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Adult-onset spinocerebellar ataxia syndromes due to *MTATP6* mutations

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Abstract

Background—Spinocerebellar ataxia syndromes presenting in adulthood have a broad range of causes, and despite extensive investigation remain undiagnosed in up to ~50% cases. Mutations in the mitochondrially-encoded *MTATP6* gene typically cause infantile-onset Leigh syndrome and occasionally have onset later in childhood. We report two families with onset of ataxia in adulthood (with pyramidal dysfunction and/or peripheral neuropathy variably present), who are clinically indistinguishable from other spinocerebellar ataxia patients.

Methods—Genetic screening study of the *MTATP6* gene in 64 pedigrees with unexplained ataxia, and case series of two families who had *MTATP6* mutations.

Results—Three pedigrees had mutations in *MTATP6*, two of which have not been reported previously and are detailed in this report. These families respectively had the m.9185T>C and m.9035T>C mutations, which have not previously been associated with adult-onset cerebellar syndromes. Other investigations including muscle biopsy and respiratory chain enzyme activity were nonspecific or normal.

Conclusions—*MTATP6* sequencing should be considered in the workup of undiagnosed ataxia, even if other investigations do not suggest a mitochondrial DNA disorder.

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Keywords

MTATP6; ATPase; mitochondria; ataxia; spinocerebellar ataxia; neuropathy; adult-onset

Mutations in the mitochondrial-encoded *MTATP6* gene typically cause severe neonatal or childhood-onset multisystem neurological disease including Leigh syndrome and neuropathy, ataxia and pigmentary retinopathy. Although late-onset forms have been described, the phenotype is usually an axonal sensorimotor neuropathy, occasionally with associated ataxia.[1-3] Screening for *MTATP6* mutations is not routinely considered in the investigative work-up in cases of adult-onset spinocerebellar ataxia. Here we describe two families with adult-onset spinocerebellar ataxia due to mutations in *MTATP6*, indicating the importance of sequencing this gene in this context.

METHODS

Institutional research ethics board approval was obtained, and patients provided written informed consent. We studied 64 pedigrees with ataxia after excluding common sporadic and inherited causes (inflammatory, metabolic, neoplastic and sporadic degenerative ataxia, spinocerebellar ataxia 1, 2, 3, 6, 7, 10, 12 or 17, dentatorubral-pallidoluysian atrophy or Friedreich ataxia). Sequencing of the *MTATP6* gene was performed on all pedigrees. In pedigrees with identified *MTATP6* mutations, the clinical records and investigations of all available family members were reviewed in detail, and the heteroplasmy level of the mutation was quantified using pyrosequencing assays.

Sequencing of MTATP6 gene

Total genomic DNA was extracted from several tissues including muscle and blood by standard procedures. Direct sequencing of the *MTATP6* gene was undertaken employing three overlapping pairs of M13-tagged oligodeoxynucleotide primers as follows: MTATP6a-Fw 5'-CGGGGGTATACTACGGTCAA-3' (m.8151-8170) and MTATP6a-Rv 5'-TCCGAGGAGGTTAGTTGTGG-3' (m.8765-8784); MTATP6b-Fw 5'-ACCACCCAACAATGACTAATC-3' (m.8656-8676) and MTATP6b-Rv 5'-GTTGTCGTGCAGGTAGAGG-3' (m.9183-9201); MTATP6c-Fw 5'-ATCCTAGAAATCGCTGTCGC-3' (m.9127-9146) and MTATP6c-Rv 5'-ATTAGACTATGGTGAGCTCAG-3' (m.9641-9661). Amplified PCR products were sequenced using BigDye Terminator v3.1 chemistries (Applied Biosystems, Warrington, UK) and compared to the revised Cambridge reference sequence (GenBank Accession number NC_012920.1).[4]

Measurement of heteroplasmy with pyrosequencing assays

The mtDNA mutation load levels were quantified using a sensitive pyrosequencing assays according to the manufacturer's standard protocol. Pyromark Assay Design Software v.2.0 (Qiagen) was used to design locus specific PCR and pyrosequencing primers for the m.9035T>C and m.9185T>C mutations. PCR of mtDNA amplicons spanning the m.9035 nucleotide was achieved using the following primers – 9035_Fw: 5'-

CCGTACGCCTAACCGCTAACAT-3' (m.8996-9018) and 9035_Rv 5'-AGGGTGGCGCTTCCAATT-3' (m.9045-9062).

