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Clinical and Molecular Genetic Characterization of a Female with Fragile X Syndrome and Two Expanded Alleles: A Case Report

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A B S T R A C T

Fragile X syndrome is a genetic condition causing a range of developmental problems, with males more severely affected compared to female patients. The main features include a long and narrow face, large ears, and a prominent jaw and forehead. Males develop enlarged testicles after puberty, and carrier females are expected to show fragile X-associated primary ovarian insufficiency (FXPOI). Fragile X Syndrome (FXS) was suspected in a consanguineous family referred to a Medical Genetics center because of a family history of intellectual disability and primary ovarian insufficiency in their small village population. The cytosine guanine guanine (CGG) repeat expansion of the *FMR1* gene in the 65-year-old proband was amplified and then analyzed by Gene Marker software. The female proband showed two expanded alleles, including one full mutation allele and one premutation allele with an accurate size of 74 (CGG) repeats. Despite having two mutant *FMR1* alleles and manifesting some symptoms of FXS, she was fertile. Consanguineous marriages and, in more unfavorable conditions, marrying Fragile X-affected or premutation-carrying males with female carriers is not uncommon in such genetically isolated populations. Therefore, the need for Fragile X syndrome examination in suspected patients with similar features and screening their relatives is highly emphasized.

Keywords: Fragile X Syndrome; *FMR1* gene; Intellectual disability; CGG repeats

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Introduction

Fragile X Syndrome (FXS) is the most common X-linked genetic cause of intellectual disability (OMIM 300624). The expansion of CGG trinucleotide repeats located at the polymorphic 5' UTR of the Fragile X messenger ribonucleoprotein 1 (*FMR1*) gene in Xq27.3 is responsible for the pathogenesis of the disease (1). According to the American College of Medical Genetics and Genomics (ACMG) guidelines for fragile X testing, there are four types of *FMR1* alleles: normal (5-44 repeats), intermediate (45-54 repeats), premutation (55-200 repeats), and full mutations (> 200 repeats) (2). FMRP, the protein product of *FMR1*, is a ubiquitously expressed RNA-binding protein with high levels of brain expression. FMRP is believed to be a translational repressor, suppressing the translation of proteins involved in the regulation of synaptic plasticity and connectivity in the developing brain. Lack of FMRP expression leads to a modest but functionally significant increase in FMRP-regulated proteins. Expansion of CGG repeats into the full mutation causes hypermethylation of *FMR1*, resulting in gene silencing and complete absence of FMRP. Premutation alleles, however, cause an upregulation in *FMR1* transcripts with a modest reduction effect on the FMRP protein product. In addition, Repeat Associated Non-AUG (RAN) translation of premutation alleles produces toxic FMRpolyG protein species, which may be responsible for neurodegenerative symptoms linked to these alleles (3).

FXS affects 1:6000–1:8000 females and 1:3000–1:4000 males in the general population. The frequency of the full mutation in the whole population appears to be about 1.4 per 10,000 males and 0.9 per 10,000 females, and the premutation frequency is about 11.7 per 10,000 males and 34.4 per 10,000 females (4). FXS can affect both males and females, but there is a gender bias in severity and phenotypic spectrum, which is thought to be due to the X-linked nature of the disease. Both female and male carriers of normal and intermediate alleles are not at risk of developing FXS and are phenotypically normal. Individuals with FXS exhibit a variety of symptoms, ranging from a normal IQ to learning difficulties, intellectual disability (ID), and autistic characteristics. Physical characteristics have been described; however, they are often nonspecific. Female FXS patients usually show milder phenotypes, which is attributed to the existence of an unaffected X chromosome (1).

About one-third of hemizygous males with premutation alleles (and, at lower rates, heterozygous females) develop late-onset fragile X-associated tremor and ataxia syndrome (FXTAS) by the age of 50. The risk of developing FXTAS increases with age as well as the size of the repeat expansion. In carriers of alleles with more than 70 repeats, for instance, the penetrance of the disease appears to be around one-third of cases. In addition to FXTAS, other neurological phenotypes such as attention-deficit/hyperactivity disorder (ADHD), depression, and anxiety—collectively known as fragile X-associated neuropsychiatric disorders (FXAND)—are reported at higher rates in females (5).

