Journal of Human Genetics (2011) 56, 87–90

www.nature.com/jhg

SHORT COMMUNICATION

# Four sisters compound heterozygotes for the pre- and full mutation in fragile X syndrome and a complete inactivation of X-functional chromosome: implications for genetic counseling

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**Fragile X syndrome (FXS) is a neurodevelopmental disorder and a leading monogenic form of cognitive impairment and autism. It is the most common form of inherited mental retardation in males and a significant cause of mental retardation in females. It is caused by the instability and subsequent expansion of the CGG repeat in the promoter region of the *FMR1* (*fragile X mental retardation 1*) gene at Xq27.3. We describe a double consanguineous family with four sisters compound heterozygotes for the full and pre-mutation CGG repeat size. The index case shows clinical features of the affected males with profound mental retardation; the other three sisters also suffer from mental retardation, ranging from mild to severe. Molecular analysis reveals very similar ranges for the CGG expansions for both chromosomes in all four sisters. The phenotypic differences observed in the index case and her sisters are the total inactivation of X premutated chromosome and the total absence of FMRP (fragile X mental retardation protein). This family case raises important issues for genetic counseling in families with consanguinity and with cases of idiopathic mental retardation.**
*Journal of Human Genetics* (2011) **56,** 87–90; doi:10.1038/jhg.2010.140; published online 25 November 2010

**Keywords:** compound heterozygote; fragile X syndrome; full mutation; mental retardation; premutation; X-inactivation

## INTRODUCTION

Fragile X syndrome (FXS) is the first inherited cause of development, intellectual and conductual problems. The estimated prevalence of affected individuals is 1/4000 males and 1/6000 females, and 1/260 women and 1/800 men would carry the genetic alteration without showing symptoms of the disease. The symptoms characterizing FXS are varied, and the most important is mental retardation, ranging from mild to severe. Severity of common symptoms depends largely on the age and sex of individuals. The most distinctive physical features are large and prominent ears, long narrow face, prominent jaw, joint hypermobility, visual and hearing impairment, heart problems and epilepsy; males in adolescence may have macroorchidism. Regarding cognitive and behavioral aspects, individuals tend to have psychomotor retardation, hyperactivity and inattention, speech reading and writing problems and autistic traits.[1] The females are usually less affected, because of the presence of the other X chromosome, compensating the lack of FMRP (fragile X mental retardation protein). Approximately 60% of women affected by the syndrome may have mild mental retardation, although most have a normal intellect quotient, but cognitive–behavioral phenotype of the syndrome is present in most of the cases, with attention problems, low self-esteem and shyness, which may be deep in adolescence and can lead to anxiety, dependency or depression.[2]

The mutation causing FXS is the instability and subsequent expansion of a CGG repeat in the *FMR1* (*fragile X mental retardation 1*) gene, located on Xq27.3. Four categories of alleles have been established based on the number of CGG copies: normal range (6–44 CGG repeats), gray zone (45–54), premutation (55–200) and full mutation ($>200$ repeats).[3] When the number of repeats exceeds 200, the individual would be affected because of the inactivation of the gene, and consequently the protein FMRP is not produced.

Recent studies change the pre-existing idea that premutation alleles do not give rise to clinical involvement. Although premutation carriers were reported initially to have normal cognitive abilities, mild emotional problems have also been reported in 20% carrier females,[4] premature ovarian failure has been observed in 24% of such females carriers[5] and 40% of premutation males and 8% of females carriers aged $>50$ years suffer from fragile X-associated tremor ataxia syndrome.[6]

Up to date, several cases of compound heterozygotes for FXS have been reported; three sisters with both chromosomes in the

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Received 14 September 2010; revised 11 October 2010; accepted 16 October 2010; published online 25 November 2010

premutation range,[7,8] two sisters with one gray-zone allele and one full mutation allele,[9] four of them are unrelated female carriers of a premutation and a full mutation.[10–13]

In the present study we describe an exceptional consanguineous Moroccan family, who has been living in Spain for 4 years, in which the four sisters show a compound heterozygote genotype for full and pre-mutation, and the index case has complete inactivation of functional X chromosome.

