

Fragile X–Associated Tremor/Ataxia Syndrome in Sisters Related to X-Inactivation

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Fragile X tremor/ataxia syndrome (FXTAS) is a recently described condition consisting of tremor, ataxia, parkinsonism, and executive dysfunction, presenting predominantly in male carriers of a fragile X mental retardation 1 premutation. In this report, we present premutation carrier sisters in whom severity of clinical signs correlated with a molecular pattern of X-inactivation favoring higher expression of the premutation allele. In these women with a common genetic background, we suggest that symptom severity may be dictated by X-inactivation, and thus a higher percentage of cells producing the premutation-containing mRNA result in increased toxicity and disease.

Ann Neurol 2005;57:144–147

Fragile X syndrome (FXS) results from a trinucleotide (CGG) repeat expansion mutation in the promoter region of the fragile X mental retardation 1 (FMR1) gene.¹ The full mutation (>200 CGG repeats) is associated with methylation and transcriptional silencing of FMR1, absence of FMR1 protein (FMRP),^{2,3} and the clinical syndrome of mental retardation. The premutation (approximately 55–200 repeats) predisposes an individual to have children or grandchildren with FXS but is not associated with hypermethylation of FMR1, transcriptional silencing, absence of FMRP, or typical FXS.^{3,4} Individuals with the premutation may display some of the physical features of FXS,⁵ and, although overall cognitive disability is not evident,^{6,7} behavioral issues seen in FXS, such as anxiety and social phobia, are more prevalent in premutation carriers than in control groups.^{8,9}

A subgroup of older male premutation carriers de-

velop a progressive neurological condition, termed *fragile X-associated tremor/ataxia syndrome* (FXTAS) and characterized predominantly by multidimensional tremor, gait and limb ataxia, and parkinsonian symptoms.^{10–13} More variable features include neuropathy, autonomic dysfunction, and psychiatric and executive function deficits, with progressive cognitive deterioration in some individuals. Magnetic resonance imaging findings in affected individuals include diffuse brain atrophy with evidence of white matter disease and characteristic high-signal lesions in the middle cerebellar peduncle.¹⁴ Associated neuropathological findings include eosinophilic intranuclear inclusions in neurons and glia and spongiform change in the white matter with mild axonal and myelin loss.¹⁵ Pathological changes are thought to result from cellular toxicity related to elevated levels of CGG repeat-containing FMR1 mRNA, which have been observed in premutation carriers.^{10–15} FXTAS is not seen in individuals with FXS and a full mutation because the FMR1 mRNA is generally reduced or absent in these individuals because of transcriptional silencing.

FXTAS initially was described only in male premutation carriers, and two studies have shown no significant differences in neurological function between groups of 50+-year-old premutation carrier female subjects and normal female controls.^{12,14} A recent report described five women with FXTAS, although their symptoms resembled the milder end of the spectrum seen in male subjects.¹⁶ It is thought that women are far less likely to develop FXTAS, and, when they do, they develop more subtle disease because of the diluting effect of the normal X chromosome. If this explanation is correct, women with nonrandom X-inactivation who express predominantly the X chromosome with the FMR1 premutation should demonstrate significant FXTAS signs. In support of this hypothesis, we present two FMR1 premutation carrier sisters discordant for FXTAS symptom severity and X-inactivation patterns.

Subjects and Methods

As part of a population study characterizing FXTAS symptoms in fragile X premutation carriers, two biological sisters, one with two children with FXS, volunteered for neurological examination and genetic testing and signed informed consent. Both sisters underwent a structured videotaped neurological examination as described previously,¹¹ from which tremor could be rated on the Clinical Rating Scale for Tremor (CRST), ataxia on the International Co-operative Ataxia Rating Scale (ICARS), and Parkinsonism on the Unified Parkinson's Disease Rating Scale (UPDRS). Ratings of videotapes were performed by an investigator (C.G.G.) blinded to any information on genetic status. FMR1 DNA analysis was performed according to a polymerase chain reaction assay described previously.¹⁷ This assay gives specific allele sizes (number of CGG repeats) and differentiates indi-

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Received Aug 9, 2004, and in revised form Oct 19. Accepted for publication Oct 20, 2004.

