Episodic ataxia and hemiplegia caused by the 8993T→C mitochondrial DNA mutation

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The m.8993T→C *MTATP6* mutation of mitochondrial DNA (mtDNA) usually causes mitochondrial disease in childhood, but was recently described in a family with adult onset ataxia and polyneuropathy. Cytochrome c oxidase muscle histochemistry, which is the standard clinical investigation for mitochondrial disease in adults, is usually normal in patients with *MTATP6* mutations. This raises the possibility that these cases have been missed in the past. We therefore studied 308 patients with unexplained ataxia and 96 patients with suspected Charcot–Marie–Tooth disease to determine whether the m.8993T→C *MTATP6* mutation is common in unexplained inherited ataxia and/or polyneuropathy. We identified a three-generation family with the m.8993T→C mutation of mtDNA. One subject had episodic ataxia (EA) and transient hemipareses, broadening the phenotype. However, no further cases were identified in an additional cohort of 191 patients with suspected EA. In conclusion, m.8993T→C *MTATP6* should be considered in patients with unexplained ataxia, CMT or EA, but cases are uncommon.

As a group, mitochondrial DNA (mtDNA) mutations cause a diverse spectrum of neurological disease.¹ Strikingly different phenotypes are often seen in different members of the same family. In general, the phenotypic variation is less extreme in families transmitting mutations that affect structural (protein-encoding) genes, than in patients with mutations that compromise intramitochondrial protein synthesis. In keeping with this, various mutations affecting the mtDNA ATP synthase subunit 6 gene (*MTATP6*) have been described in two main phenotypic groups: children presenting with Leigh syndrome (LS) or bilateral striatal necrosis (BSN),² and those with neurogenic weakness with ataxia and retinitis pigmentosa (NARP).³ The clinical diagnosis of ATP synthase defects remains a challenge because mitochondrial histochemical abnormalities are rarely apparent in skeletal muscle, and many diagnostic laboratories do not routinely measure the rate of ATP synthesis.¹ Molecular diagnosis is critically dependent on clinical intuition, with specific mutations being sought in patients with a typical phenotype. Under these circumstances, there is a danger that the limited phenotype becomes self-fulfilling. It was therefore of great interest when the m.8993T→C *MTATP6* mutation was described in a kindred with adult-onset slowly progressive ataxia and polyneuropathy,⁴ raising the possibility that other adults with progressive ataxia or an hereditary neuropathy have the same molecular defect.

PATIENTS AND METHODS

Patients

The study group comprised 308 probands who did not have inflammatory, metabolic, neoplastic and sporadic degenerative ataxia, spinocerebellar ataxia 1, 2, 3, 6, 7, 10, 12 or 17, dentatorubral-pallidolusian atrophy or Friedreich's ataxia

(suspected inherited ataxia group); 96 probands with suspected Charcot–Marie–Tooth (CMT) disease who did not have a *PMP22* rearrangement (suspected inherited neuropathy group); and 191 probands who were suspected to have episodic ataxia type 2 on clinical grounds (suspected episodic ataxia type 2 group).

Molecular genetics

For the 308 patients with suspected ataxia and the 96 with suspected CMT, mtDNA flanking m.8993 was amplified using PCR from blood DNA using a fluorescent-tagged antisense primer (8993R-5'-CCTTCCCTCTACACTTATCA-3'), an unlabelled sense primer (8993F-5'-AACCATCAGCCTACTCATTC-3') and standard cycling conditions (58°C), followed by *HpaII* digestion and sizing (Beckman CEQ 8000 Capillary DNA Analyzer; Beckman Coulter, High Wycombe, Buckinghamshire, UK). *HpaII* cuts the 140-bp fragment at CCGG sites generating 109-bp and 31-bp products in the presence of m.8993T→G, or 108-bp and 32-bp products for the m.8993T→C change. Sensitivity of the assay was determined by mixing cloned DNA fragments containing different proportions of the mutated and wild-type template. The assay reliably detected 1.8% mutated mtDNA. Mutation-positive cases were confirmed by direct sequencing of the original template, and the proportion of mutated mtDNA was determined by last-cycle fluorescent primer PCR.⁵

The synonymous m.8994G→A substitution, present in ~2.4% of the population (<http://www.genpat.uu.se/mtDB/>) removes the *HpaII* site introduced by m.8993T→G/C, potentially leading to a false negative result. To exclude this possibility, all cases were screened for m.8994G→A by primer extension of multiplex PCR products and detection of the allele-specific extension products by matrix-associated laser desorption/ionisation time of flight mass spectrometry (Sequenom, San Diego, California, USA).

