Clin Rheumatol
DOI 10.1007/s10067-012-2052-y

BRIEF REPORT

Screening for the presence of FMR1 premutation alleles in a Spanish population with fibromyalgia

Loreto Martorell · Mireia Tondo · Ferrán Garcia-Fructuoso · Montserrat Naudo · Cayetano Alegre · Josep Gamez · Jordi Genovés · Pilar Poo

Received: 17 April 2012 / Revised: 23 July 2012 / Accepted: 26 July 2012
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Abstract Fragile X mental retardation 1 (FMR1) premutation carriers, who are at risk of having children with fragile X Syndrome, were initially considered as clinically unaffected. However, recent clinical and molecular studies have shifted this point of view. The incidence of premutation in the general population is substantial. Apart from the well-documented fragile X-associated tremor–ataxia and fragile X premature ovarian insufficiency, there is a broad constellation of symptoms including depression, anxiety, muscle pain, autoimmune and thyroid disease, chronic fatigue, and fibromyalgia that has been described, particularly in females with the premutation (55–200 repeats). Fibromyalgia (FM) is the most common cause of widespread pain and comprises a heterogeneous group of patients, affecting 2–3 % of the general population. We analyzed the FMR1 gene in a cohort of females diagnosed with fibromyalgia in order to assess the incidence of premutated alleles. CGG repeat size was determined in 353 females suffering from FM and results were compared with a control group. Four premutated carriers in the FM group were detected. The observed incidence is higher than that described for a normal female population (1/88 vs 1/250). The early detection of premutation carriers for the FMR1 gene among individuals diagnosed with fibromyalgia is important and would be helpful in correct genetic counseling of patients and their families, who may be at risk of having children with fragile X syndrome, the most common known cause of inherited intellectual disability and autism. Our data should be cautiously interpreted based on just this study; nevertheless, screening for the FMR1 gene in FM patients at least with presentations suggestive of FMR1 gene-related disease seems recommendable.

Keywords Autoimmune disease · CGG repeat · Fibromyalgia, chronic pain · FMR1 gene · Genetic counseling · Premutation

L. Martorell (✉) · M. Tondo · M. Naudo · J. Genovés
Molecular Genetics Section, Hospital Sant Joan de Deu,
Edifici docent C/ Santa Rosa 39,
08950 Barcelona, Spain
e-mail: lmartorell@hsjdbcn.org

F. Garcia-Fructuoso
Rheumatology Department, CIMA Clinic,
Barcelona, Spain

C. Alegre
Rehumatology Department, Hospital de la Vall d'Hebron
and Institut Universitari Dexeus,
Barcelona, Spain

J. Gamez
Neurology Department, Hospital de la Vall d'Hebron,
Barcelona, Spain

P. Poo
Neurology Department, Hospital Sant Joan de Deu,
Barcelona, Spain

Introduction

Fragile X syndrome (FXS) is a congenital and static disorder caused by the expansion of a CGG repeat in the fragile X mental retardation 1 gene (FMR1; OMIM*309550) [1]. Persons with FXS have more than 200 CGG repeats, designated as a full mutation, and present an array of well-documented physical, intellectual, and emotional disabilities. From the discovery of the mutation causing fragile X syndrome in 1991 until very recently, premutated carriers of the CGG expansion (55–200 repeats) were considered asymptomatic. The premutation allele frequency in the general population is estimated at approximately 1/800 in men and 1/250 in women [2, 3]. Most cases are clinically unaffected. However, some persons with the premutation present

Published online: 18 August 2012

clinical manifestations such as fragile X-associated tremor–ataxia (FXTAS; 40 % of males and 8 % of females), which is a progressive disorder with onset generally in the early sixties of action tremor, cerebellar ataxia, cognitive decline, peripheral neuropathy, and autonomic dysfunction, and fragile X premature ovarian insufficiency (FXPOI; 16–20 % of females), manifested as fertility problems and early menopause. Other clinical manifestations are learning difficulties, mild mental retardation, and psychiatric illness [4–6]. Furthermore, when compared to the normal population, female premutation carriers have a higher incidence of depression, anxiety, affective and panic disorders, thyroid problems, hypertension, muscle pain, chronic fatigue, tremor, and fibromyalgia [6, 7]. Concurrently, they are at risk of having a child with FXS, the most common known inheritable cause of cognitive disability and autism.

