

ELECTRONIC LETTER

Spinocerebellar ataxia and the A3243G and A8344G mtDNA mutations

P F Chinnery, D T Brown, K Archibald, A Curtis, D M Turnbull

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The majority of pedigrees with autosomal dominant cerebellar ataxia (ADCA) harbour a pathological expansion of a trinucleotide repeat at one of five genetic loci: spinocerebellar ataxia (SCA) 1, 2, 3, 6, and 7.¹ Other loci have been associated with ADCA in a limited number of families,¹⁻³ but in a significant number of pedigrees the genetic basis remains uncertain. Mitochondrial DNA (mtDNA) defects may present with cerebellar ataxia, with or without corticospinal tract involvement.⁴ MtDNA rearrangements are usually sporadic and may cause ataxia as part of the Kearns-Sayre syndrome. MtDNA point mutations may also cause a spinocerebellar syndrome that may be transmitted down the maternal line.^{5,6} In small pedigrees, it may not be possible to distinguish between maternal and dominant modes of transmission. This raises the possibility that mtDNA point mutations may be responsible for the ataxia seen in some SCA mutation negative families. To test this hypothesis, we identified 29 independent pedigrees with ataxia and an inheritance pattern consistent with mitochondrial transmission and 54 sporadic cases of ataxia. We excluded a pathological trinucleotide expansion at the common SCA loci and then looked for the two most common mtDNA point mutations associated with ataxia, A3243G and A8344G, in a clinically affected subject from each family.

METHODS

Spinocerebellar ataxia cases were prospectively referred to the Northern Genetics Service (UK) over a 15 year period. Genomic DNA was extracted from whole blood, and standard techniques were used to identify subjects with Friedreich's ataxia (FA) and SCA 1, 2, 3, 6, 7, and 8. The remaining cases were divided into three categories by pedigree analysis: (1) independent pedigrees with at least one documented paternal transmission of the ataxia (n=16); (2) independent pedigrees with no paternal transmission but at least one documented maternal transmission (n=29); and (3) sporadic cases of ataxia with no identifiable aetiology (n=54).

MtDNA analysis was carried out on leucocyte/platelet DNA obtained from an affected subject in each maternal pedigree and on each sporadic case. People harbouring pathogenic mtDNA mutations usually have a mixture of mutant and wild type mtDNA (heteroplasmy), and the percentage level of mutant mtDNA in blood may be low in affected subjects. To detect low levels (<1%) of mutant mtDNA, we added a fluorescence labelled dNTP (FdNTP) to the last cycle of each PCR reaction.⁷

A3243G

The mtDNA tRNA^{Leu(UUR)} gene was amplified using the following primers: L3200 (5'-tataccacaccacccaag-3'), H3353 (5'-gcgattagaatgggtacaat-3'), standard cycling conditions, and an annealing temperature of 54°C. After last cycle labelling with 0.3 µl FdNTP (PE Biosystems), the products were digested with *Hae*III.

A8344G

The mtDNA tRNA^{Lys} gene was amplified using the following primers: L8155 (5'-ggatactacggtcaatgctc-3'), H8366 (5'-tttctactgtaaagggtgtgg-3'), standard cycling conditions, and an annealing temperature of 52°C. After last cycle labelling with 0.3 µl FdNTP (PE Biosystems), the products were digested with *Bar*II.

For both mutation analyses, the RFLP products were separated on a 6% acrylamide gel by electrophoresis using the ABI 373 automated sequencer (Perkin Elmer). The presence or absence of mutant peaks was determined using Genescan and Genotyper software (PE Biosystems), and the area of each peak was used to calculate the proportion of mutant mtDNA.

RESULTS

A3243G

One of the 83 subjects tested was found to have the A3243G mutation. Case 1 was a 57 year old man who presented with a five year history of progressive speech and balance disturbance, with no family history of neurological disease. On examination he had an ataxic gait, a cerebellar dysarthria, and appendicular ataxia. Eye movements and tendon reflexes were normal and plantar responses were flexor. The only abnormal clinical investigations were a random blood glucose of 10.9 mmol/l and brain MR imaging which showed mild cerebellar atrophy. Genetic analysis showed 8% A3243G mutation in his blood. The patient died from an unrelated cause before further investigation was possible.

A8344G

One of the 29 subjects with a maternal history was found to harbour the A8344G mutation. Case 2 is a 49 year old man who presented in his third decade with blackouts that responded to anticonvulsants. He then developed a slowly progressive speech and gait disturbance. Occasional myoclonic jerks were noted in his early 40s. On examination he had a full range of ocular motility but pursuit movements were broken and his saccades were hypometric. He had a cerebellar dysarthria, appendicular and gait ataxia, brisk reflexes, and flexor plantar responses. His mother developed a similar progressive ataxic syndrome in her 30s and died in her early 50s from pneumonia. A needle muscle biopsy on case 2 showed 2% cytochrome c oxidase negative fibres and 2% ragged red fibres. The level of mutant mtDNA was 95% in blood and >98% in skeletal muscle.

