Frataxin point mutations in two patients with Friedreich’s ataxia and unusual clinical features

Melissa L McCormack, Rodney P Guttmann, Matthew Schumann, Jennifer M Farmer, Catherine A Stolle, Victoria Campuzano, Michel Koenig, David R Lynch

Abstract
Two patients with a progressive ataxia are presented with clinical features consistent with classic Friedreich’s ataxia (FRDA), but also with features unusual for FRDA. Analysis of DNA showed that each patient is heterozygous for the expanded GAA repeat of FRDA, but carries a base change on his other frataxin allele. For one patient a non-conservative arginine to cysteine amino acid change is predicted at amino acid 165 whereas the other mutation is found at the junction of exon one and intron one. Muscle biopsy showed an absence of frataxin immunoreactivity in the patient harbouring the intronic mutation, confirming the pathological nature of the base change. These mutations extend the range of point mutations seen in FRDA, and agree with recent reports suggesting phenotypic variation in patients with FRDA harbouring point mutations in conjunction with an expanded GAA repeat.

Methods
GENOMIC DNA ISOLATION
GAA repeat analysis was performed by the Genetics Diagnostic Laboratory at the University of Pennsylvania. Exons 1–5a were amplified using the intronic primers described previously and sequenced using Sequenase™ polymerase chain reaction (PCR) product sequencing kit and automated sequencing.

Western blots of quadriceps biopsy tissue were performed as described previously.

Results
CASE HISTORIES
Patient 1
An 11 year old boy presented at the age of 5 with difficulty walking. This slowly progressed without complaints referable to his upper limbs. His lower limbs became stiff, and his feet progressively turned down and in. Speech, vision, and sensation were unchanged. Three cousins of his maternal grandmother had a neurodegenerative disease similar to Friedreich’s ataxia by report.

Physical examination disclosed a well developed boy without significant scoliosis but with bilateral pes cavus. Mental status and speech were normal. Optic disks were flat without temporal pallor. Visual acuity was 20/20, and visual fields were full. Extraocular movements were full without nystagmus. No muscle atrophy was present. Tone was normal in the upper limbs, and increased in his lower limbs. The increased tone was uniform with passive movement and was not velocity dependent. Power was normal, although his feet were plantar flexed and inverted from increased tone. Reflexes were symmetrically increased at his knees, but absent in his upper limbs and at his ankles. Plantar responses were extensor bilaterally. Finger to nose testing was well...
performed; no pseudoathetosis was seen. He had decreased proprioception in the feet. The boy walked on his toes with stiff legs. His Romberg’s sign was positive.

The patient was initially diagnosed with idiopathic dystonia. Electromyography and nerve conduction velocity were normal at age 7. A slight decrease in ulnar and median sensory potentials had developed by the age of 9, and sural sensory potentials were absent at that time. An ECG showed inverted T waves in the inferior and left precordial leads. An echocardiogram demonstrated a slight increase in the left ventricular end diastolic dimension for age. A DNA test for FRDA showed a single expanded allele (1000 repeats), suggestive of an apparent carrier status. His mother was shown to be a carrier of an expanded allele, whereas his father had no expanded alleles. Sequencing of the patient’s frataxin gene disclosed a single base change (C → T) at base 493, predicting an arginine to cysteine change at amino acid 165 in exon 5a (figure A). No other base changes were present in the frataxin coding region. This base change was also present in his father, showing that the point mutation and the expanded GAA repeat were inherited as distinct alleles. We analysed this region of frataxin in 37 other people and found the R165C change in none of them. This included six control subjects, six ataxic patients with no expanded alleles for frataxin, and 25 other patients with only one expansion of frataxin. Fourteen of the patients with one expanded allele had other mutations in the frataxin gene.

Patient 2

This 47 year old man was evaluated for an atypical progressive neurodegenerative disorder consisting of ataxia, sensorimotor axonal polyneuropathy, proprioceptive loss, optic neuropathy, and diabetes. Difficulty with writing and walking was noted around the age of 8, and he developed progressive weakness in his lower limbs. He was diagnosed with Friedreich’s ataxia, but this was later changed to Charcot-Marie-Tooth disease because of increasing calf atrophy and a failure to develop speech dysfunction. Progression of his weakness led to confinement to a wheelchair at the age of 21. Progressive visual difficulties were noted in early adulthood, including night blindness and diminished peripheral vision, especially in his lower visual fields. Diabetes was diagnosed at the age of 40, with subsequent need for oral hypoglycaemic agents. There was no family history of neurological disease or consanguinity. No scoliosis or cardiomyopathy was present.

