Mol Neurobiol (2007) 35:324–328
DOI 10.1007/s12035-007-0020-3

FXTAS in Spanish Patients with Ataxia: Support for Female *FMR1* Premutation Screening

Laia Rodriguez-Revenga · Beatriz Gómez-Anson · Esteban Muñoz · Dolores Jiménez · Monica Santos · Mar Tintoré · Gisela Martín · Luis Brieva · Montserrat Milà

Received: 25 September 2006 / Accepted: 8 December 2006 / Published online: 31 July 2007

Abstract Fragile X-associated tremor/ataxia syndrome (FXTAS) is a newly described disorder characterized by progressive action tremor and ataxia that occurs in premutation carriers of the *FMR1* gene. The incidence of *FMR1* premutated carriers in the general population is relatively high, and therefore FXTAS might explain a considerable number of sporadic, late-onset ataxias. To better establish the prevalence of FXTAS among undiagnosed Spanish patients with ataxia, we have performed a *FMR1* premutation screening. Our results evidenced three individuals carrying premutated alleles, giving an estimated FXTAS prevalence of 1.95% among patients with late-onset ataxia (1.15% for males and 3% for females). Molecular characterization of premutation carriers evidences lower fragile X mental retardation 1 protein levels and increased *FMR1* mRNA levels. Clinical and neuroimaging findings support FXTAS diagnosis in these patients. Because of the high prevalence of *FMR1* premutation in the general population, the description and characterization of the FXTAS syndrome is of great interest as it may represent one of the more common monogenic causes of ataxia, tremor, and dementia. The results obtained in this study demonstrate that FXTAS should be incorporated to spinocerebellar ataxia genetic screening protocols. Early diagnosis of these patients benefits not only them but also the rest of the family that should be advised for the fragile X syndrome.

Keywords Fragile X-associated tremor/ataxia syndrome · FXTAS · *FMR1* premutation · Dominant spinocerebellar ataxia · Genetic screening

L. Rodriguez-Revenga · D. Jiménez · M. Santos · M. Milà (✉)
Biochemistry and Molecular Genetics Department,
Hospital Clínic,
Barcelona, Spain
e-mail: mmila@clinic.ub.es

L. Rodriguez-Revenga · B. Gómez-Anson · E. Muñoz · M. Santos · M. Milà
IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer),
Barcelona, Spain

B. Gómez-Anson
Radiology Department, CDI, Hospital Clínic,
Barcelona, Spain

E. Muñoz
Neurology Service, Hospital Clínic,
Barcelona, Spain

M. Tintoré
Neurology Service, Hospital Vall d'Hebrón,
Barcelona, Spain

G. Martín
Neurology Service, Hospital Verge de la Cinta,
Tortosa, Spain

L. Brieva
Neurology Service, Hospital Arnau de Vilanova,
Lleida, Spain

Introduction

Fragile X syndrome (FXS) (MIM 309550) is the commonest familial form of inherited mental retardation. Molecular defect is an expansion of the CGG trinucleotide repeats in the 5′ untranslated region of the *FMR1* gene that is inherited in unstable fashion in FXS families [1]. In the general