Fragile X-associated primary ovarian insufficiency (FXPOI), the most common heritable cause of premature ovarian insufficiency, affects some premutation carrier females. Females with premutation alleles show higher levels of follicle-stimulating hormone as well as lower levels of anti-Müllerian hormone, inhibin A, and inhibin B than normal or full mutation carriers, all of which are markers for ovarian decline (6). Premutation carriers have a higher likelihood of developing FXPOI, with earlier onset in carriers of mid-range premutations (80–100 repeats) than in carriers of shorter or longer premutation alleles (7).

In FXS cases, there is evidence of the expansion of premutation alleles into full mutation alleles. The maternal transmission of these expanded alleles to offspring has been verified in various studies. This

premutation expansion occurs during oogenesis and is rarely seen in spermatogenesis. Females with two full mutation alleles are thought to be affected by the fundamental features of the disorder, while heterozygous females with one full mutation allele can be affected by mild autistic behavior, anxiety, socio-emotional difficulties, and learning impairments, although this is mostly related to the X chromosome inactivation process (8). In this study, we present a female fragile X patient with a heterozygous full mutation and premutation genotype for the *FMR1* gene.

Materials and methods

Patient information

The recruited family in this study was a consanguineous family referred to Sadra Medical Genetics Lab in Shahrekord, Iran, due to a family history of intellectual disability (ID) and premature ovarian insufficiency in their small village. Their pedigree indicated multiple affected individuals in each generation, with a male to female morbidity ratio of approximately 3:1 (Figure 1).

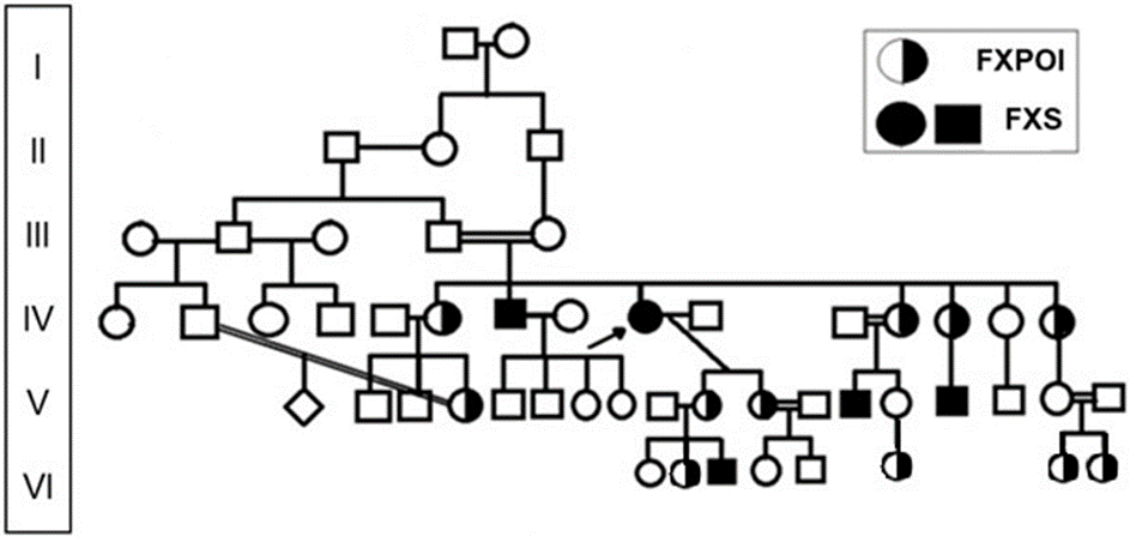

Figure 1. Six-generation pedigree of the family illustrating multiple affected individuals in each generation with characteristic and gender-specific phenotypes indicative of fragile X-associated disorders. Consanguinity is denoted by double lines between symbols. The proband is highlighted with an arrow. Abbreviations: FXPOI: Fragile X-associated Primary Ovarian Insufficiency; FXS: Fragile X Syndrome.

