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FMR1 alleles in women with idiopathic infertility

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ABSTRACT

Introduction: The frequency of the premutation alleles of the *FMR1* gene varies from 1:100 to 1:260 Israeli, Canadian, Finnish and American women, but it is unknown in Brazil. Premutation carriers may have reduced reproductive age and are at risk of transmitting the expanded allele to their offspring, and consequently fragile X syndrome. **Objective:** To observe the distribution range of the *FMR1* gene alleles in a population of women with idiopathic infertility, without symptoms of premature ovarian insufficiency. **Methods:** The presence of premutation in *FMR1* was assessed by conventional PCR, agarose, and acrylamide gel and analysis of fragments in capillary electrophoresis. Lymphocyte DNA obtained from 283 women undergoing infertility treatment was analyzed. **Results:** 169 patients had the normal heterozygous allele (59.7%), 114 had the normal homozygous allele (40.6%) and no patient had the premutation. Premature ovarian insufficiency is seen in 20 to 30% of women with the permutated allele. Thus, the condition can be asymptomatic in a large part of the premutation carriers. Brazil has a diverse population and, therefore, the allele frequencies of many gene variants are unknown. Previous Brazilian studies have shown a low frequency of the premutation allele in different patient cohorts. Corroborating these articles, the results demonstrated that the frequency of the premutation allele is low in the infertile women population studied. **Conclusion:** Tracking the size of the *FMR1* gene alleles allows the expansion of knowledge about the frequency of risk alleles associated with genetic diseases in the Brazilian population.

Keywords: Fragile X Syndrome; alleles; mutation; primary ovarian insufficiency.

INTRODUCTION

The *FMR1* (Fragile X messenger ribonucleoprotein 1) gene is located on the long arm of the X chromosome, at Xq27.3, where it encodes at least 12 different types of mRNA, originating from the alternative splicing method, with expression in several tissues, especially in the brain, testis, ovaries, and epithelium. The protein resulting from the expression of this gene is called FMRP (Fragile X messenger ribonucleoprotein 1)[1].

In 1991, an unstable and expansive CGG trinucleotide sequence was identified in the untranslated region (5'UTR) of the *FMR1* gene, resulting in variations in gene size, classified into three allelic classes[2]. The normal allele has between 6-54 CGG trinucleotide repeats, with normal FMRP production and the individual's phenotype is normal[3]. An intermediate-range of repeats, between 41 and 54 CGG, is described as the gray zone. Although this range is within the normal range, some studies point out

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that this region may increase over generations, possibly evolving into premutation[2,3].

Alleles with more than 200 repeats, known as full mutations, determine the Fragile X Syndrome, the main genetic cause of intellectual disability in boys[4,5] and originate, in general, from mothers carrying the premutation or the full mutation. In the presence of the complete mutation, *FMR1* gene inactivation occurs due to methylation of the gene promoter region and, consequently, the absence of FMRP[6].

Premutation is considered to be the range of alleles between 55-200 repeats of CGG[1]. These alleles are unstable and can expand to full mutation when transmitted by a female. The risk of expansion to a full mutation in the next generation is directly related to the size of the repeats and the absence of the AUG sequence in premutations. In general, an altered phenotype is not observed in the premutation range[4].

Female carriers of the premutation may experience symptoms such as premature ovarian insufficiency (POI), before the age of 407, fragile x-associated tremor and ataxia syndrome (FXTAS), after the age of 50[8], thyroid disease, hypertension, dizziness, peripheral neuropathy, and fibromyalgia[9], as well as menopause-related symptoms such as cardiovascular disease and reduced life expectancy[10].

Researchers suggest that POI represents a continuum of ovarian conditions. Such conditions begin with an "occult" clinical case, where in some cases reduced fertility is seen, but normal follicle-stimulating hormone (FSH) levels and regular menstrual cycle, followed by a "biochemical" stage, where fertility is indeed reduced, followed by high FSH levels, but regular menstrual cycle. The last stage, "open", approximates premature ovarian insufficiency, although with irregular menstruation[11]. Therefore, among the infertile patients selected for the research, cases of the hidden clinical stage could be found, in other words, still without climacteric or hormonal symptoms, but with a high risk of early menopause and expansion of the premutation allele.

