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FXTAS IN AN UNMETHYLATED MOSAIC MALE WITH FRAGILE X SYNDROME FROM CHILE

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Abstract

Fragile X Syndrome is caused by expansion of CGG repeats to >200 in 5'-untranslated region of fragile X mental retardation 1 (*FMR1*) gene [full mutation (FM)]. Carriers of an *FMR1* repeat expansion in premutation range (55–200 CGG repeats) often develop a syndrome similar to parkinsonism, designated fragile X-associated tremor/ataxia syndrome (FXTAS). Neurological signs of FXTAS, parkinsonism and rapid onset of cognitive decline have not been reported in individuals with an unmethylated FM. We report a Chilean family affected with FXS, inherited from a parent carrier of an *FMR1* unmethylated full mosaic allele, who presented with a fast progressing FXTAS. Our case suggests that the definition of FXTAS may need to be broadened to not only include those with a premutation and to include in addition those with an expanded allele in FM range with a lack of methylation leading to elevated *FMR1*-mRNA expression levels and subsequent RNA toxicity.

Keywords

FXTAS; Fragile X; unmethylated full mutation; parkinsonism

INTRODUCTION

Fragile X Syndrome (FXS) (OMIM# 300624) is caused by a CGG repeat expansion (>200 CGG repeats) in the 5' UTR of the *FMR1* gene [full mutation (FM)] leading to hypermethylation of the promotor region, gene silencing and a consequent deficit of the protein, FMRP [1].

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The absence of FMRP causes intellectual disabilities (ID) and behavioral problems; however the degree of severity of cognitive disability in FXS patients is not associated with the size of the FM allele but does vary with methylation status [2]. Male patients with an unmethylated FM typically have FMRP levels lower than normal and they are high functioning with an IQ > 70 in some cases [2].

Premutation (PM) alleles (55–200 CGG repeats) occur in 1 in 130–250 women and in 1 in 250–810 men [3]. Besides being unstable during transmission to the next generation they are associated with fragile X-associated tremor/ataxia syndrome (FXTAS, OMIM# 300623), which occur in more than 40% of premutation males after the age of 60. Characterization of repeat length and methylation status for each allele is a critical component of understanding fragile X-associated disorders [4].

Clinical features of FXTAS include progressive intention tremor, gait ataxia, parkinsonism, autonomic dysfunction, neuropathy, executive function and cognitive decline leading to dementia [4–6]. The clinical symptoms and progression vary among subjects with FXTAS and life expectancy is between 5 to 25 years after the onset of the symptoms [7].

Cerebral Magnetic Resonance Imaging (MRI) in patients with FXTAS show global brain atrophy, enlargement of ventricular volume, white matter disease and heightened signal intensity with lesion in the middle cerebellar peduncles (MCPs) [8–10].

Clinical and molecular evidence argue for an mRNA toxicity based mechanism leading to FXTAS. *FMRI* mRNA increases over the normal levels in premutation individuals [11]. The “high functioning” men with unmethylated FM alleles, also shown elevations on *FMRI* mRNA transcripts of 3.5 to 4.5 fold in comparison to normal controls [2, 11–13].

FXTAS has been defined as a premutation disorder [8,14] but now exceptions to this have been reported. Recently patients with the gray zone in *FMRI* (45 to 54 CGG repeats) and FXTAS have been described [15, 16]. In addition, a 65 yo male with an unmethylated full mutation with an MCP sign, symptoms of FXTAS and cognitive decline has been described [17]. More recently, Pretto et al., 2013[18] have reported symptoms, pathological and molecular evidence of FXTAS in a males full mutation size mosaic.

Finally, the presence of the *FMRI* transcript, reduced FMRP and the presence of intranuclear inclusions have been described in three individuals with FXS [19,20] and in a mouse model with an unmethylated *FMRI* allele in the FM range [19]. These studies suggest that both above and below the premutation range unmethylated alleles can lead to elevated *FMRI* mRNA levels and FXTAS [21, 22].

Here, we present a case of FXTAS in a male with an unmethylated PM/FM size mosaicism. This case and previously reported similar cases [17,18] suggest that the pathogenic mechanism thought to underlie this disorder is not only seen in premutation carriers as was originally proposed [8].