Clinical evaluations noted characteristic physical and intellectual features, and the fact that practically all the women had primary ovarian insufficiency guided us to assume that the *FMR1* gene repeat expansion caused the symptoms in the pedigree phenotypes. The proband of this family was consequently considered a candidate for fragile X molecular genetic testing following a thorough clinical evaluation and genetic counseling. The patient was a 65-year-old woman with tremor ataxia symptoms, a narrow face, FXPOI, and modest intellectual disability (Figure 2a). She was born to a consanguineous couple with mild intellectual disability (ID). This woman had two apparently normal daughters, one of whom had an affected daughter with the same symptoms as her grandmother due to consanguineous marriage. This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences, Shahrekord, Iran, and written informed consent was obtained from all participant individuals. All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki

Declaration and its later amendments or comparable ethical standards.

Figure 2. Clinical phenotypes of the family. (a) The proband patient bearing premutation and full mutation alleles. (b) Grandson of the proband affected with fragile X Syndrome carrying full mutation allele. Note the fragile X-associated facial characteristics.

The detection of repeat expansion in the 5'-UTR region of *FMR1* is performed by PCR-based fragment length analysis (9). DNA extraction from peripheral blood leukocytes was carried out using the salting-out method. DNA qualification and quantification were assessed using a Micro-Volume UV/Vis Spectrophotometer (BioSpec-nano, Shimadju, Japan). The CGG repeat expansion of *FMR1* was then investigated using the FastFrax *FMR1* Sizing Kit (The Biofactory Pte Ltd, Singapore) in a T100 Thermal Cycler (Bio-Rad, USA), followed by fragment analysis using an Applied Biosystems Genetic Analyzer (Thermo Fisher Scientific, USA). Finally, the resulting chromatograms were analyzed using Gene Marker software (V2.2.6).

Results

Molecular analysis of the proband (IV9) revealed a heterozygous genotype with two expanded alleles: a full mutation allele with more than 200 CGG repeats and a premutation allele with an accurate size of 74 CGG repeats (Figure 3).

Based on clinical manifestations, as well as known molecular mechanisms for three-nucleotide expansions and pedigree information, it was assumed that the proband inherited a premutation or full mutation allele from her hemizygous father (III-4) and a premutation or full mutation allele from her heterozygous mother (III-5). Subsequently, the proband's granddaughter (VI-2) was believed to inherit a full mutation allele from her FXPOI-affected mother and a normal or premutated allele from her father.

