, 22]. The combination of oxidative stress and overexpression of oligodendroglial α-synuclein has been reported to replicate the characteristics of this disease [23, 24].

A primary deficiency of coenzyme Q10 that is caused by COQ2 mutations has been described as an infantile-onset multisystem disorder and a nephropathy in several families [25, 26]. The clinical presentation of these affected family members differed markedly from the presentations of patients with multiple system atrophy, perhaps because the decrease in COQ2 activity associated with the mutations in patients with multiple system atrophy appears to be milder than that observed in patients with a primary deficiency of coenzyme Q10 [20].

Large mitochondrial DNA (mtDNA) deletions were first discovered in muscle of patients with mitochondrial myopathies (MM), Kearns–Sayre syndrome (KSS) (OMI M530000), Pearson syndrome (OMIM557000), and progressive external ophthalmoplegia (PEO; OMIM555000) [27–30]. MtDNA deletion syndromes have been also reported in patients with various clinical manifestations, including Addison disease, atypical Pearson presentation, cyclic vomiting, severe renal tubulopathy, hepatic dysfunction, dysarthria, organic acidopathy, hypoparathyroidism, and hypocalcemia [31].

Mitochondrial DNA Mutation Load varied widely among tissues. As a rule, DNA from urinary sediment and skeletal muscle had the highest and blood the lowest proportion of mutant genomes. In all individuals in whom the mutation was detectable in blood, it was also detected in other tissues [32, 33]. In KSS, deleted mtDNA occurs mainly in muscle and not always in leukocytes [34].

Different tissues harboring the same mtDNA mutation may be affected to different degrees or not at all, which explains the frequent occurrence of oligosymptomatic or asymptomatic individuals within the same family [35].

Mitochondrial dysfunction and oxidative stress have been implicated in cellular senescence, apoptosis, aging, and aging-associated pathologies. Mitochondrial dysfunction leads to telomere attrition, telomere loss, and chromosome fusion and breakage, accompanied by apoptosis [36].

Conclusion

Multiple system atrophy (MSA) is a fatal oligodendrogliopathy characterized by prominent α-synuclein inclusions resulting in a neuronal multisystem degeneration. Until recently, MSA was broadly conceived as a nongenetic disorder. However, within the last two decades several genes have been associated with an increased risk of MSA [37]. Furthermore, our family report reinforces again the fact that oxidative stress, mitochondrial dysfunction, and deletions have also a major role in the underlying pathogenesis of the MSA-C disorder [19, 21, 22, 24].

Acknowledgment

Ethics approval

The Research Centre Ethics Committee approval.

Conflicts of Interest

None declared.

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