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FMR1 allelic complexity in premutation carriers provides no evidence for a correlation with age at amenorrhea

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Abstract

Background Premutations in the Fragile X Messenger Ribonucleoprotein 1 (*FMR1*) gene, defined as between 55 and 200 CGGs, have been implicated in fragile X-associated primary ovarian insufficiency (FXPOI). Only 20% of female premutation carriers develop early ovulatory dysfunction, the reason for this incomplete penetrance is unknown. This study validated the mathematical model in premutation alleles, after assigning each allele a score representing allelic complexity. Subsequently, *allelic scores* were used to investigate the impact of allele complexity on age at amenorrhea for 58 premutation cases (116 alleles) previously published.

Methods The *allelic score* was determined using a formula previously described by our group. The impact of each *allelic score* on age at amenorrhea was analyzed using Pearson's test and a contour plot generated to visualize the effect.

Results Correlation of *allelic score* revealed two distinct complexity behaviors in premutation alleles. No significant correlation was observed between the *allelic score* of premutation alleles and age at amenorrhea. The same lack of significant correlation was observed regarding normal-sized alleles, despite a nearly significant trend.

Conclusions Our results suggest that the use of *allelic scores* combination have the potential to explain female infertility, namely the development of FXPOI, or ovarian dysfunction, despite the lack of correlation with age at amenorrhea. Such a finding is of great clinical significance for early identification of females at risk of ovulatory dysfunction, enhancement of fertility preservation techniques, and increasing the probability for a successful pregnancy in females with premutations. Additional investigation is necessary to validate this hypothesis.

Keywords *FMR1* gene premutation, Age at amenorrhea, *FMR1* allelic complexity, Fragile X-associated primary ovarian insufficiency, CGG repeats, AGG interspersion pattern

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Background

The *Fragile X Messenger Ribonucleoprotein 1* (*FMR1*) gene, located on the X chromosome (Xq27.3), contains a polymorphic CGG repeat on its 5' untranslated region (UTR) implicated in three disorders depending on the repeat number: Fragile X Syndrome (FXS; OMIM #300624) when CGGs >200, and Fragile X-associated Tremor/Ataxia Syndrome (FXTAS; OMIM #300623) and Fragile X-associated Primary Ovarian Insufficiency (FXPOI; OMIM #311360) [1–3] in the premutation range 55<CGG<200. The mechanism of FXPOI development is not fully understood but it is believed to be due to the toxic effect of elevated *FMR1* mRNA levels [2, 4]. Premutation carriers with FXPOI show hypergonadotropic hypogonadism and absent or irregular menstrual cycles before 40 years of age [5]. The CGG repeat length correlates unevenly with FXPOI, as females carrying 70 to 100 CGGs have an increased risk of FXPOI when compared with those with more than 100 repeats [6, 7]. Furthermore, FXPOI development is not fully penetrant. In *FMR1* normal-sized alleles (5 to 44 CGGs), the repetitive region is usually interrupted by one or more AGGs, typically occurring at every 9th or 10th CGG [8]. Premutation alleles are predominantly composed of pure CGGs; loss of AGG interruption(s) has been linked to the instability of the repetitive region and the increased risk of expansion [3, 9–13]. A formula integrating the total repeat length, and the number and pattern of the AGGs was developed to calculate *FMR1 allelic score* [14]. *Allelic score* is a metric that reflects the complexity of the *FMR1* gene CGG repetitive region. Herein, we evaluate the association between the combination of normal and premutation *allelic scores* and ovulatory dysfunction underlying FXPOI. Our formula was applied to calculate the *allelic scores* in *FMR1* premutation carriers, validating its use in samples with distinct genotypic characteristics. It was hypothesized that the combination of the AGG number and pattern from both normal and premutation alleles would associate indirectly with age at amenorrhea and hormone levels with a potential impact on FXPOI development.

Methods

FMR1 allelic scores determination

Molecular data of both alleles regarding samples from premutation carriers, previously published, were requested to the respective authors: Villate et al. [15] (Spain), Allen et al. [16] (United States of America) and Yrigollen et al. [17] (United States of America). Of all data provided by the authors, 577 results were retrieved: Villate et al. [15] (n=20, designated by set 1), Allen et al. [16] (n=59, designated by set 2) and Yrigollen et al. [17] (n=498, designated by set 3). The *allelic score*, which reflects the *FMR1* CGG/AGG substructure, was calculated separately for each allele (normal and premutation), using the formula described in Rodrigues et al. [14]. The age at amenorrhea - defined by at least 4 months of secondary amenorrhea and menopausal levels of follicle-stimulating hormone (FSH) [16] - was reported in 58 observations from set 2 (mean age 38.7±8.5 years, range 18–56) thus resulting in a slightly smaller dataset (58 observations instead of 59).