MtDNA was sequenced directly in the 191 cases of suspected EA (Applied Biosystems 3730, BigDye V.1.1; Applied Biosystems, Foster City, California, USA).

RESULTS

Neither mutation was detected in the cohort with suspected CMT. m.8993T→C was detected in one proband with ataxia (fig 1a). Eight subjects harboured m.8994G→A. The region was sequenced directly in these cases, and all eight had a wild-type allele at m.8993.

Case reports

I-1

A 76-year-old woman first presented at the age of 63 with a 23-year history of a slowly progressive gait disturbance. On

Abbreviations: BSN, bilateral striatal necrosis; CMT, Charcot–Marie–Tooth; EA, episodic ataxia; EA2, episodic ataxia type 2; FHM, familial hemiplegic migraine; LS, Leigh syndrome; mtDNA, mitochondrial DNA; NARP, neurogenic weakness with ataxia and retinitis pigmentosa

Figure 1 (a) Pedigree transmitting m.8993T→C. II-1 was the proband in this study. All three family members were clinically affected (shaded symbols). The percentage level of mutated mtDNA determined by last-cycle fluorescent PCR is shown beneath each person. (b) Sequence trace confirming the presence of the mutation in II-1. (c) T2 brain MRI from III-1. Upper, sagittal image showing cerebellar atrophy; lower, coronal image showing normal basal ganglia.

examination, she had a broad-based ataxic gait and cerebellar dysarthria. She had a full range of extraocular movements with gaze-evoked horizontal nystagmus but normal optic fundi. Limb examination revealed normal muscle tone and power, absent ankle jerks, moderate dysmetria and normal sensation. A neurophysiological study revealed chronic neurogenic changes on electromyography and evidence of a mild axonal sensorimotor neuropathy. Brain MRI revealed cerebellar atrophy. Cerebrospinal fluid examination and evoked potential studies were normal, and 86% m.8993T→C was detected in her blood.

II-1

The 50-year-old daughter of II-2 presented with intermittent speech and gait disturbance aged 35. Exacerbations were associated with headaches, nausea, vomiting and fatigue twice each month. She had had frequent hospital admissions throughout childhood with hemipareses associated with severe headache, nausea and vomiting. She subsequently developed distal paraesthesia and mild ankle dorsiflexion weakness. On examination, she had an ataxic gait, broken ocular pursuits and gaze-evoked horizontal nystagmus that fluctuated between clinic visits over a 10-year period. There was full range of external ocular movements, no ptosis, and fundoscopy was unremarkable. The remaining cranial nerves were normal. Limb tone was normal, there was symmetrical mild ankle dorsiflexion

weakness (MRC grade 4+), absent ankle jerks and bilateral extensor plantar responses. A glove-and-stockings sensory loss was first detected at the age of 48. Nerve conduction studies were normal at the ages of 39 and 43, but revealed an axonal sensorimotor neuropathy at 51 years of age. Brain and cervical spine MR imaging was normal at 47 years of age. Cerebrospinal fluid was normal with no oligoclonal bands. After 15 years of follow-up, the patient remained ambulant without a walking stick. There was 82% m.8993T→C detected in her blood.