The study aimed to investigate the distribution of *FMR1* gene alleles in women of reproductive age who sought the Ideia Fértil Institute with complaints of idiopathic infertility and to expand knowledge about the allele frequency in our population.

METHODS

The project was approved by the Research Ethics Committee of the Centro Universitário FMABC, registered under number 2.597.619.

Blood samples were collected from women of reproductive age, residents of Metropolitan area of São Paulo, who underwent assisted reproduction treatment at the Ideia Fértil Institute and who met the inclusion criteria.

Inclusion criteria were age less than 40 years, no ovarian surgery, chemotherapy or radiotherapy, normal hormone levels (including FSH), absence of thyroid alterations, absence of ovarian alterations observed on transvaginal ultrasound, and absence of complaints of menstrual irregularity. The exclusion parameters: symptoms of premature ovarian insufficiency (high FSH levels and irregular menstruation), age over 40 years, primary or secondary amenorrhea, and karyotype alterations involving the X chromosome. Clinical data of the patients were obtained from medical records and a questionnaire answered by the patients.

To obtain DNA, 5 mL of peripheral blood was collected in a tube containing EDTA. DNA was extracted from the leukocytes present by the salting-out method[12].

Determination of *FMR1* allele size was performed by DNA amplification by Polymerase Chain Reaction (PCR), according to the protocol proposed by Tassone et al.[13] This was followed by 3% agarose gel electrophoresis.

To estimate the number of CGG repetitions, from the size of the band obtained after electrophoresis, compared to a molecular weight marker and the control sample, the value of 221 bp (base pairs) that corresponds to the amplification of the non - repetitive region of the amplified DNA fragment was subtracted. The value obtained was divided by three, corresponding to the three nitrogenous bases observed in the repetitive region (CGG). Therefore, the final value corresponds to the number of repeats that the patient has, that is, the size of the alleles[13]. This methodology was applied to all samples.

The PCR product from 61 samples showing a single allele on the agarose gel was applied to a 12% polyacrylamide gel (GE Healthcare GeneGel Excel 12.5/24 Kit - GE Healthcare Bio-Sciences AB), using a heterozygous sample with two full-length alleles as a control[14].

For the samples in which there was doubt about allele size, fragment analysis was performed using the FragilEase kit (PerkinElmer). Then purification of the PCR product was performed using the Pure Link® kit The purified PCR product was placed in an Applied Biosystem 3500 Genetic Analyzer sequencer, which used the 3500 Series Data, Collection Software 3, properly configured, to analyze the number of *FMR1* gene fragments. The results were analyzed using the FraXsoft, software, available for download from PerkinElmer.

RESULTS

Twenty-four new blood samples were collected from patients who met the inclusion criteria and had their DNA extracted. The remaining 259 samples used in this research came from the Biobank of the Ideia Fértil Institute (Biobank Register CONEP B-061-Process Nº. 25000.091276/2016-95). Of these, there was no amplification for sixteen samples.

After performing the amplification techniques and briefly compiling the results, the allele size of *FMR1*, present in 283

analytes, was determined. The results are available in Table 1. The mean age among the selected patients was 32 years (Standard deviation = 3.56).

DISCUSSION

The *FMR1* gene is a gene transcription factor, expressed primarily in the brain. This gene has a CGG trinucleotide repeat region, which can vary in size between individuals and is subject to expansion to a larger allele size when transmitted from a female during meiosis[5].

The intermediate-sized allele has been associated with negative repercussions on women's reproductive life, culminating in the occurrence of premature menopause[15], observed in 20-30% of premutation carriers and imposing the risk of expansion of the allele to a full mutation in offspring[16-19]. Despite the risk, the frequency of the premutation allele in women of reproductive age without symptoms of POI is poorly known.

A Swiss study to determine the size of the *FMR1* allele was performed with 27 case samples with low ovarian reserve, high FSH levels, low anti-Müllerian hormone (AMH) levels, and/or low response to hormone stimulation, and with 32 control samples from patients without genetic or intellectual ability-related problems. Three case samples (5.6%) and one control (1.6%) showed premutation[20]. Another study in Massachusetts (USA) compared 535 samples obtained from women with a low ovarian reserve and high FSH levels with a group containing 521 samples from egg donors and women without ovarian dysfunction, to determine the frequency of premutation alleles in both groups. Among the cases, seven (1.3%) showed premutation, compared to only one control (0.19%)[21]. These studies indicated that the number of CGG repeats, and the frequency of premutation alleles are increased in women with low ovarian reserve of European and American origin[22].