METHODS

Case report

A Caucasian male, 70 yo, retired commercial trader, was identified through cascade testing in a fragile X family. The patient had no pre-morbid mental health problems and he had normal cognitive function. He studied through grade twelve. He was a good dancer and frequently he ran 3–4 times a week up to 64 yo. He has two daughters with the premutation and three grandchildren with FXS. The family pedigree is depicted in Figure 1. The grandson, patient III-1, has facial features and behaviors typical of FXS. Weschler International Performance Scale (WIPS) demonstrated a moderate range of ID (IQ of 44). His sister, patient III-2 with FXS has milder cognitive deficits, attention deficit, social anxiety, math disability, and depression. While the other grandson, patient III-3 with FXS has anxiety and hyperactivity, prominent ears and joint laxity. WIPS testing demonstrated a moderate range of ID (IQ of 40). All the family members were informed about the study protocol before signing a written consent to participate in this study.

At age 64, after the surgery of a pharynx nodule, he started having coordination and balance problems and his footsteps shortened. Thyroid and B12 levels were normal. No cognitive deterioration was identified, and no specific cognitive tests were performed at that time. Eight months later he was reevaluated and he presented with executive function deficits, although he denied memory and mood problems.

Subsequently, in the course of 2 years, he developed intention tremor and required assistance for walking. He also had mood liability, hypersomnolence, poor abstract reasoning and severe difficulties in mental calculation. Neuropsychological tests showed immediate memory problems, disorientation in time and space and constructional apraxia.

At age 68, he showed several features of FXTAS, including ataxia, left sided tremor and cramps in both feet. Neurological examination revealed hypo- and bradykinesia, intentional and positional tremor, postural instability, and mild rigidity; all symptoms of parkinsonism. Urinary incontinence and signs of peripheral neuropathy were also present.

He also had a history of diabetes mellitus type II, hypertension and dyslipidemia that were treated with nutritional and pharmacological treatment. He has no history of cerebral stroke, drug or alcohol abuse, or neurotoxin exposure.

Neuropsychological tests revealed a global cognitive performance deficit (Table 1), deficits in memory, verbal fluency, and visuospatial abilities, poor sustained attention, motor difficulties, altered capacity of cognitive flexibility, abstract thoughts and conceptual elaboration, and important difficulties in direct and differed recall. All cognitive functions declined on a fast temporal course (over one year).

Recently, (at age 70) he was treated with L-Dopa, showing slight motor improvements, especially in rigidity.

Transcranial ultrasound presented pathological echogenicity of substantia nigra up to 0.26 cm², no significant echogenicity of lenticular nuclei and conserved echogenicity of mesencephalon nuclei and periaqueductal grey substance.

Brain MRIs demonstrated severe global atrophy especially in the supratentorial region in addition to bilateral hippocampal atrophy. White matter disease was seen throughout sub-cortical and periventricular regions (Figure 2). The MCP sign was not present in this case.

Molecular Studies

Genomic DNA was isolated from the peripheral blood lymphocytes using standard methodology. Repeat size and methylation status were determined using Southern blot and PCR as previously described [23, 24]. Analysis and calculation of repeat size for Southern and PCR analysis were carried out using an Alpha Innotech FluorChem 8800 Image Detection System.

FMRI mRNA quantifications were performed as described earlier [11] with minor modifications. Briefly, total RNA was isolated from 3 ml of peripheral blood leucocytes using Tempus Blood RNA tubes (Applied Biosystems, Foster City, California, USA). All quantifications of *FMRI* mRNA were performed using a 7900 Sequence Detector (Applied Biosystems) mRNA levels were scored relative to the reference β -glucuronidase gene (β Gus).

FMRP expression levels were determined by Western blot as previously described [25].

RESULTS

Molecular measures show that the patient with FXTAS (I-1) is an unmethylated full mutation size mosaic (CGG repeat size ranging from 180 to 410). Although the *FMRI* mRNA level was 7-fold above the normal average, he showed reduced FMRP expression levels (38% of normal) in agreement with previous reports (11, 13). All the molecular measurements were performed in blood cells and no other tissue were studied thus potential differences in *FMRI* gene expression levels in other tissue, including neural cells, has to be considered.

The molecular testing results on the family are summarized in Figure 1.

