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Somatic instability of the expanded GAA triplet-repeat sequence in Friedreich ataxia progresses throughout life

Irene De Biase [a], Astrid Rasmussen [a,b], Antonella Monticelli [c], Sahar Al-Mahdawi [d], Mark Pook [d], Sergio Cocozza [c], Sanjay I. Bidichandani [a,e,*]

[a] Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA
[b] Department of Neurogenetics and Molecular Biology, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, Mexico City, Mexico
[c] Department of Molecular and Cellular Pathology and IEOS, University of Naples "Federico II," Naples, Italy
[d] Department of Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge, UK
[e] Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

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Abstract

Friedreich ataxia (FRDA) patients are homozygous for expanded GAA triplet-repeat alleles in the *FXN* gene. Primary neurodegeneration involving the dorsal root ganglia (DRG) results in progressive ataxia. While it is known that DRG are inherently sensitive to frataxin deficiency, recent observations also indicate that they show age-dependent, further expansion of the GAA triplet-repeat mutation. Whether somatic instability is progressive has not been systematically investigated in FRDA patients. "Small-pool" PCR analysis of ~2300 individual molecules from tissues of an 18-week fetus homozygous for expanded alleles revealed very low levels of instability compared with adult-derived tissues (4.2% versus 30.6%, $p<0.0001$). Mutation load in blood samples from multiple patients and carriers increased significantly with age, ranging from 7.5% at 18-weeks gestation to 78.7% at 49 years of age ($R=0.91$; $p=0.0001$). Therefore, somatic instability in FRDA occurs mostly after early embryonic development and progresses throughout life, lending further support to the role of postnatal somatic instability in disease pathogenesis.

Keywords: Friedreich ataxia; GAA triplet repeat; Instability; Progressive; Embryonic development; Somatic instability

Friedreich ataxia (FRDA) is characterized by slowly progressive sensory ataxia with onset usually before the age of 25 years, typically associated with absent tendon reflexes, loss of position and vibration senses, dysarthria, and extensor plantar responses [1,2]. These neurological manifestations result from primary degeneration of the dorsal root ganglia (DRG), associated with axonal degeneration in the posterior columns, spinocerebellar tracts, and corticospinal tracts of the spinal cord and large myelinated fibers in the peripheral nerves [3]. Although the rate of progression is variable, the mean age of loss of ambulation is 25 years, and patients often die in their fourth or fifth decade.

FRDA is inherited as an autosomal recessive trait and the vast majority of patients are homozygous for expanded GAA triplet-repeat (GAA-TR) sequences (E alleles) in intron 1 of the *FXN* gene [4]. E alleles interfere with *FXN* gene transcription in a length-dependent manner [5,6] and result in proportional deficiency of frataxin [7]. Most E alleles contain 600–1200 triplets; however, the spectrum of disease-causing alleles ranges from 66 to 1700 triplets. Disease severity and rate of progression correlate with the length of E alleles [8–12], and carriers of shorter alleles (fewer than 500 triplets) commonly show slower than average disease progression.

DRG neurons are especially sensitive to frataxin deficiency, as was demonstrated by their selective loss in neuronal-specific, conditional, frataxin-knockout mice [13]. Upon analysis of

* Corresponding author. Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA. Fax: +1 405 271 3910.
E-mail address: Sanjay-Bidichandani@ouhsc.edu (S.I. Bidichandani).

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nervous system tissue from multiple autopsies of FRDA patients we recently found that there was a progressive accumulation of large expansions in the DRG [14]. This phenomenon was not seen in other tissues and may serve as a further modulator of the progressive DRG-specific pathology seen in FRDA patients. Furthermore, in patients who are compound heterozygous for a conventional E allele and a borderline GAA-TR allele (44–66 triplets), somatic instability of the borderline allele was required for the development of the FRDA phenotype [15].

All of the above observations suggest that somatic instability of E alleles in FRDA is likely to be progressive. However, this has not been systematically investigated in patient-derived tissues. Given that DNA replication is able to induce instability of GAA-TR sequences in simple model systems [16,17], one would predict that the burst of cellular proliferation occurring in early embryonic development would be associated with enhanced somatic instability. Here we show that somatic instability of E alleles is progressive; it occurs mostly after early embryonic development and continues throughout life. These observations have important implications for the mechanism of somatic instability in FRDA and lend further support to the role of progressive somatic instability in disease pathogenesis.