DISCUSSION

We have shown that the A3243G and A8344G mutations are rare causes of spinocerebellar ataxia in our region. Only 8% A3243G mutation was detected in the blood sample from case

Abbreviations: ADCA, autosomal dominant cerebellar ataxia; SCA, spinocerebellar ataxia; FA, Friedreich's ataxia; mtDNA, mitochondrial DNA

1 and further investigation was not possible. While it is difficult to be absolutely certain that the A3243G mutation was responsible for the ataxia in this patient, similar percentage levels of mutant mtDNA in blood have been reported in some patients with neurological disease, usually in association with much higher levels of mutant mtDNA in skeletal muscle.^{8,9} This is because the percentage level of the A3243G mutation in blood decreases throughout life,¹⁰ while the level of mutant mtDNA in muscle (and other non-dividing tissues such as central neurones) may remain the same or increase during life.¹¹

The A3243G and A8344G mutations are at least twice as prevalent as the total number of other mtDNA point mutations known to cause disorders involving the central nervous system.¹² It is therefore unlikely that other mtDNA point mutations^{13–15} account for a significant number of undiagnosed cases of ataxia in our series. It would be difficult to investigate this possibility without carrying out muscle biopsies on affected subjects from each family. The percentage level of mutant mtDNA in blood may fall below the detection threshold for mtDNA sequencing.¹⁶ Based upon dilution experiments performed in our laboratory,¹⁶ we would not have detected the A3243G mutation in case 1 by using automated mtDNA sequencing of blood mtDNA. Thus, even if we had sequenced the entire mitochondrial genome using blood mtDNA from the remaining 81 families studied here, there would be no guarantee that we would detect other pathogenic mtDNA mutations. Much higher percentage levels of mutant mtDNA are usually present in skeletal muscle,¹⁷ comfortably exceeding the threshold for detection by automated mtDNA sequencing. It is for this reason that muscle mtDNA is conventionally used when searching for novel mtDNA mutations.¹⁸

It is important to consider the possibility that the presence of mtDNA mutations in patients with ataxia might be a coincidence. Hereditary ataxias affect fewer than 1 in 10 000 of the general population.^{19,20} The A3243G mutation is present in at least 1 in 70 000 of the UK population,⁸ but has been detected in up to 1 in 6000 of the Finnish population.²¹ The prevalence of the A8344G mutation is considerably less.¹² Thus, the chance association of A3243G and hereditary ataxia would be expected once in every 60 million people, and the chance association of A8344G with ataxia would be even less frequent. The Northern Genetics Service (UK) serves approximately 2.5 million, making it highly improbable that we would identify two cases of ataxia harbouring A3243G or A8344G by chance alone.

The retrospective analysis of each positive case in this study showed additional clinical features that are unusual in ADCA. For example, case 2 had myoclonic epilepsy and case 1 had evidence of impaired glucose tolerance. We carried out a retrospective study of our own cases and published families, which included 165 subjects with the A3243G mutation and 71 with the A8344G mutation. In this group, 39 cases with A3243G had ataxia (24%) and 42 cases with A8344G had ataxia (59%). In each case there were additional clinical features suggestive of mitochondrial disorder, such as external ophthalmoplegia, proximal myopathy, diabetes mellitus, epilepsy, or myoclonus. Similarly, in the family reported by Santorelli *et al.*,⁶ the proband and a maternal relative had neuropathological features of Leigh syndrome. Presumably this would have been apparent on MRI imaging had this investigation been available at the time of presentation. Similarly, in the family studied by Howell *et al.*,⁵ one subject had cutaneous lipomata and myoclonic jerks, one had hypertrophic cardiomyopathy, and two children died with a cardiomyopathy and Leigh syndrome. These findings stress the importance of thorough clinical assessment of patients with spinocerebellar ataxia before genetic testing. Although there are many clinical similarities between autosomal spinocerebellar ataxia syndromes and mtDNA disorders, there are often key clinical clues that

- Twenty-nine families with maternally transmitted spinocerebellar ataxia and 54 sporadic cases of idiopathic cerebellar ataxia were screened for two of the most common mitochondrial DNA (mtDNA) point mutations, A3243G and A8344G.
- The A3243G mutation was found in one sporadic case and the A8344G mutation was found in one maternal pedigree.
- MtDNA analysis should be considered in patients with unexplained sporadic and maternally inherited ataxia.

point to a mitochondrial aetiology. MtDNA analysis should be considered in patients with unexplained sporadic and maternally inherited ataxia.

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Authors' affiliations

P F Chinnery, D T Brown, D M Turnbull, Department of Neurology, The University of Newcastle upon Tyne, UK

K Archibald, A Curtis, Department of Human Genetics, The University of Newcastle upon Tyne, UK

Correspondence to: Dr P F Chinnery, Department of Neurology, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK; P.F.Chinnery@ncl.ac.uk

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