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FRAGILE X SYNDROME IN FEMALES – A FAMILIAL CASE REPORT AND REVIEW OF THE LITERATURE

ZESPÓŁ ŁAMLIWEGO CHROMOSOMU X U KOBIET – OPIS PRZYPADKU I ANALIZA LITERATURY

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Abstract

Background: *Fragile X syndrome (FXS), one of the manifestations of FMR1-related disorders, is one of the most frequent genetic causes of intellectual disability. In over 99% of all cases it results from the expansion of CGG repeats in the 5'-untranslated region of the FMR1 gene and presents in males and in about 50% of the females with an FMR1 full mutation, usually with a milder phenotype.*

Objective: *Although the morphologic and behavioral phenotype in males is a well-recognized entity, the presentation in females is variable and not as specific. The objective of this paper is to present a family with quite a severe expression of the disorder in two sisters with a full mutation.*

Methods: *We report on a two-generation family where both males and females were found to be affected by FXS. We also present the diagnostic pathway and methods that led to the diagnosis of fragile X syndrome in the two sisters, as well as the method that explained the normal phenotype in their mother.*

Results: *The CGG repeats analysis in the FMR1 gene showed one normal allele and one allele with a full mutation in both sisters (probands) and their mother. A full mutation was also found in three male cousins of the probands. The analysis of the X-chromosome methylation status has shown a random X inactivation in proband 1 and 2 and a non-random one in the proband's mother, with the normal allele predominantly active.*

Conclusion: *The reasons for different clinical presentations are discussed; moreover a review of the literature on females with FXS is presented. We hope that this paper will facilitate the future diagnosis of fragile X syndromes in females.*

Key words: fragile X syndrome, *FMR1* gene, X-inactivation, female, intellectual disability

Streszczenie

Wstęp: *Zespół łamliwego chromosomu X (fragile X syndrome, FXS) jest związany z mutacjami genu FMR1 i stanowi najczęstszą uwarunkowaną genetycznie przyczynę niepełnosprawności intelektualnej u chłopców. Zespół ten jest związany w ponad 99% przypadków z ekspansją powtórzeń CGG obecnych w niepodlegającym translacji regionie 5' genu FMR1 leżącego na chromosomie X. Objawy choroby obserwuje się u wszystkich mężczyzn i u około 50% kobiet z pełną mutacją, przy czym u kobiet są zazwyczaj mniej nasilone. Chociaż fenotyp morfologiczny i behawioralny tego zespołu u mężczyzn jest dobrze poznany, u kobiet jego manifestacja jest zmienna.*

Cel pracy: *Celem niniejszej pracy jest opis kliniczny dwóch pacjentek – sióstr z pełną mutacją w genie FMR1.*

Metody: *Prezentacja dwupokoleniowej rodziny z mutacją w genie FMR1, opis ścieżki diagnostycznej oraz metod, które doprowadziły do rozpoznania zespołu łamliwego chromosomu X u dwóch sióstr, a także wyjaśnienia fenotypu ich matki.*

Wyniki: *Analiza liczby powtórzeń CGG w genie FMR1 wykazała obecność jednego prawidłowego allela i jednego allela z pełną mutacją u obu badanych sióstr i ich matki. Pełną mutację stwierdzono*

również u trzech kuzynów probandów. Analiza metylacji chromosomu X w limfocytach krwi obwodowej wykazała losową inaktywację tego chromosomu u sióstr oraz nielosową u ich matki, z przeważającym aktywnym chromosomem prawidłowym.
Wnioski: *Omówiono różne postaci kliniczne zespołu FXS u kobiet wraz z przeglądem literatury. Niniejsza publikacja ma charakter edukacyjny i może ułatwić rozpoznanie łamliwego chromosomu X u kobiet.*

Słowa kluczowe: fragile X syndrome, *FMR1* gene, X-inactivation, female, intellectual disability

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INTRODUCTION

Fragile X syndrome (FXS) is the most common worldwide cause of inherited developmental disability [1, 2, 3]. This genetic disorder results from an expansion of CGG repeats present in the 5'-untranslated region (UTR) of the *FMR1* gene (locus Xq27.3; fragile X intellectual disability 1). The size of a normal allele ranges from 6 to 54 CGG repeats (intermediate alleles - 45-54), of premutation alleles - 55 to 200 (usually without clinical features of FXS but associated with FXPOF, fragile X-associated premature ovarian failure in some females or FXTAS, fragile X-associated tremor/ataxia syndrome) and over 200 CGG repeats in full-mutation alleles (wide range of clinical features) [3]. The expansion of trinucleotide repeats leads to the methylation of these repeats' tract, as well as an upstream genomic region rich in CpG islands. It results in *FMR1* gene silencing *via* preventing synthesis of mRNA and loss of the encoded fragile X intellectual disability protein (FRMP). The lack of this protein is detrimental, because of its critical role in neuronal development [3].