Results

Somatic instability in FRDA occurs mainly after early embryonic development

E alleles are commonly detected by conventional PCR or genomic Southern blot analyses, which typically use 0.06–6 μg genomic DNA, thus simultaneously analyzing 10^4–10^6 cells. These assays, commonly used for molecular diagnosis of patients, estimate the repeat length of only the "constitutional" or "most common" allele (E allele). However, using "small-pool" PCR (SP-PCR), a sensitive assay that involves the use of very low levels of genomic DNA (typically 6–600 pg), it is possible to detect GAA triplet repeats in individual *FXN* molecules (genes) [15,18]. SP-PCR analysis of DNA from tissues of an 18-week fetus, homozygous for E alleles, revealed a remarkably low level of instability in all tissues tested (Fig. 1A; Table 1). Conventional PCR showed homozygous E alleles of the same size (620/620 triplets in blood), which therefore allowed accurate determination of the magnitude of allelic size variation. Analysis of 2320 individual *FXN* molecules from multiple tissues revealed a cumulative mutation load of 4.2% (Table 1). Compared to a 24-year-old FRDA patient homozygous for two E alleles of the same size (943/943 by conventional PCR analysis), fetal tissues had 7.3-fold less instability than the corresponding adult-derived tissues (4.2% versus 30.6%, $p<0.0001$; Figs. 1B and 1C; Table 1). The overall mutation load is skewed in favor of contractions (3- to 4-fold more contractions) in both adult and fetal tissues (Table 1). Analysis of an additional 572 molecules from a primary fibroblast cell line derived from the fetus also revealed similarly low levels of instability (2.1%). Blood and cerebellum showed the highest mutation load. Interestingly, these tissues also revealed the highest frequency of large mutations (±20% of the progenitor allele length), which amounted to 15 and 31% of all mutations in blood and cerebellum, respectively (examples are indicated by asterisks in Fig. 1A). Together, these data indicate that the relatively high level of somatic instability observed in adult-derived tissues develops after early embryonic development.

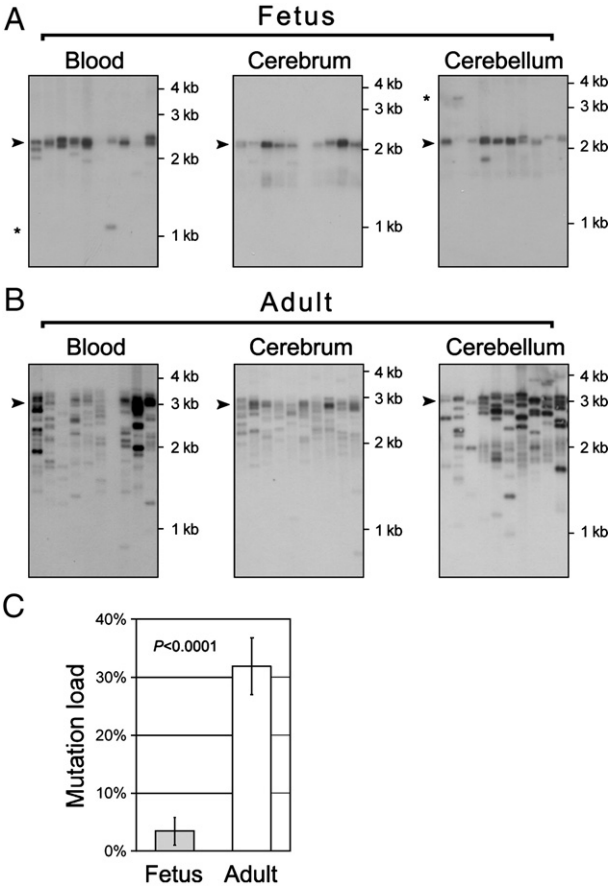

Fig. 1. SP-PCR showing significantly low levels of somatic instability in fetal tissues. (A and B) Southern blots showing limited somatic variability in representative tissues of (A) an 18-week fetus and (B) a 24-year-old adult patient, each of whom are homozygous for E alleles. Each lane contains small pools of 12–20 individual *FXN* molecules. Arrowheads indicate the position of the constitutional allele determined by conventional PCR. Asterisks indicate rare instances of large expansions/contractions despite the overall low level of somatic instability. DNA size markers are shown as dashes along the right margin, which indicate the relative positions of 1 (182 triplets), 2 (515 triplets), 3 (849 triplets), and 4 kb (1182 triplets). (C) Cumulative average mutation load of all fetal versus adult (patient A-1) tissues analyzed showing a highly significant, 7.3-fold lower level of somatic instability in fetal tissues compared with adult tissues. Error bars depict ±2 SEM.

It is known that non-GAA interruptions within an E allele can stabilize the sequence in somatic cells in vivo [17]. To rule out the possibility that similar intra-allelic interruptions may be the reason for the stability seen in fetal tissues, we analyzed blood samples from the parents of the fetus to test if the inherited E alleles were capable of developing somatic instability. In contrast to the fetal sample, SP-PCR analysis of