III-1

The 25-year-old granddaughter of II-2 developed an episodic gait disturbance with a progressive dysarthria over 2 months, which improved after sleep. When assessed she tended to invert her right ankle on walking, hyperextended the fingers of her right hand, and had a marked cerebellar dysarthria. Ocular saccades were hypometric and she had bilateral optic atrophy with no retinal pigmentation. Limb tone and power was normal, but she had pathologically brisk tendon reflexes and absent ankle jerks. The right plantar was extensor. She had impaired dorsal column sensation and mild dysmetria. Brain MRI revealed cerebellar atrophy with normal cerebral hemispheres and deep structures (fig 1b). Neurophysiology identified an axonal sensorimotor neuropathy, and 83% m.8993T→C was detected in her blood.

Suspected EA

Neither m.8993T→G nor m.8993T→C were detected in the 191 cases of suspected EA.

DISCUSSION

The family described here confirm that *MTATP6* mutations can present with adult-onset ataxia, but our observations indicate that m.8993T→G/C substitutions are rare in patients with suspected spinocerebellar ataxia or CMT. Although various family members reported sensory symptoms, had mild ankle dorsiflexion weakness and absent ankle reflexes, ataxia was the prominent phenotype in all three subjects. Based on current evidence we therefore do not recommend screening patients with an isolated axonal neuropathy for m.8993T→G/C, unless other family members have central neurological features.

Unlike most other heteroplasmic mtDNA mutations, percentage levels of m.8993T→G/C do not vary significantly between different tissues in the same subject, nor do they change over time.⁶ It is therefore likely that the levels measured in blood reflect levels in the cerebellum and peripheral nerve. Given that our assay reliably detected ~2% mutated mtDNA, it is unlikely that we missed patients who had significant levels of mutated mtDNA in clinically relevant tissues. However, different family members transmitting m.8993T→G/C often have markedly different levels of heteroplasmy,⁷ thought to be due to a germline genetic bottleneck during maternal transmission of the mutation. It is therefore intriguing that all three maternal relatives had the same percentage level of mutated mtDNA in blood. Although at first sight this seems unusual, retrospective analysis of a large number of pedigrees has shown that little or no change in the percentage level of heteroplasmy is actually more common than large changes over one generation,⁸ suggesting that the dramatic changes seen in small pedigrees are an ascertainment artefact. Precisely why >80% m.8993T→C should cause adult-onset ataxia with neuropathy in this family, but a severe childhood encephalopathy in others,⁷ remains unclear.

Our observations add to the widening phenotypic spectrum associated with *MTATP6* mutations. The periodic exacerbations described by two members of the family we describe here, accompanied by migraine-like headaches with hemiparesis in one subject (II-1) led to a clinical diagnosis of the allelic disorders episodic ataxia type 2 (EA2) and familial hemiplegic migraine (FHM). III-1 did not harbour the *CACNA1A* T666M mutation, which is present in 15% of families with FHM.⁹ Although we have not excluded other mutations in *CACNA1A*, nor other known genetic causes of EA2 or FHM such as mutations in *ATP1A2* and *SCN1A*, these three genes only account for a small proportion of FHM cases in Europeans.¹⁰ The m.8993T→C mutation is extremely rare in the general population (found in none of 2600 control mtDNA sequences, <http://www.genpat.uu.se/mtDB/>), making it the likely cause in this family. We did not detect any additional cases by screening 191 patients with suspected episodic ataxia, confirming that this is an uncommon presentation for mitochondrial disease. However, fluctuating neurological signs are common in other mtDNA disorders, including recurrent hemipareses.¹ Therefore, mtDNA mutations should be considered in families with unexplained EA and periodic limb weakness.

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Key points

- The m.8993T→C *MTATP6* mutation of mitochondrial DNA (mtDNA) usually causes mitochondrial disease in childhood, but was recently described in a family with adult-onset ataxia and polyneuropathy.
- By studying 308 patients with unexplained ataxia and 96 patients with suspected Charcot–Marie–Tooth disease, we identified a three-generation family with the m.8993T→C mutation of mtDNA.
- One subject had episodic ataxia (EA) and transient hemipareses, broadening the phenotype.
- No further cases were identified in an additional cohort of 191 patients with suspected EA.
- In conclusion, m.8993T→C *MTATP6* should be considered in patients with unexplained ataxia, CMT or EA, but cases are uncommon.

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