A study in Lucknow (India) evaluated 300 women of reproductive age with no history of ovarian complications and with healthy children, women with low ovarian function showing high FSH values (10U/L at 2-4 days of the menstrual cycle) and low anti-Müllerian hormone values (AMH: 0.2-0.7 ng/mL) and premenopausal women and found that 1.7% were carriers of alleles in the gray zone and 0.3% had premutation[23].

Table 1: Distribution of *FMR1* gene alleles in 283 DNA samples from women of reproductive age according to allelic class.

Allelic Class	Number of repetitions	Frequency
Normal	<10 CGG	0 (%)
	11-26 CGG	12 (4.12%)
	27-40 CGG	115 (40.64%)
	41-54 CGG	156 (55.12%)
Premutation	55-200 CGG	0 (0%)
Full mutation	>200 CGG	0 (0%)

Screening for *FMR1* gene premutation is not established in the minimal propaedeutic of the infertile couple and the frequency of the permutated allele is not known in Brazilian women. Since the patients had reproductive intentions, it seemed interesting to know the risk associated with the presence of the *FMR1* premutation allele, allowing to perform reproductive genetic counseling to the patients and to organize variant screening procedures, if necessary.

Previous frequency studies have estimated that premutation alleles in females in the world population range from 1/100 to 1/260 in Israelis, Canadians, and Finns[24]. *FMR1* gene premutation occurs in about 1:800 males and 1:100-200 females in the US population[14]. However, the frequency of the premutation allele in Brazil seems to be lower than in these populations.

Previous Brazilian studies have not identified premutation alleles in the general population. A study involving DNA samples from 386 men and women with intellectual disability, conducted in the Northeast region of the country, found no premutation allele[25]. Another group also found no premutation alleles among 511 men in the general population of Salvador[14]. In the Southeast, 100 men from the general population of Rio de Janeiro were evaluated and one carrier of the premutation was found[26], while in São Paulo, researchers found no premutation allele in a sample composed of 58 men and women with intellectual disability[27]. In the South, 22 men and 100 women suspected of suffering and/or being carriers of Fragile X Syndrome were evaluated, and no premutation alleles were observed[28]. Thus, the Brazilian studies previously carried out focused more on patients with intellectual disability and on men in the general population not allowing to know the allele frequency in women, or the reproductive characteristics of the population sample.

In the present study, none of the 283 female samples presented the *FMR1* gene pre- mutated allele. Therefore, the frequency of the allele in the infertile population seems to be low compared to worldwide data but corroborates the findings of other Brazilian samples investigated. However, it is important to highlight that 54.9% of the samples evaluated were in the gray zone, which can increase with the passing of generations giving rise to premutation[29]. This shows the need to monitor the size of the *FMR1* gene alleles in the families of these patients, especially in women with reproductive desires.

In our sample, we observed amplification failure of 16 DNA samples. A possible cause for the amplification failure is the degradation of the stored DNA. In this project, we used samples from a Biobank. Such samples were collected over 10 years and the quality of some samples may have been compromised over time. In addition, the salt extraction methodology (salting-out method) used to obtain DNA from these samples is a technique that leaves residues that can compromise DNA amplification. Additionally, the repetitive regions, rich in GC have difficult amplification and demand excellent quality samples. However, we

cannot completely exclude the possibility of amplification failure due to the presence of expanded alleles.

Despite the low frequency in the population, the identification of premutation alleles in the infertile population allows the proper genetic counseling to families, helping the couple in the decision about their reproductive future, since, in case a risk allele is identified, they will be informed about the chances of transmission to their descendants and ways to prevent the continuity of the allele transmission. This investigation should be mandatory in women with high FSH and low AMH levels due to idiopathic causes, in patients with symptoms of premature menopause, and mothers, sisters, and maternal aunts of patients with an idiopathic intellectual disability or with a diagnosis of Fragile X Syndrome. Among the reproductive options available to these couples are in vitro fertilization with donated eggs or *in vitro* fertilization associated with embryo genetic analysis (PGT – preimplantantion genetic testing) allowing the selection of embryos of both sexes, free of the mutation.