Published online Dec 27, 2004, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20360

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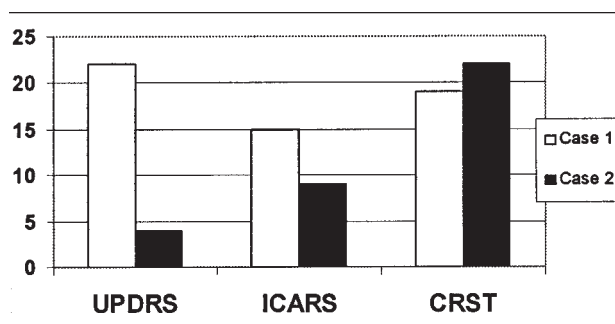


Fig 1. Comparison of overall scores for the sisters on the Unified Parkinson's Disease Rating Scale (UPDRS; parkinsonism), International Co-operative Ataxia Rating Scale (ICARS; ataxia), and Clinical Rating Scale for Tremor (CRST; tremor).

viduals with a normal *FMR1* gene (<55 repeats) from those with a premutation (55–200 repeats). Southern blot analysis of *FMR1* with probe StB12.3, after *EcoRI/EagI* digestion,¹⁸ was also done to assess methylation status of the premutation and normal alleles to give an estimate of X-inactivation ratios. Activation ratios for the premutation containing allele were quantified by densitometric scanning of bands corresponding to unmethylated (active) DNA on the Southern blot. Activation ratio for the premutation allele was calculated as signal from the premutation-containing band divided by total signal in both the premutation-containing and normal bands.

Results

Clinical Descriptions

CASE 1. The younger sister exhibited normal development, cognition, and function until age 75 years. At age 75 years, she noted a postural tremor which progressed to rest tremor. A neurologist diagnosed Parkinson's disease and instituted treatment with L-dopa, which did not improve symptoms. A second neurologist diagnosed essential tremor and instituted primidone treatment, which resulted in substantial improvement in the tremor. At age 76 years, she noted gait and balance problems and subsequently fell and broke her hip. She also noted problems with stress incontinence. Neurological examination at age 77 years while receiving primidone treatment showed significant postural tremor, mild rest tremor, a slight increase in upper extremity tone, hyperreflexia, distal sensory loss to pin and vibration in the lower extremities, ataxia with mildly broad-based gait, inability to do tandem gait, and difficulty with turns. There was mild disability from tremor, particularly with writing and from gait problems. Standardized motor scores were: UPDRS = 22; ICARS = 15; CRST = 19 (Fig 1). A magnetic resonance image of the brain showed abnormal high signal diffusely throughout the subcortical white matter on T2 and flair images, some increased signal in the cerebellar white matter including the middle cerebellar

peduncle, and atrophy involving gray and white matter (Fig 2). The medical history was significant only for rheumatic fever. This sister had never married and did not have children.

CASE 2. The older sister described normal development, cognition, and function throughout life. At age 81 years, she noted onset of intermittent postural tremor, mostly when lifting things. She had no gait or balance problems. Neurological examination at age 83 years showed mild postural tremor in the hands, more on the right, distal sensory loss to pin, and mild hyperreflexia in the lower extremities. There was no disability from tremor including no problems with writing. Standardized motor scores were: UPDRS = 4; ICARS = 9; CRST = 22 (see Fig 1). Neuroimaging had not been done because of the minimal nature of the symptoms. The medical history was significant for atrial fibrillation and hypertension. This individual had two children with FXS and cognitive disability. The father of the two sisters was said to have had a severe tremor starting in his 50s and gait problems that were diagnosed as Parkinson's disease. The father's brother also had a severe tremor that developed and worsened in his 50s and 60s. Other women in the family were not known to have had neurological symptoms, although there were no other biological sisters.

DNA Analyses

DNA analyses showed that both sisters were *FMR1* premutation carriers with 69 (Subject 1) and 83 (Subject 2) CGG repeats. Both sisters had nonrandom X-inactivation (Fig 3) with the active X chromosome being predominantly the premutation X in Subject 1 and the normal X in Subject 2. The activation ratio for the premutation allele (fraction of cells with the premutation X active) was 0.71 for Subject 1 and 0.22 for Subject 2.