population, individuals carry 6–52 repeats and the triplet number is usually stably transmitted. Individuals with alleles between 55 and 200 CGG repeats are called premutated carriers. This range of repeats is unstable through transmission to the next generation and they tend to expand. Affected individuals carry alleles with more than 200 repeats (full mutation). Under these circumstances, the *FMR1* gene becomes silenced, leading to the absence of the fragile X mental retardation 1 protein (FMRP) [2]. Fragile X-associated tremor/ataxia syndrome (FXTAS) is a newly described disorder that occurs among premutation carriers of *FMR1* gene. It is characterized by progressive intention tremor, ataxia, and hyperintensities of the middle cerebellar peduncles on T2-weighted magnetic resonance imaging (MRI) [3]. FXTAS was first described in families with children affected with FXS with a suggested age-related penetrance higher than 75% in male carriers 80 years of age or older [4]. Studies of the expression of *FMR1* have provided conclusive evidence that CGG expansions in the premutation range cause dysregulation of gene expression, but it is surprising to note that the reduced FMRP levels are not because of reductions in gene transcription. Elevated mRNA levels have been detected among premutated carriers, particularly in males, proposing a compensatory mechanism to overcome the mild protein deficit [5]. The incidence of *FMR1* premutated carriers in the general population is relatively high, and it has been estimated at 1:411 females and 1:1,233 males in the Spanish population [6]. Thus, FXTAS might explain a considerable number of sporadic, late-onset ataxias. To better establish the frequency of FXTAS in those patients, we have performed a screening among 154 unrelated individuals referred to our laboratory for genetic testing of spinocerebellar ataxia (SCA). To our knowledge, this is the first study performed in the Spanish population, and the results obtained demonstrate that FXTAS should be incorporated to SCA genetic screening protocols.

Materials and Methods

Subjects

We selected 154 unrelated and sporadic patients (87 males and 67 females) suspected of having SCA. All patients tested negative for the SCA 1, 2, 3, 6, 7, 8, and DRPLA, and they all were older than 45 years of age. Parkinsonian features were excluded in all of them.

Molecular Analysis

Molecular analysis of the *FMR1* CGG repeat region was performed by PCR and/or Southern blotting. PCR amplification was carried out using fluorescent-labeled primers f and c previously described by Fu et al. [1]. The reaction product was analyzed on an ABI310 (Applied Biosystems, Foster City, CA, USA). Double restriction digestion with *Eco*RI and *Eag*I and Southern blotting with probe Stb12.3 was used for women in whom only one allele was detected by PCR. Allele sizes were estimated using the Seqaid II™ version 3.81 program. All data presented in this study have been performed and evaluated in our center, following the same procedures, avoiding possible between-center variability.

FMR1 mRNA Detection

Total RNA was isolated from 2.5 ml of peripheral blood by standard method (Purescript kits, Gentra). cDNA synthesis reaction was performed by using the high-capacity cDNA kit (Appield Biosystems, Foster City, CA, USA) and following the manufacturer's protocol. Three concentrations of total RNA (400, 200, and 100 ng per 50 μl reaction) were used for each sample to ensure linearity of the RT-PCR response. Quantitative (fluorescence) PCR for determination of relative *FMR1* mRNA levels were performed with the use of TaqMan probes and Universal PCR master mix obtained from Applied Biosystems (Foster City, CA, USA). *FMR1* mRNA levels are relative to control (*GUS*) mRNA levels. For each sample, quantitative PCR reactions were performed in duplicate with the use of a standard RNA sample obtained from a sex-matched individual harboring a normal CGG repeat range.

FMRP Detection

FMRP expression in hair root was determined using the immunohistochemical approach of Willemsen et al. [7]. An amount of 10–15 hair roots was analyzed in each case. The number of FMRP-positive hair roots was expressed as a percentage of the total hair roots examined. A positive control was included in each assay (>90% normal range of FMRP expression).

Neuroimaging Assessment

MRI studies, including T1- and T2-weighted images in all cases, were evaluated by an experienced observer. The following features were assessed: presence of cerebellar and/or pontine atrophy, hyperintensities of the middle cerebellar peduncles and/or in the brainstem, cerebral atrophy (seen as sulci enlargement) and its location, including more specifically, middle temporal lobe atrophy, ventricular dilatation, thinning of the corpus callosum, and white matter hyperintensities and their extent (percentage of cerebral white matter involved).