DISCUSSION

We have identified a male with a fully unmethylated full mutation allele ranging from 180 to 410 CGG repeats, with 7-fold over expression of *FMRI* mRNA and a reduced level of FMRP. Although he does not have a history of ID he presents with late onset neurological symptoms consistent with the diagnosis of FXTAS. Clinical features are ataxia, mild incoordination, rapid cognitive decline/dysexecutive syndrome, and some elements of parkinsonism with increased tone. MRI changes include global brain atrophy with massive white matter hyperintensities. These clinical features fulfill criteria for definite FXTAS [4, 8, 9].

This is the second case reported in the literature of an unmethylated full mutation male with FXTAS and the first Chilean case. Under-diagnosis of FXTAS is possibly due to the limited knowledge about this syndrome and also because of the clinical variability. Recognizing FXTAS in patients from various ethnic backgrounds and international locations would contribute to our understanding of the phenotypic variation of this disease [26].

The rare occurrence of FXTAS among the carriers of an unmethylated FM allele might be explained by the lowered level of FMRP combined with elevated *FMR1* mRNA levels. Previous reports of those with a completely unmethylated full mutation have had similar high elevations of mRNA unless partial methylation is seen [2]. A recently reported case of an unmethylated full mutation allele with 3.5-fold increased *FMR1* mRNA levels and FXTAS provides strong evidence that there are some cases with large CGG expansions coupled by a considerable elevation of the *FMR1* transcript, which can lead to FXTAS [17]. In that case [17], a history of alcohol and drug abuse was considered to be a partial contributor to the early onset and rapidity of progression of neurological and cognitive impairments of FXTAS. In our patient, although there was no drug or alcohol abuse, perhaps diabetes, the effect of the anaesthesia after surgery, secondary medical conditions or other environmental exposures could have also contribute to the clinical features observed in this case [27]. In a recent report [18] intranuclear inclusions, the neuropathological hallmark of FXTAS, were identified, in addition to white matter disease, intermittent tremor and parkinsonism, in a full mutation size mosaic male.

It is important to consider that the actively transcribed *FMR1* CGG expanded allele is much larger in the unmethylated FM than in PM carriers. Rare intranuclear inclusions, have been observed in three full mutation adults expressing very small amounts of *FMR1* mRNA and FMRP [17]. As FXTAS has been linked to an RNA toxicity mechanism due to the excess of CGG repeats on transcribed mRNA molecules, the increased mRNA levels in this patient would predispose him to RNA toxicity.

In conclusion, this report documents that FXTAS may also occur in those with an unmethylated full mutation. As individuals with a gray zone allele have also been reported to be affected by FXTAS [15, 16] we propose that the definition of FXTAS should be revised to not only include those with a premutation, but in addition those with an expanded allele which size and lack of methylation leads to RNA toxicity.

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Subject	III-1	II-1	III-2	I-1 (FXTAS)	II-2	III-3
Category	Full mutation	Premutation	Full mutation, size mosaic	Unmethylated, full mutation size mosaic	Premutation	Full mutation, size mosaic
CGG Repeat Number	505, 637	29, 114	282, 386, 428, 549, 596 (30, 135*)	180-410	29, 112, 128	491, 620, 704 (124, 190)
% Meth				0%		85%
FMR1 mRNA	0.01 ± 0.001	2.35 ± 0.06	1.16 ± 0.03	7.65 ± 0.98	4.45 ± 0.22	0.95 ± 0.09
FMRP Expression Level	0.00	0.90	1.09	0.38	0.89	0.31

* (CGG)n detectable only by PCR

Figure 1.
Genealogy of FXTAS patient family and results of molecular outcomes of the FXTAS patient and his affected relatives.

Figure 2. Axial fluid-attenuated inversion recovery (FLAIR) MRI showing extended compromise of white matter (A) and bilateral hippocampus atrophy (B).

Table 1

Standard cognitive test data

Test	Score/Reference
ACE-R	29 / 100
MMSE	10 / 30
WMS-III	7 / 40
GBT	16 / 48
FRWT	13 / 25
FAB	6 / 18
WCST	1 / 6 categories

ACE-R, Addenbroke's cognitive examination;

MMSE, Mini Mental State Examination;

WMS-III, Wechsler Memory Scale-3rd edition;

GBT, Grober and Buschke Test;

FRWT, Face Recognition of Warrington Test;

FAB, Frontal Assessment Battery;

WCST, Winsconsin Card Sorting Test