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. Other loci have been associated with ADCA in a limited number of families, 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. 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. 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 clinically affected subjects from each family.

RESULTS

A3243G
The mtDNA tRNALeu(UUR) gene was amplified using the following primers: L3200 (5'-ttttcactgtaaagaggtgtgg-3'), H3353 (5'-ggtatactacggtcaatgctc-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 BanII.

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.

A8344G
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.

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
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 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, 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 finding. Hereditary ataxias affect fewer than 1 in 10 000 of the general population. The A3243G mutation is present in at least 1 in 70 000 of the UK population, but has been detected in up to 1 in 6000 of the Finnish population. The prevalence of the A8344G mutation is considerably less. Thus, the chance association of A3243G and hereditary 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, 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, one subject had cutaneous lipomatous 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.

ACKNOWLEDGEMENT

PFC is an Advanced Wellcome Clinical Research Fellow.

REFERENCES