Conclusion

The current research has concluded that the frequency of premutation alleles in asymptomatic infertile women is low. However, the severity of symptoms associated with their presence warrants investigation of *FMR1* gene premutation in a reproductive age population, with or without symptoms of premature menopause and infertility.

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REFERENCES

1. Ferreira JFB, Batista JS, Fantin C. Screening for *FMR1* expanded alleles in patients with Autism Spectrum Disorders in Manaus, Northern Brazil. An Acad Bras Cienc. 2019;91(3):e20180882. https://doi.org/10.1590/0001-3765201920180882

2. Villate O, Ibarluzea N, Maortua H, de la Hoz AB, Rodriguez-Revenga L, Izquierdo-Álvarez S, et al. Effect of AGG Interruptions on *FMR1* Maternal Transmissions. Front Mol Biosci. 2020;7:135. https://doi.org/10.3389/fmolb.2020.00135

3. Zhao C, Liu Y, Wang Y, Li H, Zhang B, Yue Y, et al. A Chinese case of fragile X-associated tremor/ataxia syndrome (FXTAS) with orthostatic tremor: case report and literature review on tremor in FXTAS. BMC Neurol. 2020;20(1):145. https://doi.org/10.1186/s12883-020-01726-z

4. Nolin SL, Glicksman A, Tortora N, Allen E, Macpherson J, Mila M, et al. Expansions and contractions of the FMR1 CGG repeat in 5,508 transmissions of normal, intermediate, and premutation alleles. Am J Med Genet A. 2019;179(7):1148-56. https://doi.org/10.1002/ajmg.a.61165

5. Mila M, Mora MIA, Madrigal I, Rodriguez-Revenga L Fragile-X syndrome: An Overview and Update of the FMR1 Gene. Clin Genetic. 2018;93(2):197-205. https://doi.org/10.1111/cge.13075

6. Tseng E, Tang HT, AlOlaby RR, Hickey L, Tassone F. Altered expression of the *FMR1* splicing variants landscape in premutation carriers. Biochim Biophys Acta Gene Regul Mech. 2017;1860(11):1117-26. https://doi.org/10.1016/j.bbagrm.2017.08.007

7. Huang J, Zhang W, Liu Y, Liu Y, Wang J, Jiang H. Association between the *FMR1* CGG repeat lengths and the severity of idiopathic primary ovarian insufficiency: a meta analysis. Artif Cells Nanomed Biotechnol. 2019;47(1):3116-22. https://doi.org/10.1080/21691401.2019.1645153

8. Hwang YT, Aliaga SM, Arpone M, Francis D, Li X, Chong B, et al. Partially methylated alleles, microdeletion, and tissue mosaicism in a fragile X male with tremor and ataxia at 30 years of age: A case report. Am J Med Genet A. 2016;170(12):3327-32. https://doi.org/10.1002/ajmg.a.37954

9. Hunter JE, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K, et al. Depression and anxiety symptoms among women who carry the FMR1 premutation: impact of raising a child with fragile X syndrome is moderated by CRHR1 polymorphisms. Am J Med Genet B Neuropsychiatr Genet. 2012;159B(5):549-59. https://doi.org/10.1002/ajmg.b.32061

10. Tassanakijpanich N, Cohen J, Cohen R, Srivatsa UN, Hagerman RJ. Cardiovascular problems in the fragile X premutation. Front Genet. 20208;11:586910. https://doi.org/10.3389/fgene.2020.586910

11. Welt CK. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin Endocrinol. 2008;68(4):499-509. https://doi.org/10.1111/j.1365-2265.2007.03073.x

12. Lahiri DK, Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 1991;19(19):5444. https://doi.org/10.1093/nar/19.19.5444

13. Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. A rapid polymerase chain reaction-based screening method for identification of all expanded alleles of the fragile X (*FMR1*) gene in newborn and high-risk populations. J Mol Diagn. 2008;10(1):439. https://doi.org/10.2353/jmoldx.2008.070073

14. Goméz MKA, Acosta AX. Estudo dos alelos da região 5´UTR no gene FMR1 (Fragile X Mental Retardation 1) em homens da população geral de Salvador-BA. [master´s thesis]. [Salvador]: Fundação Oswaldo Cruz; 2011.