Neurological examination at the age of 40 disclosed a visual acuity of 20/30 OD and 20/40 OS with central scotomas OU and
Frataxin point mutations and Friedreich’s ataxia

Persistence of intervening introns. To confirm that in sequencing exons 1–5 and the adjacent portions of intervening introns. No other abnormalities were found at the fifth base of intron 1 on one allele (figure A and B). No other abnormalities were found in sequencing exons 1–5 and the adjacent portions of intervening introns. To confirm that this base change was on a distinct allele from the expanded repeat, we sequenced the frataxin gene in his brother, who has no neurological, cardiac, or endocrinological abnormalities. This man had the same base change, but lacked an expanded triplet repeat, suggesting that these mutations are inherited as distinct alleles.

DNA sequencing of the frataxin gene found a single base substitution (G→C transversion) at the fifth base of intron 1 on one allele (figure A and B). No other abnormalities were found in sequencing exons 1–5 and the adjacent portions of intervening introns. To confirm that this base change was on a distinct allele from the expanded repeat, we sequenced the frataxin gene in his brother, who has no neurological, cardiac, or endocrinological abnormalities. This man had the same base change, but lacked an expanded triplet repeat, suggesting that these mutations are inherited as distinct alleles.

However, as this base change is found in an intronic portion of the frataxin gene, it could theoretically represent a silent polymorphism. We assessed this possibility by determining if the base change was found in a significant number of control subjects. Direct sequencing of the frataxin gene from 11 controls found this base change in none. We also assessed amplified exon 1 with the adjacent intronic region for the presence of Mae III sites as this base transversion adds one site. Frataxin exon 1 from 24 controls was cleaved by this enzyme into segments of 154 and 47 bases indicating the absence of an extra Mae III site in all controls. This suggests that this abnormality is not often found within the population.

Finally, to ensure that the base change found causes a pathological abnormality, we confirmed that this transversion produces a change in frataxin expression. No immunologically detectable frataxin was found in muscle using a monoclonal antibody against frataxin, whereas frataxin was found in muscle from a GAA expansion of the naturally occurring GAA repeat within the frataxin gene on the other allele, are associated with the production of FRDA. We have shown that both base changes are on different alleles from the GAA expansion, consistent with their being causative mutations, although we cannot rule out the possibility that they are uncommon polymorphisms. In patient 1, the base change predicts a non-conservative amino acid substitution, similar to other missense mutations reported in patients with FRDA.7 Arginine 165 is also present in mouse and yeast frataxin, showing its high level of evolutionary conservation and arguing that it is a necessary amino acid.7 In the other patient, the intronic base change is associated with a loss of frataxin on muscle biopsy. This base change lies at a similar position to a previously reported intronic base change in frataxin between exons 4 and 5, and conceivably could disrupt RNA splicing or transcription.7

The use of genetic testing has increased the range of phenotypic features associated with FRDA. These features include later age of onset, spasticity rather than dorsal column sensory loss as a major manifestation, pure sensory ataxia, and generalised chorea.8,9 One recent report shows that unusual phenotypic features of FRDA are more common in patients having point mutations rather than two expanded triplet repeats.7 These unusual features include lack of dysarthria (40% of compound heterozygous patients), retained reflexes (28%), lack of bilateral extensor plantar responses (24%), and optic nerve pallor (28%).7 Missense mutations in the amino terminal portion of frataxin have been specifically associated with milder phenotypic features.7 The phenotypic features of the two patients presented here agree with these results. Our first patient differs from many patients with FRDA in the increased tone in his lower limbs, which directed evaluation away from FRDA. This patient is early in his course, and may develop more typical features later. Our second patient differs from many patients with FRDA in his lack of dysarthria, the significant motor neuropathy, and the significant optic atrophy, all of which caused difficulty in making a definitive diagnosis before the use of molecular approaches. Optic nerve abnormalities are seen on visual evoked responses in up to 64% of patients with FRDA, but only 5%-15% of patients have optic nerve dysfunction which is clinically significant.1,11 Thus, the unusual phenotypic features in our two patients agree with recent genotype-phenotype correlations.
Note added in proof
While this work was in press we became aware of one reported patient with FRDA with the R16SC mutation and two sisters with the mutation R16SP. Consistent with the present work all three have atypical phenotypes.

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