Pyrosequencing was carried out on the Pyromark Q24 platform according to the manufacturer's protocol, using mutation specific pyrosequencing primers 9035_Pyro: 5'-ACTGCAGGCCACCTAC-3' (m.9019-9034). The primers used to quantify the m.9185T>C mutation are as follows: 9185_Fw: CGCCTTAATCCAAGCCTACGTT (m.9144-9165) and 9185_Rv: GTGATTGGTGGGTCATTATGTGTT (m.9199-9222) and 9185_Pyro: CGTTTTTCACACTTCTAGTAA (m.9162-9181). The reverse PCR primers for both assays were biotinylated. Pyromark Q24 software was used to quantify the m.9035T>C and m.9185T>C heteroplasmy levels through comparison of wild-type and mutant peak heights.[5]

RESULTS

Three families were identified, each with different *MTATP6* mutations (m.8993T>C, m.9035T>C, and m.9185T>C). One family was reported previously.[2] The two families who have not been previously reported are presented below, and pedigree structure is illustrated in Figure 1.

Family A: all five siblings in the same generation presented with a spinocerebellar syndrome in late teenage or early adult life (Table 1). Each tested sibling harboured very high levels of the m.9185T>C mutation in blood or muscle. Their parents lived into their 70's and were not known to have a neurological disorder.

Family B: all three siblings in the same generation have a spinocerebellar syndrome (Table 1). Each tested sibling harboured very high levels of the m.9035T>C mutation in blood. The parents were not known to have any neurological illness. Patient I-3 has two children aged 11 and 8 years, who are presently healthy, and had a stillbirth at 37 weeks. Patient I-1 has one child aged 4, identified as II-1 in the table, who developed symptoms at 2 years of age.

DISCUSSION

The m.9185T>C mutation (predicted to cause amino acid substitution p.L170P) has been described previously in three families. Ataxia was a prominent feature in several family members from two of these reports, but onset was in childhood.[6,7] One report demonstrated broad phenotypic variability between family members carrying this mutation at similar levels of heteroplasmy.[6] Our patients from Family A who had this mutation, all had onset of disease in adulthood, with ataxia as the predominant feature, despite high levels of heteroplasmy (95-99%) in all family members tested.

The m.9035T>C mutation (causing amino acid substitution p.L220P, which was present in Family B) has been reported in one family with childhood ataxia and developmental delay.[8] With the exception of one patient (designated II-1 in Table 1), our patients with this mutation have a much milder phenotype, with adult-onset of a spinocerebellar syndrome and no developmental delay, expanding the phenotypic spectrum for this mutation, and again

indicating that onset of disease may occur in adulthood despite high heteroplasmy levels (90-96%).

With the cases described here, three separate *MTATP6* mutations have now been shown to cause adult-onset spinocerebellar ataxia syndromes (m.8993T>C[1,2], and m.9035T>C or m.9185T>C), with a broad range of phenotypes. In our families, this most characteristically included cerebellar dysfunction (midline, appendicular, ocular and dysarthria), pyramidal dysfunction (spasticity and/or abnormal reflexes), neuropathy (sensorimotor or motor axonal neuropathy), or proximal myopathy. Routine clinical diagnostic tests were nonspecific: Brain MRI was normal in 3/5 patients, and one patient each had cerebellar atrophy, or cerebellar and cerebral volume loss. Nerve conduction studies and electromyography indicated the presence of mild sensorimotor axonal neuropathy in 3/6 patients, and one patient each with motor axonal neuropathy, myopathic changes, and radiculopathy. Muscle biopsy was performed in three patients and respectively demonstrated normal results, nonspecific abnormalities, and minor myopathic changes. Respiratory chain enzyme analysis was performed on muscle from two patients in Family A, with normal activity in complexes I-IV. Although the number of patients is small, the data indicate that diagnostic investigations appear to be nonspecific in these two families with ataxia due to *MTATP6* mutations. Of particular importance is that none of the three patients with muscle biopsy had the typical findings of mitochondrial disease, with normal respiratory chain enzyme activities in frozen muscle homogenates. These features are typical for adult onset spinocerebellar ataxia and when presented with these investigations the level of suspicion may be low for a mtDNA disorder.

Genetic testing results, and measurement of heteroplasmy levels using pyrosequencing, have demonstrated high mtDNA heteroplasmy levels in all patients, including those from other reports[1-3] In our families, six patients had their genetic testing performed on leucocyte DNA, with heteroplasmy levels ranging from 90-99%. Two patients who had testing on DNA extracted from muscle had similar heteroplasmy levels (96-98%). Therefore, unlike many mtDNA disorders, *MTATP6* sequencing can be reliably performed on leucocyte DNA, and should be considered as a diagnostic test in the workup of ataxia and neuropathy, even when the routine clinical investigations for mitochondrial disease are normal. Testing for this gene, unlike numerous other SCA genes, is easily undertaken as it is only 680 bp and contains no introns.

