European Journal of Human Genetics (2009) 17, 1359–1362

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SHORT REPORT

Penetrance of *FMR1* premutation associated pathologies in fragile X syndrome families

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Within the past few years, there has been a significant change in identifying and characterizing the *FMR1* premutation associated phenotypes. The premutation has been associated with elevated *FMR1* mRNA levels and slight to moderate reductions in FMRP levels. Furthermore, it has been established that ~20% of female premutation carriers present primary ovarian insufficiency (POI) and that fragile X-associated tremor/ataxia syndrome (FXTAS) occurs in one-third of all male premutation carriers older than 50 years. Besides POI and FXTAS, new disorders have recently been described among individuals (especially females) with the *FMR1* premutation. Those pathologies include thyroid disease, hypertension, seizures, peripheral neuropathy, and fibromyalgia. However there are few reports related to FXTAS penetrance among female premutation carriers or regarding these disorders recently associated to the *FMR1* premutation. Therefore, we have evaluated 398 fragile X syndrome (FXS) families in an attempt to provide an estimation of the premutation associated phenotypes penetrance. Our results show that signs of FXTAS are detected in 16.5% of female premutation carriers and in 45.5% of premutated males older than 50 years. Furthermore, among females with the *FMR1* premutation, penetrance of POI, thyroid disease and chronic muscle pain is 18.6, 15.9 and 24.4%, respectively. The knowledge of this data might be useful for accurate genetic counselling as well as for a better characterization of the clinical phenotypes of *FMR1* premutation carriers.
European Journal of Human Genetics (2009) **17,** 1359–1362; doi:10.1038/ejhg.2009.51; published online 15 April 2009

Keywords: *FMR1* premutation; primary ovarian insufficiency; FXTAS

Introduction

Fragile X syndrome (FXS) (OMIM #300624), which is the most common familial form of mental retardation, is caused by the silencing of the *FMR1* gene, because of the presence of a full mutation (>200 CGG repeats) in the 5′UTR. The incidence of the syndrome is not known, but epidemiological studies indicate that it is responsible for mental retardation in 1 in 4000–6000 males and in 1 in 7000–10 000 females of the European descendent. In a study conducted in Catalonia we found an incidence of 1:2466 male and 1:8333 females.[1] This difference between males and females is because of the reduced penetrance of FXS in females. It is also important to highlight the high incidence of premutation carriers (55–200 CGG repeats) in the general population, which has been estimated in 1 of 813 males and 1 of 259 females.[2,3]

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Received 15 July 2008; revised 19 February 2009; accepted 24 February 2009; published online 15 April 2009

Individuals with the premutation are usually unaffected intellectually; however at least two different disorders, primary ovarian insufficiency (POI), causing menopause before 40 years, and the fragile X-associated tremor/ataxia syndrome (FXTAS), have been described among them.[4–6] Both disorders do not share any clinical features with the FXS. Within the past few years, there has been a significant change in identifying and characterizing the *FMR1* premutation associated phenotypes. For example, the premutation has been associated with elevated *FMR1* mRNA levels and slight to moderate reductions in FMRP levels.[7,8] Moreover, among women with *FMR1* premutation, an expanded clinical phenotype has been described which, besides the POI and FXTAS pathologies, also includes thyroid dysfunction, hypertension, and fibromyalgia or chronic muscle pain.[9]

We have evaluated 398 Spanish FXS families in an attempt to provide an estimation of the premutation associated phenotypes penetrance among premutation carriers. This data might be useful for providing accurate genetic counselling as well as for better characterize the nature of clinical phenotypes of *FMR1* premutation carriers.

Materials and methods

Subjects

A total of 398 FXS families molecularly diagnosed in the Genetics laboratory of the Hospital Clínic in Barcelona have been evaluated. Among them, 151 families were composed of at least three generations which presented individuals that fulfill the study inclusion criteria (female premutation carriers over 40 years and male premutation carriers over 50 years). The rest correspond to families in which only one generation was studied, and thus no data about grandparents was available. In total, 280 female premutation carriers and 44 male premutation carriers were included. A classification based on the gender and the age of the enrolled participants is summarized in Table 1. Therefore, all the carriers described herein have been recruited exclusively through families with known members affected with FXS and all of them have a Caucasian ethnicity.