FXS is inherited in an X-linked dominant manner with reduced penetrance [2]. Because the deleterious mutation is linked to the X chromosome, FXS is more frequent in males than in females and its incidence is estimated at 1/4000-1/6000 in males and 1/8000 in females [2,4]. The carrier rate is approximately 1 in 750 men and 1 in 250 women [1, 5, 6]. The risk of transmitting the *FMR1* alteration by a female carrier to offspring, male and female, is 50%. All the daughters of FXS men inherit the alteration from their father. The expansion from an intermediate allele to a premutation, as well as from premutation to full mutation may take place during oogenesis [7].

The symptoms of FXS may be observed in both males and females. A dysmorphic phenotype is not very characteristic in males before puberty. The most frequent features include: a high forehead, prominent ears, a high palate, soft skin, connective tissue dysplasia, hyperflexible joints, flat feet. After puberty the classical phenotype is more prominent, especially in adulthood and includes: a larger than average head circumference, a narrow elongated face, a large jaw, long anteflexed ears, macroorchidism. Most females with FXS do not present particular dysmorphic features or have discrete features independent of puberty [5, 6, 8, 9].

Common features in patients with FXS include a wide range of developmental, behavioral or emotional dysfunctions. In boys, FXS usually manifests itself in the form of intellectual disability, behavioral problems, poor speech and language development. Behavioral and emotional characteristics include: attention deficit disorders with or without hyperactivity, anxiety, extreme reaction or aversion to sensory stimuli such as touch, smells, autism-like features, such as poor eye-contact, stereotypic movements (hand flapping and biting), adherence to routine. In adult men moderate to severe intellectual disability, predisposition to seizures, autism-like disorders (ASD - autism spectrum disorders) are observed. Only 20% of the men are diagnosed with mild intellectual disability or borderline deficits [3,4].

Heterozygous for the full mutation females in *FMR1* gene, the patients usually display normal or borderline IQ (the nonverbal IQ score of 85 or less), learning disability, verbal deficits and behavioral and emotional problems, such as social anxiety and gaze avoidance, shyness, moodiness, attention-deficit hyperactivity disorder (ADHD) [4]. Females usually present with milder intellectual impairment than males with FXS [5, 6, 8, 9]. The presence of premutations in the *FMR1* gene increases the risk of a late-onset (after 50 years of age) FXTAS [2, 6]. FXTAS is a neurodegenerative disorder with core features of action tremor and cerebellar gait ataxia. Some frequently associated findings include: parkinsonism, executive function deficits and dementia, neuropathy, and dysautonomia. FXTAS affects males more often and is more severe than in females, moreover symptoms worsen with age [6].

Fragile X premutation female carriers are at high risk of FXPOF and menopause before the age of 40, but without the occurrence of diseases usually associated with menopause such as cardiovascular incidences and osteoporosis [10].

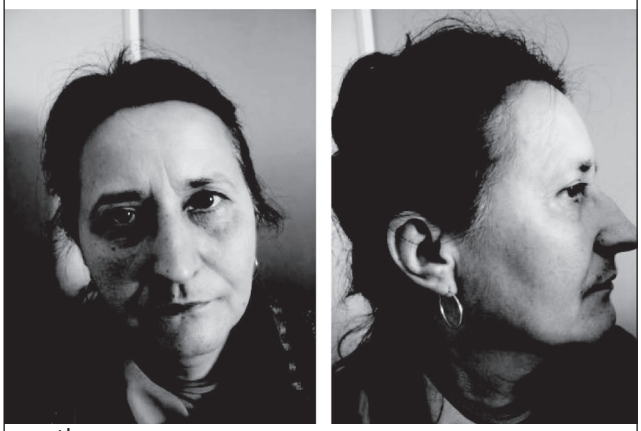
Our report describes two sisters with FXS caused by a full mutation in one allele of the *FMR1* gene and presents an overview of the literature on clinical symptoms in women with this syndrome.

CASE REPORT

We report on a two-generation family where both males and females were found to be affected with FXS. Two sisters (aged 14 and 29) were referred to the Genetic Department because of intellectual disability and similar dysmorphic features (fig. 1a, b, c, d).

younger sister
młodsza siostra

older sister
starsza siostra



mother
matka

Fig. 1. The patient's phenotype.
Ryc. 1. Fenotyp pacjentek.