## MATERIALS AND METHODS

### Patients' samples

Biological samples from all family members were collected with the appropriated signed informed consent. Genomic DNA was prepared from peripheral blood leukocytes using standard procedures.[14]

### Determination of CGG repeat size

Large expansions and methylation status of the *FMR1* gene were detected by Southern blot analysis, as previously described.[15] PCR analysis to determine the number of CGG repeats in normal range and for small expanded alleles was carried out as previously described.[16]

### Protein study and FMR1 mRNA levels

Western blot analysis was performed as previously described.[17] Obtention and relative quantification of mRNA for *FMR1* gene was performed as previously described.[18]

### X-inactivation analysis

As a second method to asses the methylation status, we used the androgen receptor gene methylation assay described previously.[19]

## RESULTS AND DISCUSSION

We report the study of a family with four sisters all compound heterozygotes for FMR1 mutation, where the index case was referred to the laboratory for carrier testing of fragile X on the basis of the mental retardation and physical characteristics of the patient. To our knowledge, this is the largest kindred reported with all members carrying simultaneously pre- and full-mutation alleles, and with the exceptional complete inactivation of X-functional chromosome in the index case.

Figure 1 represents the pedigree of the family reported, showing the molecular results. All family members live in Morocco, except the nine members analyzed.

### Intellectual and neuropsychological performance

The proband IV2, the youngest of the sisters, shows autistic-like features that include weak understanding of verbal and non-verbal communication, non-expressive language, echolalia, gaze avoidance, shot attention spam, hyperarousal to sensory stimuli, impulsivity and in occasions aggressive behavior. She was not schooled. The Kaufman brief intelligence test, and the VMI-5 (Test of Visual-Motor Integration, Fifth Edition) to measure visual-motor skills, were administered. The results show severe mental retardation (see Table 1). The other

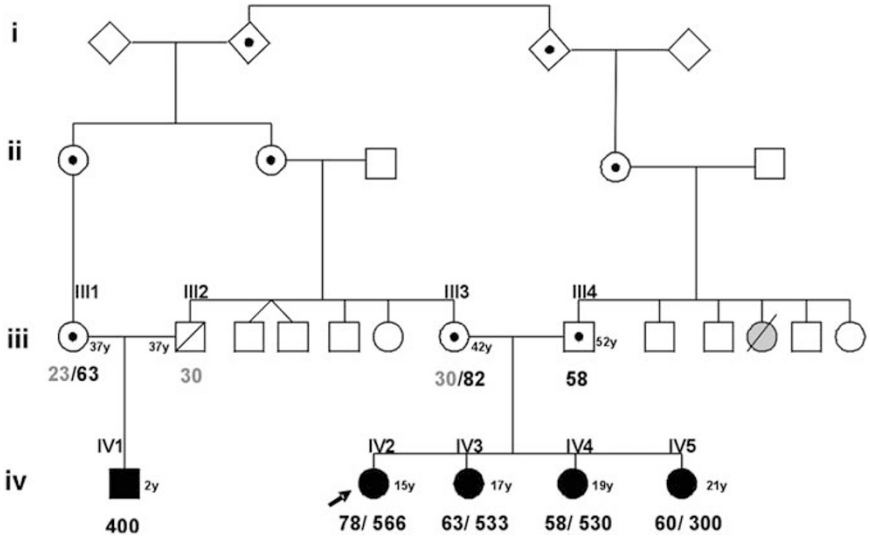


**Figure 1** Family pedigree. The arrow indicates the proband (IV2). Two consanguinities are seen in the family. The sex of the obligate carriers in the first (i) generation is not known, as the clinical status of all members living in Morocco. The age of each individual analyzed is indicated beside the symbol. Under each symbol the number of repeats for the normal (gray), pre- and full mutation (black) is indicated.

**Table 1 Psychological, molecular and phenotypic evaluation in the siblings**

| *Case* | *Age* | *Psychiatric disorders (MINI)* | *(Global IQ)/ VMI* | *Phenotypic traits* | *Genetic profile (CGG repeats)* | *X inactivation* | *FMRP protein* |
|---|---|---|---|---|---|---|---|
| IV2 | 15 | Not possible | K-BIT<50 | Long face, prominent forehead and ears, joint laxity | 78/566 | Total | No |
| IV3 | 17 | Social phobia | 61 | Long face, no joint laxity | 63/533 | Random skewed | Yes |
| IV4 | 19 | Social phobia, dysthymia | 70 | Long face, no joint laxity | 58/530 | Random skewed | Yes |
| IV5 | 21 | Social phobia, panic disorder | 60 | Long face, prominent forehead and ears, no joint laxity | 60/300 | Random skewed | Yes |

Abbreviations: FMRP, fragile X mental retardation protein; MINI, Mini-International Neuropsychiatric Interview; VMI, Test of Visual-Motor Integration.