Table 1 Clinical findings

	Patient 1	Patient 2	Patient 3
Age at onset	60	72	40
Resting tremor	–	–	–
Postural tremor	–	–	+
Intention tremor	–	+	+
Bradykinesia	–	–	–
Rigidity	–	–	–
Ocular pursuit	Jerky vertical movements	–	+
Ocular saccades	Normal	–	Slow
Nystagmus	–	–	+
Dysarthria	+	–	+
Dysmetria finger-to-nose	+ (slight)	+	+
Dysmetria heel-to-shin	+	+	+
Urinary/bowel incontinence	–	–	+
Gait difficulties	–	+	+
Memory deficiency	–	–	+
Tandem	Slightly abnormal	Cannot attempt	Cannot attempt
Sensory	Decreased vibration sense in the lower limbs	–	–

Results

One hundred fifty-four patients, referred to our molecular laboratory by different neurologists who had diagnosed a sporadic cerebellar atrophy syndrome, have been tested for the CGG repeat tract of *FMR1* gene. *FMR1* normal allele sizes ranged from 15 to 50 CGG repeats. The mean age of symptom onset was 62±11.17 years for women and 60.23± 10 years for men (average±SD). Three premutated carriers, one male and two females, were detected among these patients, giving a frequency of FXTAS of 1.95% in our SCA population. As we routinely perform the *FMR1* expansion test in all patients with clinical suspicion of dominant ataxia, we were able to inform and evaluate these patients.

Patient 1 is a male premutation carrier showing an expansion of 113 CGG repeats. He presented at the age of 64 with a clinical history of progressive dysarthria and hypoacusia. Neurological reexamination 1 year later revealed that these symptoms had progressed, while new ones had appeared (Table 1). MRI scan, performed at the age of 64, showed marked cerebellar atrophy, involving both hemispheres and vermis, and less marked brainstem atrophy, and middle cerebellar peduncles hyperintensities, among other findings (Table 2; Fig. 1a, b). The study of *FMR1* expression evidenced slightly reduced levels of FMRP (87.5%), while mRNA levels were elevated 2.7-fold (Table 3).

Patient 2 is a woman with a tract of 60 CGG repeats. She is 79 years old with a 7-year history of balance problems that required help for the past 5 years. Neurological examination at this time showed unsteady gait with weakness of the lower limbs, mild dysarthria, bilateral Babinski, and limited vertical ocular movements. She was unable to tandem-walk, and dysmetria was present in the finger-to-nose and heel-to-shin tests (right>left) (Table 1). Brain MRI demonstrated marked cerebellar atrophy, involving both hemispheres and vermis, and moderate brainstem and cerebral atrophy but no hyperintensities in the middle cerebellar peduncles or in the cerebral white matter (Table 2; Fig. 1c, d). *FMR1* mRNA analysis evidenced a 1.3-fold elevation (Table 3). Unfortunately, the sample received to perform the FMRP expression study was not suitable.

Patient 3 is an 82-year-old woman who harbors a 107 CGG repeat tract. She reports a large clinical history of a cerebellar syndrome. Intention tremor and extremity weakness began at the age of 40, and although there was no impairment in daily living activities she needed help to walk down the street. A falling episode led to a fractured secondary right malar bond. Neurological examination at

Table 2 MRI findings

	Patient 1	Patient 2	Patient 3
Global cerebellar atrophy	Marked	Marked	Marked
MCP hyperintensities	Marked	None	Minor
Brainstem atrophy	Moderate	Moderate	Mild/moderate
Brainstem hyperintensities	Minor	None	Marked
Cerebral atrophy (sulcal enlargement)	Moderate	Mild	Moderate
Location of cerebral atrophy	F, P	F, P	F, P
MTL atrophy	Minor/none	None	Minor/none
Ventricular dilatation	Moderate	None	Marked
Thinning of the corpus callosum	Marked	None	Marked
WM hyperintensities (% of total WM)	Minor (<10%)	None	Marked (>10%)

MCP Middle cerebellar peduncles, *F* frontal, *P* parietal, *MTL* medial temporal lobe, *WM* white matter