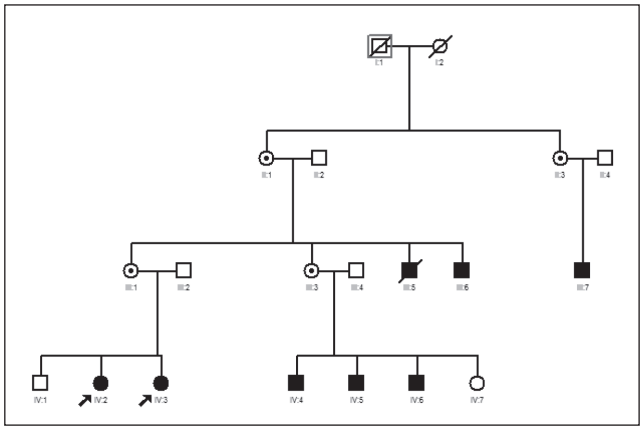


Fig. 2. Family pedigree.
III:1, IV:2, IV:3, IV:4, IV:5, IV:6 – tested, full mutation
IV:1 – tested, no mutation
II:1, II:3, III:3, III:5, III6, III:7, IV:7 - not tested
II:1, II:3, III:1, III:3 - obligatory carrier

Ryc. 2. Rodowód.
III:1, IV:2, IV:3, IV:4, IV:5, IV:6 – badany, pełna mutacja
IV:1 – badany, bez mutacji
II:1, II:3, III:3, III:5, III6, III:7, IV:7- niebadany
II:1, II:3, III:1, III:3 - obligatoryjny nosiciel

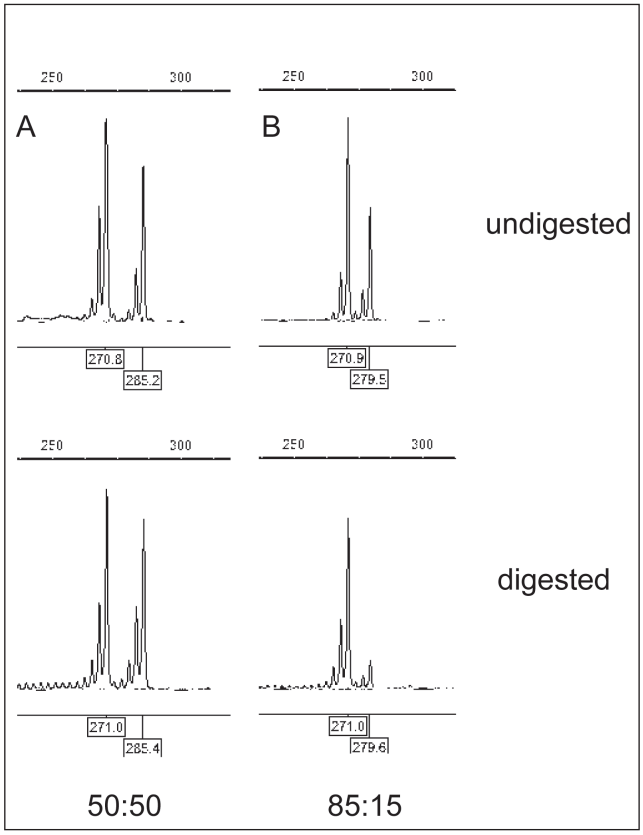


Fig. 3. X inactivation analysis by genotyping of the CAG repeat in the AR gene before and after digestion. A) an example of the random X inactivation (younger sister) B) an example of the highly skewed X inactivation (probands' mother).

Ryc. 3. Analiza inaktywacji chromosomu X, obraz genotypowania powtórzeń CAG genu AR przed i po trawieniu. A) przykład losowej inaktywacji chromosomu X (młodsza siostra) B) przykład nielosowej inaktywacji chromosomu X (matka probandów).

The probands were the first (GI, PI) and the third (GIII, PIII) of three children (GII, PII – healthy son) born to nonconsanguineous, healthy parents. Both probands were born at term by vaginal delivery with a birth weight of 3600g and 4900g and a birth length of 53cm and 65 cm, respectively. Apgar scores in the first minute were 10 and 9, respectively. Prenatal and perinatal histories in both cases were not remarkable. No congenital defects were found. Both girls reached their developmental milestones on time, but with age emotional dysfunctions, such as an inadequate response to a situation, attention and concentration difficulties, anxiety and mild social withdrawal, language delay, or sensory integration dysfunction became apparent. At the time of examination they presented similar phenotypes. Both of them were diagnosed with moderate intellectual disability (in the older proband – of regressive form), social anxiety, perseverative and repetitive language (in the younger proband). Similar dysmorphic features (more pronounced in the older proband) included: a long face, high anterior hairline, hypotelorism, low-set posteriorly rotated and anteverted ears, prominent nosolabial folds and narrow nasal bridge. Additionally, in the younger proband there are short and narrow palpebral fissures, a marked jaw, a long and large nose. In the elder sister: adnate earlobes, mild proptosis, a short prominent nose, a long philtrum, full lips and a marked chin. Both sisters had soft skin and hyperflexible joints. Their general and neurological examinations were normal.