As in a previous report,[8] the proportion of affected family members in affected generations was very high in the two families described here (all patients in a single generation in each of our families). This is intriguing, given that the percentage level of mtDNA heteroplasmy generally shows dramatic differences between siblings due to the germ-line mtDNA bottleneck. The sample size is small (and selection bias may be a factor), although in Family A, 4 separate individuals have heteroplasmy ranging from 95-99%. This highly restricted range of heteroplasmy suggests the possibility that the inheritance of mtDNA heteroplasmy in these families is different from other mtDNA mutations (for further discussion refer to the on line supplement). For the mutations described here, the clinical recurrence risk appears to be extremely high, and the proportion of affected family members is much higher than that expected for an autosomal dominant disorder. This provides a

further clinical clue that the underlying molecular diagnosis is likely to involve the mitochondrial genome.

The identification of *MTATP6* mutations has management implications, to provide guidance for genetic counselling, preimplantation/prenatal genetic diagnosis,[9] and potential disease prevention by nuclear transfer.[10] There is also preliminary evidence demonstrating improved survival of cells having the 8993T>G mutation, when treated with α -ketoglutarate and aspartate, although further work will need to demonstrate benefit in vivo, and in other *MTATP6* mutations.[11]

It is important to note that various mitochondrial disorders may include ataxia as a component of the clinical presentation, as either a background or principal feature. A detailed discussion of the various aetiologies is beyond the scope of this work although this topic was previously reviewed in detail.[12]

We conclude that sequencing of *MTATP6* should be considered in the diagnostic work-up of undiagnosed adult-onset spinocerebellar ataxia syndromes. Our finding that three pedigrees have mutations in this gene, from a pool of only 64 pedigrees, suggest that this finding is not isolated, and may be a relatively common aetiology for these syndromes. However, we cannot exclude the possibility that referral bias may in part explain the frequency that we have discovered. Testing of this gene is rapid and inexpensive, and as we have demonstrated, this disorder cannot be excluded by normal or nonspecific muscle biopsy investigations, and can be performed on leucocyte DNA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

- (1). Rantamaki MT, Soini HK, Finnila SM, et al. Adult-onset ataxia and polyneuropathy caused by mitochondrial 8993T-->C mutation. *Ann Neurol.* Aug; 2005 58(2):337–340. [PubMed: 16049925]
- (2). Craig K, Elliott HR, Keers SM, et al. Episodic ataxia and hemiplegia caused by the 8993T->C mitochondrial DNA mutation. *J Med Genet.* Dec; 2007 44(12):797–799. [PubMed: 18055910]
- (3). Tsao CY, Mendell JR, Bartholomew D. High mitochondrial DNA T8993G mutation (<90%) without typical features of Leigh's and NARP syndromes. *J Child Neurol.* Jul; 2001 16(7):533–535. [PubMed: 11453454]
- (4). Andrews RM, Kubacka I, Chinnery PF, et al. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet.* Oct.1999 23(2):147. [PubMed: 10508508]

- (5). Alston CL, Lowe J, Turnbull DM, et al. A novel mitochondrial tRNA^{Glu} (MTTE) gene mutation causing chronic progressive external ophthalmoplegia at low levels of heteroplasmy in muscle. *J Neurol Sci.* Nov 15; 2010 298(1-2):140–144. [PubMed: 20810132]
- (6). Childs AM, Hutchin T, Pysden K, et al. Variable phenotype including Leigh syndrome with a 9185T>C mutation in the MTATP6 gene. *Neuropediatrics.* Dec; 2007 38(6):313–316. [PubMed: 18461509]
- (7). Castagna AE, Addis J, McInnes RR, et al. Late onset Leigh syndrome and ataxia due to a T to C mutation at bp 9,185 of mitochondrial DNA. *Am J Med Genet A.* Apr 15; 2007 143A(8):808–816. [PubMed: 17352390]
- (8). Sikorska M, Sandhu JK, Simon DK, et al. Identification of ataxia-associated mtDNA mutations (m.4052T>C and m.9035T>C) and evaluation of their pathogenicity in transmitochondrial cybrids. *Muscle Nerve.* Sep; 2009 40(3):381–394. [PubMed: 19626676]
- (9). Steffann J, Gigarel N, Corcos J, et al. Stability of the m.8993T->G mtDNA mutation load during human embryofetal development has implications for the feasibility of prenatal diagnosis in NARP syndrome. *J Med Genet.* Oct; 2007 44(10):664–669. [PubMed: 17545557]
- (10). Craven L, Tuppen HA, Greggains GD, et al. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature.* May 6; 2010 465(7294):82–85. [PubMed: 20393463]
- (11). Sgarbi G, Casalena GA, Baracca A, et al. Human NARP mitochondrial mutation metabolism corrected with alpha-ketoglutarate/aspartate: a potential new therapy. *Arch Neurol.* Aug; 2009 66(8):951–957. [PubMed: 19667215]
- (12). Finsterer J. Mitochondrial ataxias. *Can J Neurol Sci.* Sep; 2009 36(5):543–553. [PubMed: 19831121]

Figure 1.