Fibromyalgia (FM) is a common chronic pain syndrome affecting 2–3 % of the general population comprising of a heterogeneous group of patients. FM is characterized by chronic widespread musculoskeletal pain mainly manifesting as fatigue, headache, stiffness, paresthesia, restless legs syndrome, depression, and anxiety [8, 9]. Today, FM is considered to be part of a broad spectrum of clinical features called central sensitivity syndromes [10]. The pathogenesis of fibromyalgia is not entirely understood, and both environmental and psychological factors are suspected as contributors to the symptomatology of FM in genetically predisposed individuals, as the symptoms can be observed in several members of the same family [9]. The FMR1 gene is one of the hypothesized genetic factors for developing FM in some individuals.

Female carriers of a premutation in the FMR1gene frequently have been described with widespread musculoskeletal pain and sometimes have been diagnosed with fibromyalgia [6, 8]. The aim of the present study was to screen a cohort of Spanish females with a clinical diagnosis of fibromyalgia to search for the presence of FMR1 premutated alleles.

Material and methods

Patient samples Associations of patients with fibromyalgia throughout Spain were contacted in order to find for a group of affected women. This study was prospective, in that all subjects were recruited solely for this project. Ultimately, 353 Caucasian females from different regions in Spain (age range 27–78 years; mean 54.0; SD 11.1) participated in the study (706 analyzed chromosomes). All females were unrelated and met the American College of Rheumatology 1990 criteria for the diagnosis of FM [11]. All patients underwent periodical neurological and rheumatologic examinations. Samples were obtained with the appropriate signed informed consent and the study was carried out in accordance with the principles of Declaration of Helsinki. A parallel study with a matched control group of 125 Spanish females (age range 27–74 years; mean 56.9; SD 8.6) without a personal history of FM, muscle pain, or muscular or autoimmune involvement was also performed. Genomic DNA was prepared from peripheral blood leukocytes using standard procedures [12].

Determination of CGG repeats size Polymerase chain reaction analysis to measure normal alleles and Southern blot for detection of large expansions and methylation status of the FMR1 gene were performed as previously described [13].

X-inactivation analysis The androgen receptor gene methylation assay was used as a second method to assess the methylation status of the X chromosome, as previously described [14]. The results are expressed as the percentage of cells that carry the normal allele on the active X chromosome, called the activation ratio (AR).

Statistical analysis Chi square was used to search for association between the studied population (affected females and control females) and CGG allele's length, classified as categorical variables (normal (<45 repeats) or impaired (>55 repeats)). Statistical calculations were performed with the SPSS.19.0 program.

Results

Four carriers of the premutated allele were found among the 353 affected females with fibromyalgia, leading to a frequency of one every 88 women. No premutated female carrier was found among our control group of patients. Despite these data, our results have no statistical significance (incidence of premutation carriers in control population (1/250) vs incidence of premutation in females with fibromyalgia (4/353) $p=0.4091$, compared with incidence of premutation in our control group (0/125) vs incidence in females with fibromyalgia (4/353) $p=0.577$).

Patient 1 Patient 1 is a 52-year-old female. She carries an expanded allele with 63 CGG repeats and a normal allele with 36 repeats. She showed onset of pain at the age of 24, with rheumatism, fibromyalgia, chronic fatigue, insomnia, and dysthymia. She showed premature ovarian failure from the age of 22, with migraines, a slipped disc, carpal tunnel syndrome, sphincter control problems, and high cholesterol. Medical history revealed chronic depression, panic, obsessive behavior, several attempted suicides, anxiety, and a conversion disorder. She had no history of alcohol abuse but she was a compulsive smoker. Cranial MRI was carried out 1 year ago and showed cerebellar mild atrophy with

minor abnormalities in hemispheric white matter, suggesting gliotic changes. Cranial MRI showed T2 hyper-intensities of the middle cerebellar peduncles, the "MCP sign", which is considered to be the most distinct radiological sign of FXTAS. Neuropsychometric tests showed abnormalities compatible with mild frontosubcortical disturbances. Physical exam revealed no tremors. Both parents were dead but the patient described a family history of depression and tremors. She had two male siblings without the FMR1 mutation who related personal histories of depression and anxiety. This female has an AR of the normal X chromosome of 30 %.