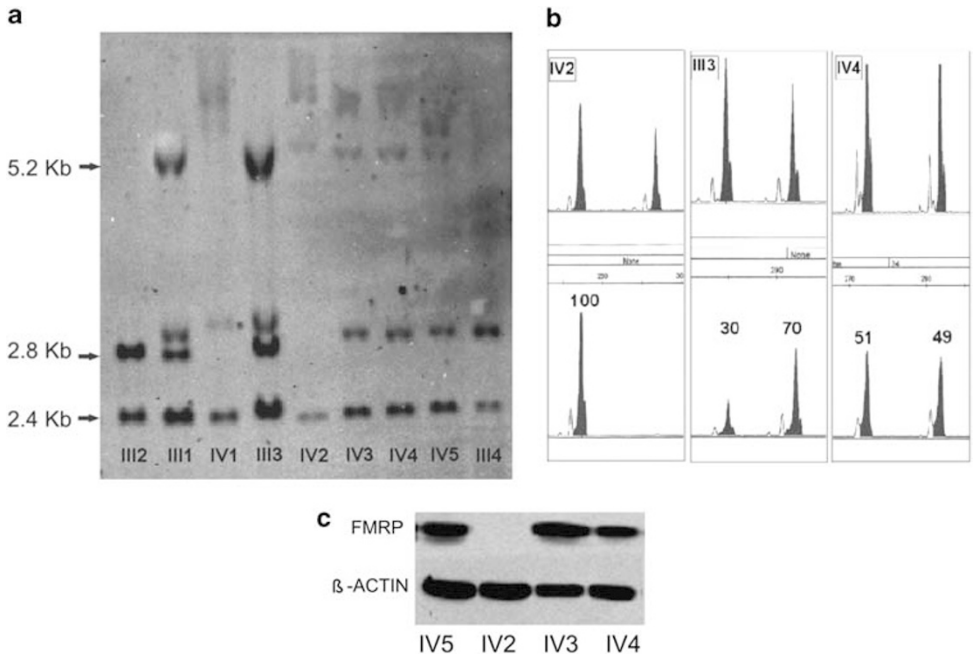


**Figure 2** (**a**) Southern blot analysis of *FMR1* (*fragile X mental retardation 1*) gene. Sizes of normal unmethylated (2.8 Kb), normal methylated (5.2 Kb) and a control band (2.4 Kb) are indicated. (**b**) X-inactivation study on *AR* gene. Upper panel is PCR before *Hpa*II DNA digestion, and lower panel corresponds to the PCR after enzyme digestion. Percentages of inactivation are indicated: total skewed normal X-chromosome inactivation for index case (IV2), partially skewed in mother, III3, and random skewed in sister IV4, as representation of the rest of females analyzed. (**c**) Western blot analysis of FMRP protein of four sisters. As a control, the β-actin protein was used.

three sisters have cognitive impairments with learning disabilities and emotional problems. We can describe them as displaying significant shyness, and they express important anxiety in social situations with avoidance behaviors associated. We used the Intellectual and Neuropsychological Wechsler Adult Intelligence Scale (WAIS) achievement tests that reported a mildly affected IQ (60–70) without significant discrepancy between verbal and performance tasks (Table 1). The Mini-International Neuropsychiatric Interview (MINI) was also used, for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition) and ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th Revision), to explore psychiatric disorders.

**Molecular analysis**

Haplotype analysis of both chromosomes was identical in the family, and no AGG interruptions were detected in all mutated alleles (data not shown). As reported in the literature, and knowing the pattern of inheritance of the mutated alleles in FXS (males transmit only premutations, and the expansions to full mutation are through females), we conclude that all the premutations detected in the four sisters are paternally inherited, and the full mutations come from their mother (Figure 1).

The results obtained in Southern blot, with reference to the pattern of inactivation, were confirmed with a second method, the analysis of the androgen-receptor methylation sensitive locus. The index case has a totally skewed chromosome X inactivation. Her mother has slight skewed inactivation pattern, and the other sisters have random X-inactivation patterns (Figure 2).

The mRNA studies and the western blot analysis confirmed the absence of mRNA and protein in the proband and her full mutation cousin (IV1), and a normal pattern in the other family members analyzed (Figure 2).

We conclude that the severe phenotype observed in the index case, identical to what is observed in the full mutated males, is because of the complete inactivation of the X-functional chromosome and total absence of FMRP protein, not only by the fact of having a full mutation chromosome, as it was seen in the other sisters and in a reported case of two monozygotic sisters with the fragile X mutation, but with different phenotypes.[20]

Our results are consistent with earlier reports indicating a higher risk of females with either the full or pre-mutation for social phobia and schizotypical as well as avoidant personality disorders. Molecular analysis of *FMR1* gene in families with individuals with idiopathic mental retardation is recommended, and in cases with consanguinity genetic counseling is essential.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

We are indebted to the family for their cooperation in performing this study and thank Dr Sylvie Guiroux, from the Center de Reserche en génétique humanine et moléculaire, CHUQ, Québec, Canada, for kindly supplying us the StB12.3 probe.

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