Methods

Participants answered a survey over the phone or in person (20 *vs* 80%) that comprised questions focused on identifying individuals with FXTAS symptoms and/or POI, thyroid problems and muscle pain. The neurological symptoms questionnaire was derived from Jacquemont *et al* (2004)[10] and includes questions regarding presence and time-of-onset of tremors, balance problems, recent falls, and gait ataxia. Symptoms were scored as present if noticed by the respondent. Overall, FXTAS encompasses patients that meet criteria in any of the all three categories of involvement: definitive, probable, and possible.[10] Regarding POI, thyroid dysfunction (diagnosed by an endocrinologist) and chronic muscle pain (persistent myalgia for more than 2 months unrelated to injury or surgery), questions were asked related to the presence and the onset of the disorders.

Table 1 Classification of the premutated individuals enrolled in the study

	Age		
Gender	*>50 years old*	*<50 years old*	*Total*
Male (n)	44	Not included in the study	44
Age mean	72.8		
SD	9.4		
Female (n)	85	195[a]	280
Age mean	70.3	43.5	
SD	13	3.1	

[a]These females are between the ages of 40 and 50 years.

Statistical analysis

Data were analyzed by Fisher's exact test and significance was accepted for P-value <0.05. Statistical analyses were carried out using commercially available software (SPSS-PC, Version 16.0; SPSS Inc, Chicago, IL, USA).

Results

The results showed that among the 151 FXS families that fulfill the inclusion criteria for the study, 20.5% (31/151) presented at least one female with POI, 13.2% (20/151) showed at least a male or female presenting FXTAS symptoms (defined primarily in terms of its core features of gait ataxia and/or intention tremor)[9] and in 6% (9/151) of the families both disorders were present. Interestingly, one family showed familial aggregation for FXTAS syndrome (two premutation carrier sisters evidenced symptoms). In nine families, FXTAS and POI coexisted in different individuals, and we have also identified three women with POI and FXTAS symptoms.

If we consider premutation carriers older than 50 years, FXTAS symptoms were present in 14 females (three with a FXTAS diagnosis of definitive) out of 85 and in 20 males (six with a FXTAS diagnosis of definitive and one of possible) out of 44. Therefore FXTAS penetrance is 16.5% among female premutation carriers and 45.5% among male premutated carriers (Table 2). The percentage of *FMR1* premutated male and female carriers with self-reported FXTAS symptoms is summarized in Table 3 based on age range. Overall, the mean age and the mean of CGG repeat number for the self-reported FXTAS men group is of 72.05 ± 6.85 and 85 ± 21.5 (mean $\pm$ SD), respectively. Similarly it is of 75.8 years old $\pm$ 10.2 and 82 CGG repeats $\pm$ 18 (mean $\pm$ SD) for the self-reported FXTAS women group.

Regarding other clinical phenotypes of females with the *FMR1* premutation, in our cohort we observed POI in

Table 2 Clinical phenotypes associated to premutation *FMR1* alleles

Phenotype	*Sample size*	*Onset*	*Penetrance (%)*
POI	280 females	Adulthood	18.6
FXTAS	85 females	>50 years	16.5
	44 males	>50 years	45.5
Thyroid disease	88 females	Adulthood	15.9
Chronic muscle pain	90 females	Adulthood	24.4

Table 3 Percentage of *FMR1* premutated carriers with self-reported FXTAS symptoms

	FXTAS symptoms		
	Male	*Female*	*Total*
50–59 years	0% (0/1)	3.7% (1/27)	3.6% (1/28)
60–69 years	52.9% (9/17)	20% (3/15)	37.5% (12/32)
70–79 years	53.3% (8/15)	26.3% (5/19)	38.2% (13/34)
≥80 years	27.3% (3/11)	20.8% (5/24)	22.9% (8/35)

18.6%, thyroid disease in 15.9% and chronic muscle pain in 24.4% (Table 2). These percentages are statistically significant for POI and chronic muscle pain when comparing with general population ($P<0.00001$ and $P=0.00002$, respectively). Among the 14 premutation carriers with thyroid problems, nine reported a history of hypothyroidism, one indicated hyperthyroidism, one a thyroid nodule, one a Graves Disease, and two have not a specific diagnosis.

