Malaysian Siblings with Friedreich Ataxia and Chorea: A Novel Deletion in the Frataxin Gene

Siân D. Spacey, Blazej I. Szczygielski, Sean P. Young, Juliette Hukin, Kathy Selby, Terrance P. Snutch

ABSTRACT: Background: Friedreich ataxia (FRDA1) is most often the result of a homozygous GAA repeat expansion in the first intron of the frataxin gene (FRDA gene). This condition is seen in individuals of European, North African, Middle Eastern and Indian descent and has not been reported in Southeast Asian populations. Approximately 4% of FRDA1 patients are compound heterozygotes. These patients have a GAA expansion on one allele and a point mutation on the other and have been reported to have an atypical phenotype. Objective: To describe a novel dinucleotide deletion in the FRDA gene in two Malaysian siblings with FRDA1. Setting: Tertiary referral university hospital setting. Patients and Methods: A previously healthy 10-year-old Malay boy, presented with fever, lethargy, headaches, dysarthria, dysphagia, vertigo and ataxia which developed over a one week period. His neurological exam revealed evidence of dysarthria and ataxia, mild generalized weakness and choreiform movements of the tongue and hands. His reflexes were absent and Babinski sign was present bilaterally. A nine-year-old sister was found to have mild ataxia but was otherwise neurologically intact. Results: Molecular genetic studies demonstrated that both siblings were compound heterozygotes with a GAA expansion on one allele and a novel dinucleotide deletion on the other allele. Conclusions: We describe a novel dinucleotide deletion in the first exon of the FRDA gene in two siblings with FRDA1. Additionally this is the first report of FRDA1 occurring in a family of southeast Asian descent, it demonstrates intrafamilial phenotypic variability, and confirms that atypical phenotypes are associated with compound heterozygosity.

The frataxin gene (FRDA gene) has been localized to chromosome 9q13 and codes for the frataxin protein, a putative iron transporter that regulates mitochondria iron content. In approximately 96% of cases the mutation in FRDA gene is an unstable homozygous expansion of a GAA repeat in the first intron. The number of repeats in normal individuals ranges from 7 - 60 and in affected individuals from 66 to greater than 1700. Somatic mosaicism of the GAA expansion has been demonstrated in FRDA1 patients. The GAA expansion that causes FRDA1 has only been reported in individuals of European, North African, Middle Eastern or Indian origin (Indo-European and Afro-Asiatic speakers), it has not been reported in the southeast Asian population. Unusual phenotypic features are more commonly seen in the approximately 4% of patients with FRDA1 who are compound heterozygotes with a GAA expansion on one allele and a point mutation in the FRDA gene on the other allele. Phenotypic variation between family members with 2 GAA expansions is seen but is rarely reported in compound heterozygote families.

METHODS

GAA repeat analysis
Genomic DNA was isolated from peripheral blood of the two siblings and their parents. The region encompassing the GAA repeat in the FRDA gene was polymerase chain reaction amplified under conditions previously described. Expanded alleles were then resolved and sized using agarose gel electrophoresis.

Sequence analysis of the FRDA gene
Exons 1-5a and associated intron/exon boundaries were polymerase chain reaction amplified using intronic primers previously described. The amplification products were then subjected to automated sequencing using an ABI377 DNA sequencer.

RESULTS

Case 1
Patient 3:1 (Figure) a previously healthy 10-year-old Malaysian boy, presented with fever, lethargy, headaches, dysarthria, dysphagia, vertigo and ataxia developing over a one week period. His neurological exam revealed mild choreiform movements of the tongue and hands, mild generalized weakness, absent reflexes and bilateral Babinski signs. Sensory examination demonstrated decreased temperature perception in a glove-stocking distribution, however pinprick, vibration and proprioception were normal. He had dysarthria, marked finger-nose and heel-shin ataxia. He walked with a broad based ataxic gait and was unable to tandem walk. There was evidence of thoracolumbar scoliosis and pes cavus. As the fever remitted there was mild but not significant improvement in the ataxia and the chorea remained unchanged. Nerve conduction studies demonstrated a severe generalized peripheral sensory and motor neuropathy with both axonal and demyelinating features. An electrocardiogram (ECG) was normal, but an echocardiogram demonstrated left ventricular hypertrophy. An MRI of the head was normal, however the caliber of the cord from the foramen magnum to the conus was significantly smaller than normal. There was no evidence of glucose intolerance. Levels of very long chain fatty acids, arylsulfatase A, phytanic acid, vitamin E, vitamin B12, cholesterol, triglycerides, hexosaminidase A&B were all normal, as was the cerebral spinal fluid.