Patient 2 Patient 2 is a 47-year-old female. She presented 62 CGG repeats and a normal allele with 30 repeats. She underwent left ovarian ovariectomy at the age of 10 and left nephrectomy due to hydronephrosis at the age of 17, suffered right ovarian tumor at the age of 22, and chronic neck, shoulder and lower back pain from the age of 30. Other medical records reveal fibromyalgia, chronic diarrhea, irritable bowel syndrome, surpraventricular arrhythmia, and heightened eye pressure. She suffers insomnia, phobias and anxiety, and she is under medication for chronic depression. She has a brother suffering from mild schizophrenia, social phobias, and limited intellectual capacity. Her mother died of cancer at the age of 70 and suffered from depression, phobias, fibromyalgia, arthritis, and breast and vulva cancer. She also described a maternal uncle with similar symptoms. The AR of the normal X chromosome is 40 %.

Patient 3 Patient 3 is a 71-year-old female. She presented 127 CGG repeats and a normal allele with 27 CGG repeats. She was diagnosed with FM by her primary-care physician based on her symptoms. She presented a long and complex history of illness together with a broad constellation of symptoms, including fibromyalgia, chronic pain, chronic venous insufficiency, arthritis and chronic fatigue, among the most relevant ones. Currently, she complains of action tremor that started 6 years ago and suffers frequents falls. Medical report includes irritable colon, cardiomegaly, osteopenia, hypercholesterolemia, and FXPOI. She suffers anxiety and depression. Unfortunately, no MRI was performed on this patient. She has a grandson with intellectual handicap. DNA shows a total inactivation pattern of the normal X chromosome, with an AR of 4 %.

Patient 4 Patient 4 is a 43-year-old female. She presented 56 CGG repeats and a normal allele with 39 CGG repeats. She describes joint pain and migraines from the age of 20. She also described carpal tunnel syndrome in her right hand, tension headaches, numbness in extremities, severe fibromyalgia, intense physical fatigue, irritable colon, increased sensitivity to odors and hyperparathyroidism. Medical history includes regular menses. She suffers from anxiety, depression, and insomnia. Neuropsychological examination showed cognitive deficits due to attention difficulties, impaired verbal fluency, and low scores on visual orientation tests. Cranial MRI showed no abnormalities. She does not complain of tremors. She presents decreased serotonin levels in plasma. She has two healthy daughters and a history of one miscarriage; however, no fertility problems could be proven. Her mother has essential arterial hypertension and fibromyalgia but no history of tremors. The activation ratio for the normal X chromosome is 30 %.

A pattern of methylation of the FMR1 gene obtained with the Southern blot analysis is represented in Fig. 1.

Discussion

To our knowledge, this is the first attempt to carry out molecular study of the *FMR1* gene in a group of females affected with FM in Spain. A previous study carried out in 2011 described the clinical features of female *FMR1* premutation carriers with symptoms of FM. The authors conclude that screening females with FM for the presence of the premutated allele is worthwhile, as an increase in the prevalence of females with the premutation would lend support to genetic testing for *FMR1* in this population [8]. Other studies screening for the presence of *FMR1* premutation

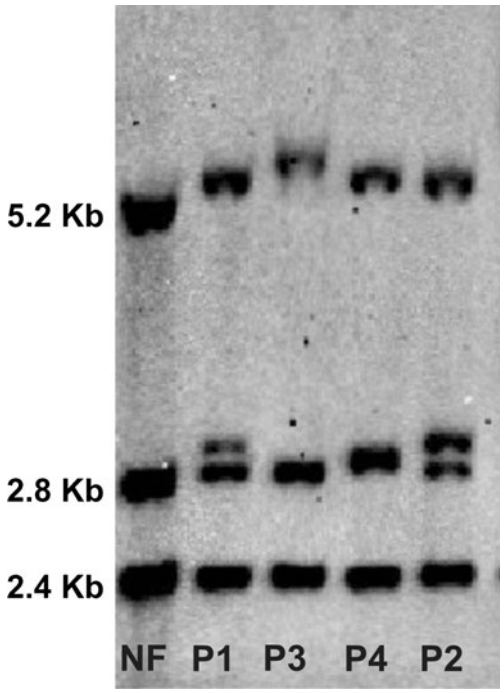

Fig. 1 Southern blot analysis of the FMR1 gene. Sizes of normal unmethylated (2.8 Kb), normal methylated (5.2 Kb), and a control band (2.4 Kb) are indicated. Each patient is indicated with the corresponding number, and a NF (normal female) is represented

alleles were carried out in women with Parkinsons as the authors thought that the presence of Parkinson in FXTAS raised the possibility that some affected individuals were actually carriers of a premutation FMR1 allele. The observed frequency did not differ from that observed in a matched population concluding that this screening was unlikely to be productive [15].