Discussion

The sisters described are discordant for symptoms of FXTAS. Both individuals had tremor but Subject 1 had more generalized parkinsonian and ataxic symptoms leading to more disability despite her younger age. Also, Subject 1 likely had artificially low tremor scores because of the use of primidone; she reported that her tremor was much milder and less impairing since initiating the medication. Her clinical picture and disability meets criteria for "definite FXTAS,"¹² whereas her sister (Subject 2) was much less affected. Symptom severity in these sisters corresponded to a strong nonrandom X-inactivation pattern: the sister whose normal allele was predominantly active had only mild tremor, whereas the sister whose premutation X was predominantly active had the more severe syndrome. Both women, however, had milder symptoms

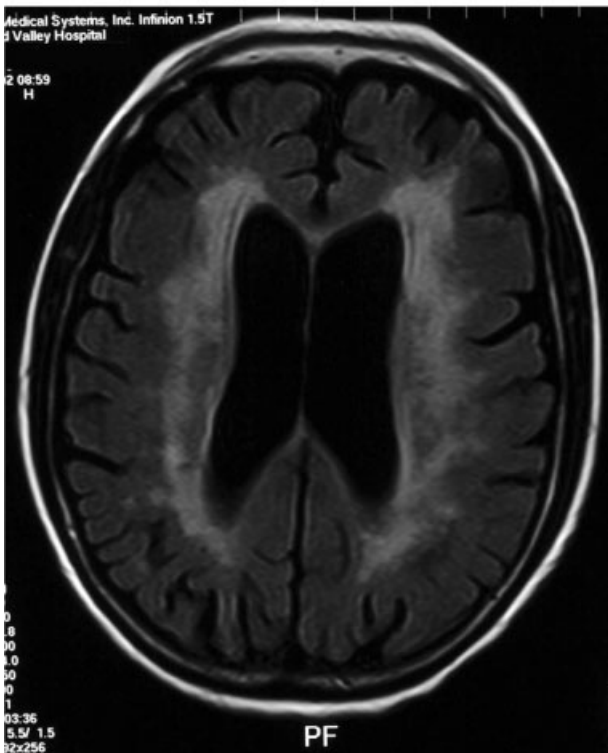
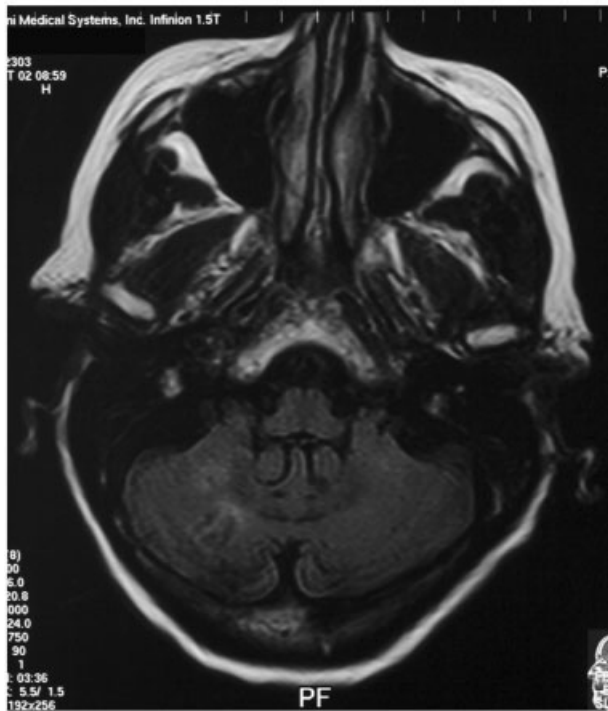


Fig 2. Cranial magnetic resonance image of Subject 1 was performed using a 1.5T magnet (Marconi Medical Systems, UK). Fluid-attenuated inversion recovery images shown were obtained utilizing a TR, 1,800 milliseconds and TE, 96-millisecond weighted sequence and demonstrated high signal in the white matter of the cerebral hemispheres (bottom) and cerebellum (top), and gray and white matter atrophy in the cerebral hemispheres (bottom).