Reference set

The reference set, composed of one hundred and thirty-one female samples with normal (n=127) and intermediate genotypes (n=4), was previously described and characterized in Rodrigues et al. [14] (the summary of the results can be found in Supplementary Table 1).

Statistical analysis

A linearized form of a logarithmic model [i.e., regression of ln(score 1) against score 2] was used to obtain a functional model to relate the complexity of both alleles in each set. Covariance analysis (ANCOVA) compared the reference set with premutation sample set regression models, following the methodology outlined by Zar [18]. SigmaPlot version 14.0 (Systat Software®Inc., Chicago, IL, USA) was used for One-Way ANOVA on ranks (Kruskal-Wallis test) to compare separately *allelic score* and the size of each allele (normal and premutation). Dunn's method was used for multiple comparisons after conducting a Kruskal-Wallis test, comparing sets based on median allele size and *allelic score*. The relationship between the age at amenorrhea and *allelic score* was assessed by Pearson correlation coefficient. R sofware version 4.3.0 by R Core Team [19] with the ggplot2 package [20] was used to generate contour plots to display the relationship between independent variables normal and premutation *allelic scores*, and the dependent variable, age at amenorrhea. All statistical tests were carried out for a significance level of 0.05.

Results

FMR1 CGG repeat characterization

FMR1 molecular data of 1154 alleles are summarized in Supplementary Table 2. In a set 3 sample both alleles are in the premutation (PM) range, a rare event previously reported in seven cases [21]. The most frequent repeat length among normal-sized alleles is 30 CGGs, despite the significant differences among allele sizes (Kruskal-Wallis test: H=12.3; df=2; p=0.002) (Supplementary Fig. 1a). The majority of normal-sized alleles contained one or two AGG interruptions (93.8%, n=540) while pure alleles occurred in 4.7% of samples (n=4, set 2, n=23, set 3), and the remaining 1.5% of samples showed three AGGs (n=9, set 3). In total, ninety-seven different AGG patterns (n=8/ 20, set 1, n=23/ 59, set 2 and n=75/ 498,

Table 1 Summary of the *FMR1* allelic complexity (*allelic score*) results

		A1 - Shorter CGG repeat length allele			A2 - Longer CGG repeat length allele		
		Set 1	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**
Number of alleles		40	118	996	40	118	996
Allelic score	Mean (± S.D.)	159.0 ± 66.5	165.8 ± 79.8	159.7 ± 105.5	152.7 ± 81.8	150.2 ± 81.7	131.0 ± 61.6
	Median	205	207	193	217	214	109
	Range	49–206	16–234	9–829	56–242	63–288	55–313
	Most frequent (%, n)	205 (30%, *n* = 6)	223 (27.1%, *n* = 16)	205 (34.9%, *n* = 174)	231 (15%, *n* = 3)	133 (5.1%, *n* = 3)	103 (2.4%, *n* = 12)
		206 (25%, *n* = 5)	207 (18.6%, *n* = 11)	189 (12.7%, *n* = 63)	217 (15%, *n* = 3)	83 (5.1%, *n* = 3)	100 (2.4%, *n* = 12)
Allelic scores Kruskal-Wallis Test		H = 33.20; df = 2; $p < 0.001$			H = 1.45; df = 2; $p = 0.484$		

S.D. = Standard deviation; % = Frequency; n = Number of alleles

p values represent significant levels between sets 1, 2 and 3 *allelic scores*; Multiple Comparison (Dunn's Method) results in Supplementary Fig. 1b and d

Data published in Villate et al. [15] (set 1), Allen et al. [16] (set 2) and Yrigollen et al. [17] (set 3)