As previously described in several reports, a wide range of symptoms other than FXTAS and FXPOI must be taken into account when screening for the premutation in a population affected by FM [6, 16]. For females with the premutation, symptoms such as mood instability and anxiety were initially attributed to having a child with mental retardation [17], coupled with the social and familial handicaps that this situation frequently incorporates. However, these behavioral symptoms have also been described in premutated females without affected children or familial history of mental retardation [5]. Furthermore, these symptoms have not been detected in mothers of children with Down syndrome, indicating that the premutation itself may produce the behavioral disorders previously described in the literature. One possible explanation is aberrant hypothalamus–pituitary–adrenal (HPA) function, which would lead to an altered hormone response. Previous studies demonstrate that the HPA axis in mice carrying a CGG repeat expansion revealed dramatically elevated cortisone levels in serum in response to a mild stressor [4]. We believe that this mechanism could also occur in female premutation carriers as cortisone is a hormone released in response to stress, leading to some of the observed clinical signs. Concerning symptoms related to muscular pain and chronic fatigue in FM, a possible cause would lie in the alteration of pain neurotransmission, as the pathophysiology of FM involves hyperexcitability of central neurons through various synaptic and neurotransmitter mechanisms [18], as well as an imbalance in neurotransmitter metabolism in the case of dopamine (which, among other mechanisms, controls the perception of pain intensity) [19]. Finally, we hypothesize that the FMR1 premutation may be a genetic factor predisposing females to develop FM for several reasons. Some authors have showed that in patients with FXTAS, the premutation is associated with increased levels of CGG repeat-containing FMR1 mRNA, leading to cytotoxic effects for the cells [20, 21]. Furthermore, other authors demonstrated that there was a highly significant association between the number of CGG repeats in the FMR1 gene and the number of intranuclear inclusions [22]. As observed in other pathologies such as oculopharyngeal muscular dystrophy, increased number of intranuclear inclusions can cause muscular weakness [23].

Among our 353 studied individuals, there exists a high incidence of female premutation carriers, more than double what has been routinely described for the general population: 1/88 vs 1/250 [8, 24]. Despite this incidence, our results have no statistical significance, probably due to our reduced number of females carrying the premutation among the studied group, but these results are likewise strong enough. In light of this, the molecular test for FMR1 expansion should be recommended in patients with FM as well as in patients with other described symptoms (anxiety, depression, primary ovarian insufficiency, muscle pain, autoimmune disease, family history of mental retardation or intellectual disability, among others). Nevertheless, FM still seems to be a collection of odds and ends that includes patients with atypical and complex clinical histories, such as myotonic dystrophy type 2 [25]. For this reason, the importance of detecting an FMR1 premutation depends on different points. First of all, many females with FM have relatives with similar symptoms, and a positive result could impact the whole family's medical history. Secondly, the risk of inheriting the mutant allele is greater in descendants of females carrying the premutation [2]. Therefore, the risk of having a child with mental retardation rises too. Finally, screening would help to predict the possibility of developing FXTAS or other medical problems with aging. The study of the FMR1 gene in other populations would be of interest in order to corroborate our observed results.

Our finding that the prevalence of premutation carriers in a FM population is more than double than expected needs confirmation. We conclude that molecular testing for FMR1 premutation should be considered in FM patients, especially in those cases where the affected female has a history of ovarian insufficiency, tremor/ataxia, or a family history of FXS, FXTAS, or FXPOI, as the correct diagnosis has important implications for the genetic counseling in affected families.

Acknowledgments We are indebted to all the patients who participated in our research for making this study possible. Thank to Dr. Sylvie Guiroux, form the Center de Reserche en génétiquehumanine et moléculaire, CHUQ, Québec, Canada, for kindly supplying us the StB12.3 probe. We also want to thank Katherine Andrews for assisting in language support.

Disclosures None.

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