Case 2
After diagnosis of 3:1, the other siblings were assessed. Patient 3:3 is the nine-year-old sister. Retrospectively, her
parents had noticed that she appeared clumsy over the preceding year. Her neurological exam revealed evidence of finger-nose and heel-shin ataxia and she had difficulty with tandem walking and single leg hopping. Her neurological examination was otherwise normal. There was no evidence of scoliosis or pes cavus. There was no evidence of glucose intolerance. An ECG showed normal sinus rhythm, the ST segments were strikingly flattened and inverted across the precordial leads. An echocardiogram demonstrated concentric left ventricular hypertrophy without outflow obstruction.

**Parents**

The siblings are the product of a nonconsanguinous relationship. Both father (2:2) and mother (2:6) are Malaysian and were clinically unaffected.

**Molecular genetic analysis**

Analysis of the GAA repeat in patients 3:1, 3:3, and their father (2:2) revealed single allele expansions of approximately 273 repeats, 256 repeats, and 306 repeats, respectively. All other alleles including those of the mother (2:6) were of normal length.

In patients 3:1 and 3:3 direct sequence analysis of the amplification products revealed a heterozygous TC deletion in the first exon on the unexpanded allele. The deletion of nucleotides 11 and 12 results in a frame shift and a predicted truncated frataxin protein. The TC deletion was also found to be present in the mother but to be absent in the father.

**Discussion**

This case is notable for four reasons: it reports a novel dinucleotide deletion in the *FRDA* gene, it is the first case of *FRDA1* described in a family of southeast Asian descent and demonstrates unusual phenotypic features as well as intrafamilial phenotypic variation.

The novel mutation is a TC deletion located in codon 4 of exon 1 resulting in the deletion of the 11th and 12th nucleotides, a frame shift and a predicted truncated protein. This is the first frame shift dinucleotide deletion reported in the *FRDA* gene. To date, six other mutations have been described in exon 1. Four involve the first codon and cause disruption of initiation of translation, and two involve either the insertion or deletion of a single nucleotide (C) at position 158 producing a truncated protein. Another mutation in intron 1 has been associated with a loss of frataxin on muscle biopsy. Friedrich ataxia has not been previously described in the Malaysian population. The GAA expansion in the *FRDA* gene underlying FRDA1 is thought to arise from a common origin. Expanded alleles (>66 GAA) and long normal alleles (< 60 GAA) have not been observed in Southeast Asian populations and, therefore, FRDA1 would be very rare. As GAA expansions are so uncommon it is not surprising that the affected individuals in this family have compound heterozygote mutations. The proband in this study presented with chorea, an atypical feature of FRDA1 not observed in the sibling, supporting the observation of atypical presentations in compound heterozygotes and intrafamilial phenotypic variability in FRDA1. Sudden progression of ataxia on the background of a febrile illness was observed in the proband. Whether this sudden progression is the result of genetic factors, environmental factors or a combination of the two remains to be determined. We hypothesize that an increase in mitochondria demand provoked by a febrile viral illness may have resulted in decompensation of the mitochondria, accelerated cell death and acute presentation of ataxic features.

**Acknowledgements**

This work was supported by a grant from the Canadian Institute of Health Research (CIHR) of Canada to T.P. Snutch. TPS is supported by a CIHR Senior Scientist Award.

**References**