Discussion

New advances in FXS have resulted in the description of an emerging phenotype associated to adult premutation carriers. Besides POI and FXTAS, new disorders have recently been found among individuals (especially females) with the *FMR1* premutation.[9] Those pathologies include thyroid disease, hypertension, seizures, peripheral neuropathy and fibromyalgia or chronic muscle pain. In a recent study, Coffey and co-authors (2008)[9] reported that female premutation carriers have an increased prevalence of developing these disorders compared with control individuals. It is well established that between 12–20% of female premutation carriers have POI, whereas only 1% of women in the general population presents it.[11,12] On the other hand, it has been estimated that FXTAS affects one-third of all male fragile X premutation carriers older than 50 years,[13,14] although the penetrance increases with age, exceeding 50% for men aged 70–90 years.[15] Regarding females, to our knowledge there is only one study that reports a FXTAS prevalence of 8.3% in a sample of female carriers assessed through family studies.[9] Furthermore, few data have been published about the recently described spectrum of clinical involvement in female premutation carriers. Therefore, the aim of this study was to estimate the penetrance of *FMR1* premutation associated disorders in our cohort of FXS families.

Our results showed that FXTAS symptoms have been detected in 16.5% of female premutation carriers and in 45.5% of premutated males older than 50 years. This penetrance is age related for both premutation carrier groups, as it increases together with the age of the self-reported FXTAS individuals (Table 3). However and contrary to the earlier published data,[13] the highest FXTAS penetrance is found in the group aged 70–79. We speculate that FXTAS patients die earlier than same-aged non-FXTAS patients and probably due to the disorder. Moreover and as expected, FXTAS penetrance is lower in females than males, presumably because of the protective effect provided by the expression of *FMR1* from the normal allele on the active X chromosome in a percentage of cells.[16]

Similarly to what was reported earlier,[11–14] we have found that POI occurs in 18.6% of female carriers. Prevalence of thyroid disease and chronic muscle pain among the general population is highly variable as it increases with age. However, in individuals over 50 years, it has been estimated to be around 10 and 2%, respectively.[17,18] In contrast, the penetrance of these pathologies among our female premuation carriers cohort is higher (15.9 and 24.4%, respectively), although the difference is statistically significant only when comparing the prevalence of chronic muscle pain ($P=0.00002$). The cause of these penetrance differences is still unknown, although it has been hypothesized that both disorders may be related to a dysfunction in the hypothalamic–pituitary–adrenal axis,[19,20] that in the premutation status might be caused by a direct effect of RNA toxicity.[9] Furthermore, it has been described that psychiatric symptoms, such as depression and anxiety, are often associated with fibromyalgia,[20,21] and interestingly, it has been shown that female premutation carriers have a distinctive neuropsychiatric profile with higher tendency to depression.[22,23]

Taken together, the existence of these findings should be considered when offering genetic counseling for FXS, as they should be mentioned to premutation carriers. Although these disorders occur by a completely separate mechanism from FXS and affects different individuals, they are caused by defects in the same gene, therefore their study and characterization might help to better understand how the *FMR1* gene works.

Acknowledgements

We thank the Fragile X families, the 'Associació Catalana Síndrome X fràgil', and the Federación Española del Síndrome X Frágil for their cooperation. This work was supported by MARATO TV3 (TV06-0810). The CIBER de Enfermedades Raras is an initiative of the ISCIII.

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