15. Tang R, Chen R, Luo M, Lin S, Yu Q. Chinese women with 29-30 *FMR1* CGG repeats have an earlier menopause. Climacteric. 2020;23(3):298-305. https://doi.org/10.1080/13697137.2020.1727877

16. Asadi R, Omrani MD, Ghaedi H, Mirfakhraie R, Azargashb E, Habibi M, et al. Premutations of FMR1 CGG repeats are not related to idiopathic premature ovarian failure in Iranian patients: A case control study. Gene. 2018;676:189-94. https://doi.org/10.1016/j.gene.2018.07.034

17. Murray A, Schoemaker MJ, Bennett CE, Ennis S, Macpherson JN, Jones M, et al. Population-based estimates of the prevalence of FMR1 expansion mutations in women with early menopause and primary ovarian insufficiency. Genet Med. 2014;16(1):19-24. https://doi.org/10.1038/gim.2013.64

18. Bussani C, Papi L, Sestini R, Baldinotti F, Bucciantini S, Bruni V, et al. Premature ovarian failure and fragile X premutation: a study on 45 women. Eur J Obstet Gynecol Reprod Biol. 2004;112(2):189-91. https://doi.org/10.1016/j.ejogrb.2003.06.003

19. Allen EG, Sullivan AK, Marcus M, Small C, Dominguez C, Epstein MP, et al. Examination of reproductive aging milestones among women who carry the FMR1 premutation. Hum Reprod. 2007;22(8):2142-52. https://doi.org/10.1093/humrep/dem148

20. Streuli I, Fraisse T, Ibecheole V, Moix I, Morris MA, Ziegler D. Intermediate and premutation FMR1 alleles in women with occult primary ovarian insufficiency. Fertil Steril. 2009;92(2):464-70. https://doi.org/ 10.1016/j.fertnstert.2008.07.007

21. Karimov CB, Moragianni VA, Cronister A, Srouji S, Petrozza J, Racowsky C, et al. Increased frequency of occult fragile X-associated primary ovarian insufficiency in infertile women with evidence of impaired ovarian function. Hum Reprod. 2011;26(8):2077-83. https://doi.org/10.1093/humrep/der168

22. Pastore LM, Johnson J. The *FMR1* gene, infertility, and reproductive decision-making: a review. Genet. 2014;5:195. https://doi.org/10.3389/fgene.2014.00195

23. Dean DD, Agarwal S, Kapoor D, Singh K, Vati C. Molecular Characterization of FMR1 Gene by TP-PCR in Women of Reproductive Age and Women With Premature Ovarian Insufficiency. Mol Diagn Ther. 2018;22(1):91-100. https://doi.org/10.1007/s40291-017-0305-9

24. Filipovic-Sadic S, Sah S, Chen L, Krosting J, Sekinger E, Zhang W, et al. A novel *FMR1* PCR method for the routine detection of low abundance expanded alleles and full mutations in fragile X syndrome. Clin Chem. 2010;56(3):399-408. https://doi.org/10.1373/clinchem.2009.136101

25. Silva RG, Silva LM. Detecção de expansões CGG na população do estado de Pernambuco e verificação de sua relação com a síndrome do x-frágil. [dissertation]. [Recife]: Universidade de Pernambuco; 2004.

26. Sucharov CC, Silva R, Rondinelli E, Moura-Neto RS. Fragile X trinucleotide repeats from a normal population in Rio de Janeiro, Brazil. Hereditas. 1999;130(2):189-90. https://doi.org/10.1111/j.1601-5223.1999.00189.x

27. Mingroni-Neto RC, Rosemberg C, Vianna-Morgante AM, Pavanello RC. Fragile X frequency in a mentally retarded population in Brazil. Am J Med Genet. 1990;(35):22-7. https://doi.org/10.1002/ajmg.1320350106

28. Queiroz MA. Avaliação de Pré-mutação por PCR na Síndrome do X Frágil, 2006. [master´s thesis]. [Florianópolis]: Universidade Federal de Santa Catarina; 2006.

29. Rosot N, Franco VDF, Riechi TIJS. A Síndrome do X Frágil e o estabelecimento de fenótipos cognitivo-comportamentais: uma revisão sistemática de literatura. Cienc Cognição. 2017;22(1):30-40.