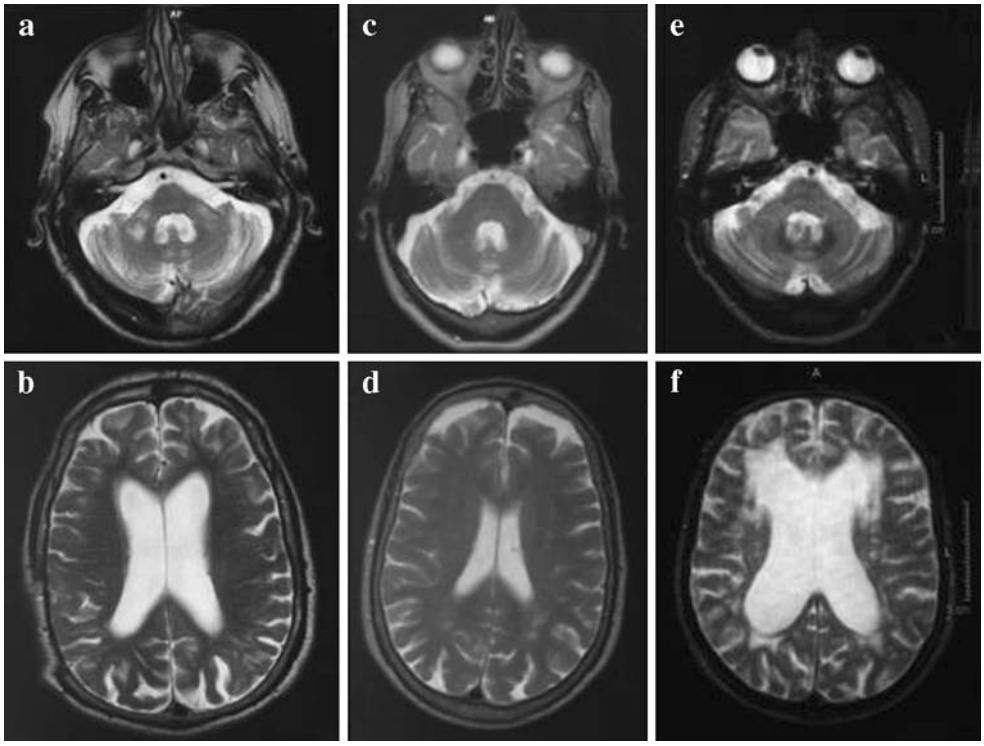

Fig. 1 Magnetic resonance imaging findings on T2-weighted FSE in the cerebellum, cerebellar peduncles, and cerebrum in the three patients. **a** Patient 1, marked cerebellar atrophy, moderate brainstem atrophy, and hyperintensities in the middle cerebellar peduncles. **b** Patient 1, moderate cerebral atrophy, seen as sulci enlargement. No white matter hyperintensities. **c** Patient 2, marked cerebellar atrophy but no middle cerebellar peduncles hyperintensities. **d** Patient 2, mild cerebral atrophy, and no white matter hyperintensities. **e** Patient 3, marked cerebellar and mild/moderate brainstem atrophy with minor/none middle cerebellar peduncles hyperintensities. **f** Patient 3, moderate cerebral atrophy with marked ventricular dilatation, and marked white matter hyperintensities

the age of 82 revealed dysarthria, severe ocular dysmetria, dysmetria in all four extremities, bilateral Babinski, and bladder and bowel incontinence (Table 1). MRI features are shown in Table 2 and Fig. 1e, f. FMRP production was reduced (75%) and *FMR1* mRNA levels were increased to 2.4-fold (Table 3).

