Spinocerebellar ataxia and the A3243G and A8344G mtDNA mutations

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

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).

MitDNA 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 fluorescently labelled dNTP (FdNTP) to the last cycle of each PCR reaction. 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 A8344G, suggesting that these mutations are at least uncommon in our region.

Abbreviations: ADCA, autosomal dominant cerebellar ataxia; SCA, spinocerebellar ataxia; FA, Friedreich’s ataxia; mtDNA, mitochondrial DNA
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. It is therefore unlikely that other mtDNA point mutations 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. Based upon dilution experiments performed in our laboratory, 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 sequencing of blood mtDNA, 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, 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.

It is important to consider the possibility that the presence of mtDNA mutations in patients with ataxia might be a coincidental hereditary disorder, rather than a mitochondrial disorder, such as external ophthalmoplegia, pigmentary retinopathy, diabetes mellitus, myopathy, or myoclonus. Similarly, in the family reported by Santorelli et al., the proband and a maternal relative had neuropathological features of Leigh syndrome. Presumably this would have been apparent on MRI imaging had there been investigation available at the time of presentation. Similarly, in the family studied by Howell et al., 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 point to a mitochondrial aetiology. MtDNA analysis should be considered in patients with unexplained sporadic and maternally inherited ataxia.

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**REFERENCES**


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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. This is because the percentage level of the A3243G mutation in blood decreases throughout life, 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.

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.
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