Complete pedigrees for Families A and B. All members of generation I are affected by adult-onset ataxic disorders. The family members of generation II are unaffected (except for B:II-1), although because of their young age some or all may yet develop the condition in adulthood.

Clinical features and testing results

Table 1

	Age/sex	Onset age and symptom	Clinical features	Status at last assessment	Genetic mutation	Heteroplasmy by pyrosequencing (tissue tested)	Muscle biopsy	Respiratory chain enzyme analysis***	MRI	EMG/NCS
Family A										
I-1	58/M	Gait ataxia in adolescence (in retrospect, not athletic in childhood)	Ataxia, saccade dysmetria, proximal myopathy, cognitive impairment (58/100 Addenbrooke's test), distal leg wasting, absent leg DTR, sensorimotor neuropathy	Mobilises independently but occasional falls	m.9185T>C	98% (muscle)	Minor nonspecific abnormalities	Normal	Normal	Sensorimotor axonal neuropathy
I-2	55/F	34, gait ataxia	Ataxia, slow extraocular movements, optic pallor, dysarthria, distal leg wasting, spasticity, dystonia, sensorimotor axonal neuropathy	Mobilises by wheelchair	m.9185T>C	99% (blood)	Mild myopathic/neurogenic changes	Normal	Moderate panceerebellar atrophy	Mild sensorimotor axonal neuropathy
I-3	51/M	32, gait ataxia	Ataxia, dysarthria, Broken ocular pursuit, Distal leg muscle atrophy, brisk DTR except absent DTR at ankles, extensor plantar reflexes, Pes cavus bilaterally.	Mobilises with cane, wheelchair for long distances	m.9185T>C	99% (blood)	N/A	N/A	Cerebellar and cerebral atrophy	Pure motor axonal neuropathy
I-4**	49/F	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Radiculopathy
I-5	43/F	37, gait ataxia	Ataxia, dysarthria, short stature, distal weakness with foot drop, extensor plantar reflexes, decreased dorsal column sensation in ankles. Mild phenotype.	Mobilises unaided	m.9185T>C	95% (blood)	N/A	N/A	N/A	Sensorimotor axonal neuropathy
Family B										
I-1	48/F	20, slurred speech and gait imbalance	Cerebellar dysarthria, limb and gait ataxia. Increased motor tone, brisk DTR, proximal weakness 4/5.	Mobilises unaided	Declined	N/A	N/A	N/A	N/A	N/A
I-2	45/F	20, ataxia, brainstem syndrome	Sub-acute onset ophthalmoplegia and ataxia with long tract signs in lower limbs. Spontaneous improvement but residual midline ataxia, disordered	Mobilises unaided	m.9035T>C	96% (blood)	Normal (at age 20)	N/A	Normal	N/A

Age/sex	Onset age and symptom	Clinical features	Status at last assessment	Genetic mutation	Heteroplasmy by pyrosequencing (tissue tested)	Muscle biopsy	Respiratory chain enzyme analysis***	MRI	EMG/NCS
eye movements, dysarthria, extensor plantar responses and absent DTR at ankles. eye movements, dysarthria, extensor plantar responses and absent DTR at ankles. eye movements, dysarthria, extensor plantar responses and absent DTR at ankles.									
I-3	43/F	16, leg cramps post-exercise Cramping/soreness on activity, gait ataxia. Soreness post-exercise up to hours/days. Intermittent paraesthesiae. Cerebellar dysarthria, limb, midline ataxia. Hypometric saccades. Leg spasticity. Pyramidal weakness distribution of 4/5 in legs, worse proximally. Brisk DTR, extensor plantar reflexes.	Can walk independently but develops symptoms within 10 metre walk.	m.9035T>C	90% (blood); 96% (muscle)	N/A	N/A	Normal	Minor myopathic abnormalities
II-1	4/F	2 Short stature and speech delay	N/A	m.9035T>C	95% (blood)	N/A	N/A	N/A	N/A

N/A = data not available

** Limited clinical information was available, and reported from patient’s siblings. An EMG/NCS report was available for review.

*** Complexes I, II, III, and IV were tested