The family history revealed intellectual disability in men (*see* family pedigree, fig. 2). The photos of men with FXS in this family are not available.

The proband's mother is healthy and exhibits normal intelligence. Some discrete dysmorphic features such as a long face, a large nose, long ears, a prominent chin, thin upper and lower lips were observed (Fig. 1e,f).

MATERIAL AND METHODS

Cytogenetic analysis

The metaphase chromosomes from blood lymphocytes were prepared according to standard procedures [11]. Chromosome analysis was performed using the GTG banding technique on 15 methaphases at the 450-550 band level according to ISCN 2013 [12]. Chromosome banding was analyzed using the Imager.M1 (Zeiss) microscope and Ikaros software (Metasystems DE).

Molecular testing

DNA for molecular tests was isolated from whole blood using the QIAamp DNA mini kit (Qiagen) following the manufacturer's protocol.

Southern blot

The analysis of CGG repeats in *FMR1* gene was performed using Southern blot according to the standard protocol. DNA samples were digested with EcoRI and NruI and for hybridization and detection pFxa1NHE probe and the Sure Blot Chemi Hybridization & Detection kit (Millipore Corporation, Billerica, MA, USA) was used.

Inactivation of X-chromosome – analysis of the methylation status of the androgen receptor gene.

DNA methylation assay for X-chromosome inactivation was prepared in the following manner: 250 ng of DNA was digested with 10U HpaII with the appropriate buffer (Yellow, Fermentas) in 10µl of total volume at 37°C overnight. The enzyme was subsequently inactivated at 65°C for 20 minutes. The PCR mixture was prepared using: 10µl of the digested DNA, 0.5µl of each primer (10mM) with a forward primer labeled with FAM at 5'end (Forward AR: 5' FAM-TCCAGAATCTGTTCCAGAGCGTGC and Reverse AR: 5' GCTGTGAAGGTTGCTGTTCCTCAT), 2µl dNTPs (40mM, Fermentas), 2.5µl buffer (10x, Qiagen), 0.3µl HotStart DNA Polymerase (5µ/µl Qiagen) and dH_2O in the total volume of 25 µl.

The cycle parameters were: 95°C 15min, 95°C 30sec, 65°C 30sec, 72°C 30sec for 35 cycles, and 72°C 5min of final extension in an MJ thermocycler.

PCR products were diluted 20 times with water and separated using ABI 310 Genetic Analyser with GeneScan Analysis software version 3.1.2 (Applied Biosystems) with POP-4 Polymer (Applied Biosystems) and LIZ 500 size standard (Applied Biosystems). The X chromosome inactivation rate was calculated using GeneMarker software, version 1.85 (SoftGenetics LLC) as the ratio of the height of the shorter peak of the two peaks after digestion. For each patient the analysis was performed twice (see fig. 3).

RESULTS

The cytogenetic analysis of patients' chromosomes revealed normal karyotypes [46,XX].

The CGG repeats analysis in the *FMR1* gene using PCR and Southern blot showed one normal allele with 18 (±1) repeats of CGG and one allele with a full mutation (>200 CGG) in both sisters. In the probands' mother one normal allele with 27(±1) repeats of CGG and one allele with a full mutation was found.

A full mutation was also found in three male cousins of the probands (see fig.2; IV:4, IV:5, IV:6). The family's medical history has also shown that the uncle of the probands (their mother's brother) had intellectual disability but there is no molecular diagnosis (see fig.2; III:6).

The analysis of the X-chromosome methylation status has shown two different PCR products for the *AR* gene: 273bp and 286bp for both probands and 273bp and 281bp for their mother. It was proven that the X inactivation was random in proband 1 (50:50%) and proband 2 (50:50%) and non-random in the probands' mother (15:85%), with the normal allele predominantly active.