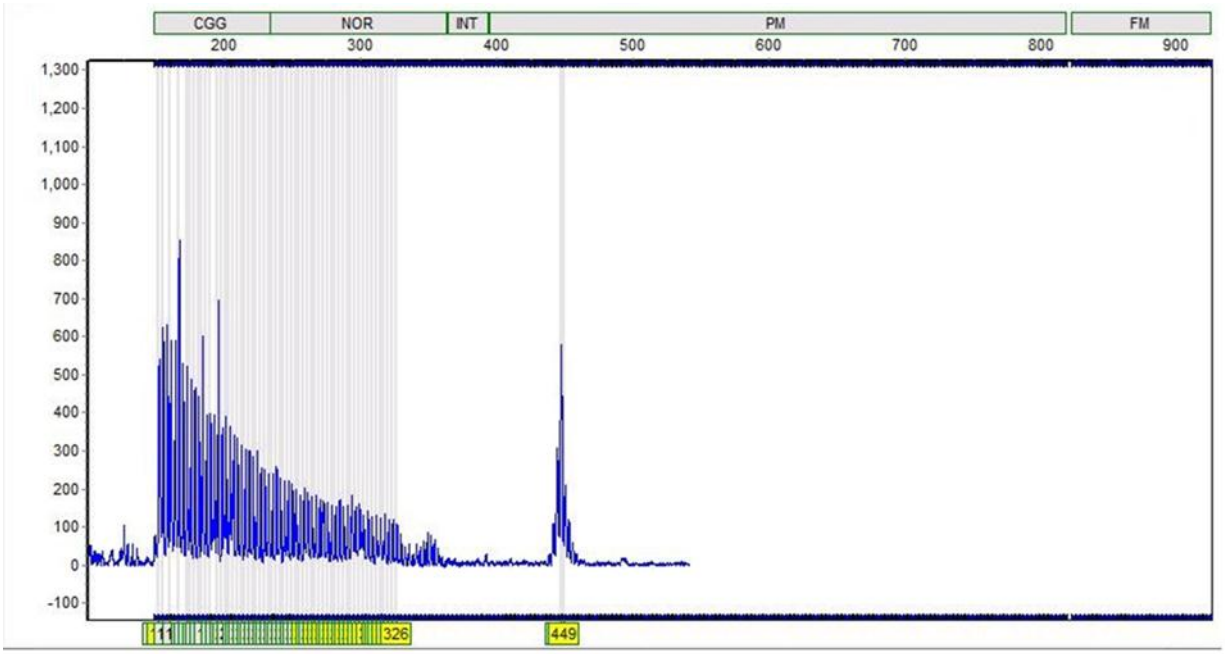

Figure 3. Chromatogram of the proband showing heterozygosity for a premutation allele with accurate sizing of 74 CGGs and an expanded full mutation.

Discussion

A diverse variety of phenotypic spectra, ranging from facial characteristics to premature ovarian insufficiency, intellectual disability, and autistic behavior, have been linked to FXS. Nonetheless, affected females with pre- or full mutation alleles usually present milder phenotypes due to having a second X chromosome with a healthy *FMR1* gene. Three patients with fully methylated premutation alleles have been recently described by Tabolacci et al. (10). This epigenetic alteration is the most likely reason for the milder intellectual phenotype reported in females harboring the *FMR1* premutation allele. Females with mutations in both *FMR1* alleles are rarely reported. Unless a mutation arises in paternal transmission, this rare incidence can only occur if both parents are mutation carriers. Here, we report a consanguineous family with a proband female diagnosed with mild ID, carrying both *FMR1* premutation and full mutation alleles. Some other studies have also reported patients with compound mutations in the *FMR1* gene, with one allele having 55-200 repeats premutation and the other with more than 200 repeats full mutation. Most reported cases, including the case described in the current study, were due to inbreeding or marriages within small and isolated geographical areas with high rates of intellectual disability (ID) among the population (11-12). However, some patients have been reported to be born to non-consanguineous and unaffected parents (4, 13, 14), and some cases were unclear in this regard (15, 16). The high rate of inbreeding in our affected family indicates that consanguineous relationships are the main cause of the compound *FMR1* mutations identified in the female proband. A review of reported cases of female patients with fragile X-associated phenotypes is provided in Table 1.

Table 1. Reported Patients Carrying Both Premutation and Full Mutation Alleles in the *FMR1* Gene.

Row	Age (years)	Consanguinity yes/no	Repeat size	Symptoms	References
1	NA*	NA	S/L**	NA	[38]
2	11	NO	130/730	Borderline ID, long face, prominent occiput and other typical FXS patients' dysmorphisms	[36]
3	15	NO	103/363	Mild ID and FXS	[35]
4	Newborn	NA	55/>200	NA	[37]
5	11	NO	53/480	Mild ID	[28]
6	16	NO	53/650	Severe ID and FXS	
7	15	YES	78/566	Severe ID, long face, prominent forehead and ears, joint laxity	[33]
8	17	YES	63/533	Social phobia and long face	
9	19	YES	58/530	Social phobia, dysthymia and long face	
10	21	YES	60/300	Social phobia, panic disorder, long face, prominent forehead and ears	
11	5	YES	73/670–965	Prader Willi-like phenotype and FXS	[34]
12	65	YES	74/>200	FXPOI, mild ID and FXS	Present study

*NA not available, **S the insert <0.5 Kb (premutation), L the insert >0.6 Kb (full mutation)

Of note, it is widely reported that females with a full mutation in *FMR1* are not usually affected with significant intellectual disability (ID). Extremely skewed inactivation of the X chromosome is probably responsible for the variety of different and distinct characteristics described in females with a fully mutated *FMR1* (17). Using triplet-primed PCR followed by fragment length analysis, we found that the proband had FXS with heterozygous *FMR1* pre- and full mutation alleles.