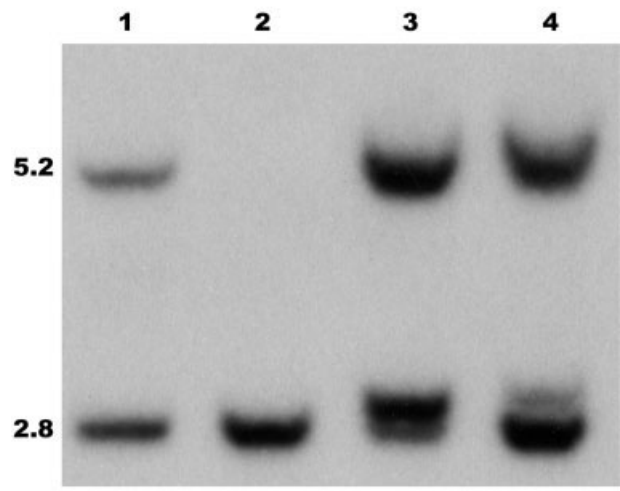


Fig 3. Southern blot analysis of fragile X mental retardation 1 (FMR1) methylation pattern in DNA from a normal female control (lane 1), normal male control (lane 2), Subject 1 (lane 3), and Subject 2 (lane 4). The 5.2kb band represents methylated FMR1 signal from inactive X chromosome in normal female subjects, whereas the 2.8kb band represents unmethylated active X chromosome in normal female and male subjects. The roughly 3kb extra band in Subjects 1 and 2 represents the premutation. Note that for Subject 1 most of the active (unmethylated) X signal is in the premutation band, whereas for Subject 2 most of the signal is in the normal band.

than typical male subjects affected with FXTAS, and, had much milder symptoms than those described in the men in the family in the prior generation, supporting the concept that women develop FXTAS with milder features than men. Furthermore, neither of these women had dementia, a feature reported in approximately 20% of men with FXTAS.¹³ Dementia was not seen in the five female FXTAS subjects presented previously.¹⁶

FXTAS is believed to result from a gain-of-function mechanism in which accumulated mRNA containing the expanded CGG repeat exerts a toxic effect by sequestering and perturbing function of nuclear proteins.¹⁰⁻¹⁵ Studies in *Drosophila* expressing an expanded CGG repeat have supported the hypothesized RNA toxicity mechanism.¹⁹ It has been proposed that FXTAS is milder and less prevalent in female FMR1 premutation carriers because of a protective effect due to expression of the normal allele in a proportion of neurons, or possibly a palliative effect of estrogen acting on the disease mechanism.¹⁶

Our clinical-molecular correlation supports the concept of a protective effect from expression of the allele on the normal X chromosome. Because FXTAS penetrance and severity is quite variable even in men, it is likely that genetic background has an influence on expression. Overall, the family presented here may have a

genetic background with particular susceptibility to manifestations of FXTAS, because the father and his brother also had symptoms characteristic of FXTAS. Because the women described in this report are sisters and thus have a relatively constant genetic background, they present a unique opportunity to observe the effects of variation in X-inactivation more clearly than can be done in groups of unrelated individuals. Identification of identical twin carriers discordant for X-inactivation pattern would provide the ideal setting for studies of the effects of X-inactivation on FXTAS manifestations.

Correlation between FXTAS severity and X-inactivation patterns in women may be obscured in cross-sectional studies because of differences in X-inactivation patterns in blood and brain for some individuals, differences in genetic background, and possible hormonal effects. The sisters presented in this report support the concept that manifestation of FXTAS in women is likely to relate at least in part to X-inactivation patterns. Because the FMR1 premutation is present in 1 in 250 women²⁰ in the population, this report also underscores the need to consider FXTAS as a possible genetic cause of undefined syndromes of tremor or ataxia in older women.

This work was supported by grants from the NIH (National Institute of Neurological Disorders and Stroke, NS43532, E.B.K.) and the Spastic Paralysis Research Foundation of the Illinois-Eastern Iowa District of Kiwanis International (E.B.K.).

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