DISCUSSION

Fragile X syndrome an X-linked disorder that may be diagnosed in both males and females with variable clinical phenotype, often with non-specific clinical features and IQ that ranges from normal or borderline (in women) to intellectual impairment. Usually FXS, especially in men, is connected with intellectual disability and might

also be associated with different spectrum of autism-like features, developmental delay, learning disability and dysmorphic features, which become characteristic in adulthood [4]. Because of a relatively high prevalence of FXS and variable clinical phenotype, the molecular test for FXS is a standard procedure in genetic evaluation of all patients with developmental delay [4].

Early diagnosis allows the implementation of an appropriate therapeutic intervention program for children with FXS and genetic counseling for the family.

Females presenting FXS with full mutation are usually relatively mildly affected. They have non-specific clinical features, and may have normal IQ, however approximately 50-70% of the females are intellectually impaired (IQ less than 85) [1, 2, 13]. Other studies found that about 25% of the girls with FXS present with IQ less than 70, and about 28% girls with IQ between 70 and 84 (borderline range) [14]. More often emotional and behavioral features dominate the clinical picture [13]. In females with full learning disability, such features as school-based anxiety, social anxiety and avoidance, excessive shyness, withdrawal and/or emotional problems, depression, autistic behaviors and many others are observed [8,13]. These problems become apparent when taking family history but typically are not the reason for genetic counseling of female patients. Because of this, the FXS diagnosis in females may be overlooked during clinical testing [1]. One of the symptoms present in about 20% of women with the premutation of the *FMR1* gene is premature ovarian failure, so it is irrelevant during genetic counseling of the younger patients [4].

We report a case of two sisters with FXS referred for genetic counseling because their healthy brother was seeking advice on the risk of having intellectually impaired offspring. Our patients, finally diagnosed with FXS, presented with moderate intellectual disability and facial dysmorphic features. The molecular analysis of the family revealed that both the proband's mother and the mother's sister are full mutation carriers.

Because of the lack of FXS symptoms in the mother, and the presence of FXS in both her daughters, we analyzed the methylation status of the X chromosome and found random and nonrandom methylation patterns in both probands and their mother, respectively.

The variability of the phenotype in *FMR1* full mutation carrier females results from the protection of the normal *FMR1* allele: the amount of expressed protein and X inactivation skewing in various tissues. The higher the expression of the normal allele in more tissues, the milder the symptoms that are present [15, 16, 17]. In most studies the level of inactivation of the X chromosome is analyzed in peripheral blood lymphocytes.

We assessed the X-chromosome inactivation patterns in DNA peripheral blood lymphoctes in these families using the polymorphism of the CAG repeats of the androgen receptor (*AR*) gene. In the proband's mother a nonrandom methylation pattern was observed. The normal allele predominantly remained unmethylated and this is responsible for the normal phenotype without clinical features characteristic for FXS. Both her daughters presented a random methylation pattern of the analyzed sequence. Phenotypic differences among female patients with full mutation in the *FMR1* allele are the consequence of skewed X-chromosome inactivation as reported previously [1, 16, 17]. For example Heine-Suner et al. reported two sisters, both with full mutation in one allele of the *FMR1* gene. In the first sister with intellectual disability, the normal copy of the *FMR1* gene was totally methylated (non-random methylation pattern), in the second sister with mild delay, about 70% of the cells had an unmethylated normal copy of the *FMR1* gene and normal protein level [17].

In contrast to these results, Chaste at al. [2012] described two sisters without intellectual deficits and with full mutation in the *FMR1* gene. A thirty year-old female was diagnosed with autism spectrum disorder, IQ in the low normal range and a long face with a high forehead and normal ears, obesity and hirsutism. Her sister (a twenty-eight-year-old female) had major difficulties in social interactions, speech disturbance, no dysmorphic features and obesity. Although the X inactivation profile, assessed at the AR locus, was normal in these sisters (45:55 and 47:53), the authors suggested that the lack of intellectual disability in the probands could be linked to skewed X inactivation in favor of the allele without mutation [15]. Our findings are consistent with their results, which may suggest a wider genetic background of clinical features and mental disabilities in FXS female patients [15]. Tissue mosaicism, mosaicism of *FMR1* repeats expansion or incomplete *FMR1* promoter methylation and also other rare mutations/CNVs undetectable in patients or various environmental factors may influence the phenotype of FXS patients [15].

CONCLUSIONS

Fragile X syndrome as a pathology in which the cognitive and behavioral phenotype plays a role in children's education and social life, should be diagnosed in early childhood to allow adequate management and genetic counselling for the family [4]. Because of the non-typical dysmorphic features and autism or autistic behavior in girls affected with FXS, the diagnosis of fragile X syndrome should be considered during genetic counseling of every female with mental disability or ASD.

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