Discussion

Nowadays and contrary to the original beliefs, it is well known that premutation alleles of *FMR1* give rise to a characteristic late-onset neurodegenerative disorder, which is known as FXTAS. These alleles contribute directly to clinical manifestations as elevated levels of *FMR1* mRNA have been demonstrated in premutation carriers [5]. Hagerman and Hagerman have proposed an RNA 'toxic gain of function' model in which the mRNA itself is causative of the neurological disorder [8]. The same mechanism has been proposed for myotonic dystrophy (DM1 and DM2) in which either the expanded CUG repeat tract in DM1 or CCUG in DM2 sequester CUG-binding proteins that disrupt mRNA processing of other genes or transport of other mRNAs [9]. Apart from this finding, FXTAS and myotonic dystrophy have another important similarity, which supports the RNA gain-of-function mechanism for the FXTAS syndrome. Both disorders show nuclear inclusions produced as a result of the binding proteins sequestered by the corresponding mRNA with a cytotoxic effect that leads to cell death. In a study performed by Greco et al. [10], eosinophilic intranuclear inclusions in neurons and astrocytes throughout the cortex and in the deep cerebellar nuclei were reported in postmortem samples of patients with FXTAS.

In spite of all previous studies performed to better define FXTAS, its frequency among sporadic, late-onset ataxia patients still remains unclear. To our knowledge, we report the first large Spanish group of late-onset ataxia patients negative for SCA 1, 2, 3, 6, 7, 8, and DRLPA that have been screened for *FMR1* premutated alleles. Of them, we have detected three premutated alleles, and remarkably two being females. Based on the proposed diagnostic clinical and neuroimaging criteria [11], FXTAS diagnosis in our premutated carriers is probable for patient 1, possible for patient 2 (the youngest woman with 60 CGG repeat), and

Table 3 Molecular findings

	Patient 1	Patient 2	Patient 3
Current age (years)	66	79	82
CGG repeat	113	60	107
FMRP levels (%)[a]	87	ND	75
FMR1 mRNA level[b]	2.7	1.3	2.4

ND Not determined

[a] Percentage of hair roots positive for FMRP by immunohistochemical staining

[b] RNA levels are reported as fold elevated over those in normal sex-matched control individuals.

definite for patient 3. Among men, the estimated frequency of FXTAS in our SCA-suspected population is 1.15%, while it rises to 3% in women. Previously reported frequencies for men are quite variable, ranging from 0.6 to 5% [12–15]. Our estimation seems to agree with that obtained by Seixas et al. [16] who found one premutation expansion among 64 male patients having movement disorders (1.7%). On the other hand and because of the fact that FXTAS occurs less frequently in females than in males, having a milder phenotype, and an older age of onset, it has been questioned if one should screen for the *FMR1* premutation in those older females presenting with ataxia [17]. In our study, the premutation was found in 2 out of 67 females. Although it might be a small sample, in light of our results, we recommend the *FMR1* premutation screening in any patient presenting with ataxia because of its high cost–benefit relationship. Early diagnosis of these FXTAS patients not only benefits them but also the rest of the family that should be advised regarding the risk of having FXS-affected offspring.

To date, the allele sizes reported among premutated female carriers are higher than 80 CGG repeats, and although it has not been ascertained, it has been suggested that shorter tracts might be less toxic, leading to a milder phenotype and to a lower penetrance of the clinical manifestations [13, 18]. Although we have to be cautious, our results in the two female cases seem to reinforce this hypothesis, as the premutated female with the longer CGG repeat tract (case 3) shows a more severe phenotype than the other female (case 2), both having similar age. Moreover, there seems to be a correlation between the amount of *FMR1* mRNA and the severity of the clinical and MRI manifestations of FXTAS, as the RNA gain-of-function model suggests [8].

To summarize, we recommend testing for the *FMR1* premutation in all patients (males and females) with late-onset ataxia. Finally and because of the risk of having children with fragile X syndrome, one must be aware of the ethical and genetic counseling requirements that premutation carriers identified through such a screening may need.

Acknowledgements We wish to thank Dr. R. Willemsen (Rotterdam, ND) for the kind donation of the antibody. We also thank the patients for their cooperation and consent to publish this study. This work has received financial support from SAF 2004-03083, PI050159, and PI050776 granted by the "Fondo de Investigación Sanitaria" Spain.

Disclosure The authors have reported no conflicts of interest.

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