It is worth noting that some premutation alleles, particularly those with 61-200 repeats, are unstable and could potentially progress to full mutations, capable of transmission from the carrier mother to the offspring during oogenesis. *FMR1* premutations have been shown to be linked to two distinct disorders, including FXPOI and FXTAS. Females with a premutation, particularly those with FXTAS, are at a higher risk of developing reproductive, cognitive, and mental problems than the general population (18). Due to the paucity of reported cases and contributing factors such as methylation status, the expression pattern in somatic cells, CGG repeat numbers, AGG interruptions, genetic background, and environmental effects, all of which complicate genotype-phenotype correlations, no definitive conclusion can currently be drawn regarding the prognosis of affected females with two expanded alleles. However, it has been demonstrated that carriers of double allelic premutations are more likely to develop early-onset FXPOI, which is consistent with findings in the present study.

Patients with compound premutations typically exhibit earlier onset and more severe psychological issues than their parents. The majority of *FMR1* premutation carriers at the same age show psychiatric symptoms, suggesting that compound premutation alleles may predispose to psychiatric disorders. However, since the onset of FXTAS symptoms often occurs around the age of 60, studying this phenotype may be limited to older ages. In the present study, for example, the proband showed late-onset FXTAS, making further follow-

up examinations necessary to comprehensively investigate the phenotype in the next generations.

An affected female with compound heterozygous alleles is the product of two mutant alleles inherited from different parents. The proband's full mutation allele could be the result of a highly unstable premutation allele inherited from either the mother or the father, expanded through gametogenesis. In this scenario, an individual with the unstable premutation allele married their cousin or a member of the isolated village harboring another premutation allele.

The transmission pattern of maternal and paternal *FMR1* alleles to the offspring is not similar. First, even in the case of having a full mutation, males can only carry premutation alleles in their sperm (19). Second, paternal transmission is generally associated with constriction. Therefore, in this family, the affected proband could originate from an FXS male married to a heterozygous female carrying premutation and normal-range alleles; or most likely, from the marriage of a male harboring a premutation allele with a heterozygous female carrying full mutation and normal-range alleles. The proband's male offspring could either be FXS-affected or premutation carriers, which exposes them to FXTAS.

The genotype and consequent clinical picture in the proband's daughters depend on paternal *FMR1* expansion length; outcomes could range from mild ID and FXPOI with premature/early menopause in premutation homozygous individuals to significant ID, FXPOI, autistic features, and other characteristic symptoms in full mutation homozygous patients. Heterozygous female carriers of premutation and full mutation alleles fall between the two mentioned genotypes in respect to clinical manifestations (20).

Conclusion

The expansion of CGG trinucleotide repeats in the non-coding region of *FMR1* causes diverse fragile X-associated phenotypes, including FXS, FXTAS, FXPOI, and related neuropsychiatric features, expressing in a gender-biased pattern. As females with FXS are usually identified by non-typical dysmorphic characteristics as well as autistic behavior and intellectual disability, the diagnosis of FXS should be considered during genetic counseling for affected females with corresponding features, particularly intellectual deficits.

The importance of *FMR1* analysis in females with intellectual phenotypes, especially in the presence of a family history of FXS, is highlighted in this case study. It is also worth considering whether the parents are carriers of *FMR1* aberrant alleles, especially if they are consanguineous or from small, isolated areas. Our findings, in line with literature review, suggest that premutation and full mutation female carriers may experience earlier onset of FXPOI and more pronounced mental issues compared to individuals with only the mutant allele. To further understand the phenotypic profile of compound *FMR1* premutation and full mutation carriers, more extensive research on a considerable number of such patients is required.

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Conflict of interest

Authors declare no conflict of interest.

Authors' contributions

AN: Genetic counseling, Whole Exome Sequencing bioinformatics analysis, primer designing, and Sanger sequencing bioinformatics analysis. MH: Drafting and revising the manuscript. JKH, AS, NJ: Blood sampling, DNA extraction, PCR, Sanger sequencing, and contributing to the writing of the manuscript.

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