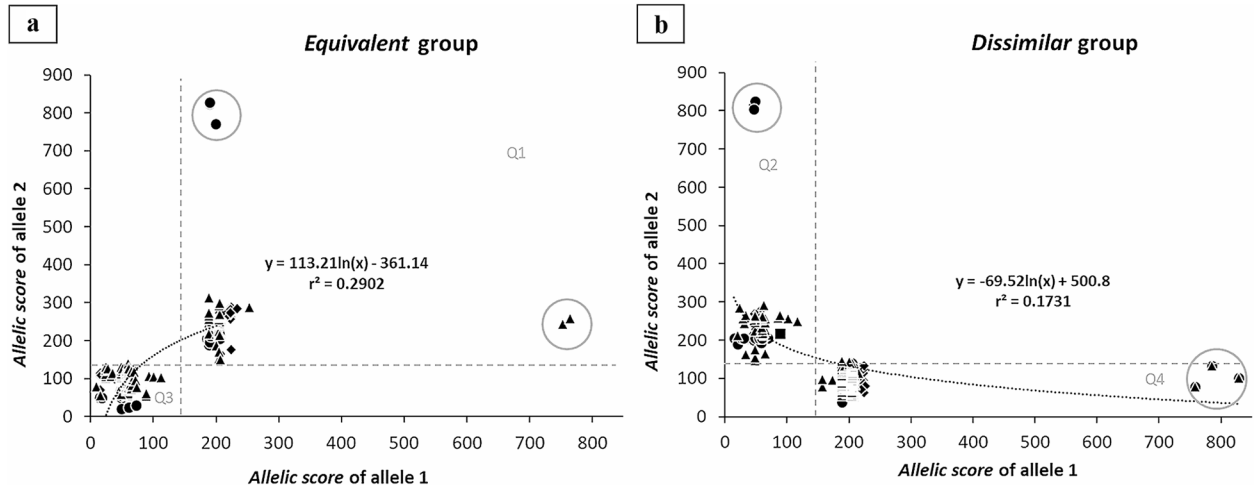

Fig. 1 Correlation between the *FMR1* allelic complexity (*allelic score*) of each allele in all sets, according to groups: *equivalent* (**a**) and *dissimilar* (**b**). The graph was partitioned into four quadrants based on the dispersion of the data: Q1 (quadrant 1), Q2 (quadrant 2), Q3 (quadrant 3), and Q4 (quadrant 4). The reference set is denoted by circle symbols, while set 1, set 2, and set 3 are represented by squares, lozenges, and triangles, respectively. Samples marked with a grey circle represent alleles with an *allelic score* above 700

set 3) were identified. The most common AGG interspersion pattern in sets 1 and 3 is $(CGG)_{10}AGG(CGG)_9AGG(CGG)_9$. On the contrary, a very rare pattern was identified as commonest among set 2 samples $(CGG)_{11}AGG(CGG)_{10}AGG(CGG)_7$. Around half of the PM alleles had no AGGs (50.3%, *n* = 8, set 1, *n* = 26, set 2, *n* = 257, set 3), and approximately 49.7% showed one or two AGG interruptions (*n* = 287). Two hundred and eleven different patterns were identified (*n* = 13/ 20, set 1, *n* = 45/ 59, set 2 and *n* = 177/ 498, set 3) in PM alleles revealing very exclusive CGG/AGG structures.

Mathematical model validation

Descriptive statistics and frequency analyses of premutation *allelic scores* are shown in Table 1. The median PM *allelic scores* did not show statistically significant differences between sets (Kruskal - Wallis test: H = 1.45; df = 2; $p = 0.484$) (Supplementary Fig. 1d); despite the sets having significantly different normal median *allelic scores* (Kruskal-Wallis test: H = 33.20; df = 2; $p < 0.001$) (Supplementary Fig. 1b). To compare these PM samples with previously published data using the same mathematical model, a reference set was built from that publication [14]. All sets distributed *allelic scores* into four quadrants, separated by a value of 150 as previously observed in the reference set, revealing similar compositions (Fig. 1): samples with alleles showing a similar complexity (*equivalent* group, quadrants 1 and 3) (Fig. 1a) and samples where alleles have a different complexity (*dissimilar* group, quadrants 2 and 4) (Fig. 1b). Thus, the correlation between the *allelic score* of each allele (Fig. 1a and b) was described following a logarithmic model. Significant correlations were found in both groups from all sets: reference set – *equivalen*t group: $r = 0.551$; df = 71; $p < 0.0001$ and *dissimilar* group: $r = 0.466$; df = 54; $p < 0.0001$ (Fig. 1a and b, represented by circles); set 1 – *equivalen*t group: $r = 0.994$; df = 8; $p < 0.0001$ and *dissimilar* group: $r = 0.991$; df = 6; $p < 0.0001$ (Fig. 1a and b, represented by squares); set 2 – *equivalent* group $r = 0.933$; df = 26; $p < 0.0001$ and *dissimilar* group: $r = 0.938$; df = 27; $p < 0.0001$ (Fig. 1a

and b, represented by lozenges), and set 3 – *equivalent* group: $r=0.912$; df=187; $p<0.0001$ and *dissimilar* group: $r=0.882$; df=297; $p<0.0001$ (Fig. 1a and b, represented by triangles). An exponential growth of the *allelic score* was observed, particularly in alleles having more than two AGGs (Supplementary Table 3); due to the relevance attributed to the AGG number by the formula. For instance, samples with three AGGs show scores above 700 ($n=6$, reference set, and $n=8$, set 3; represented by a grey circle in Fig. 1a and b). To validate the mathematical model in expanded alleles a covariance analysis between the reference and PM sample sets logarithmic models was performed separately for each group (Supplementary Fig. 2). Supplementary Table 4 shows the individual models resulting from each set. Coincident regression lines demonstrate the absence of statistically significant differences in each *equivalent* and *dissimilar* groups from PM samples sets when compared with those of the reference set. This result supports a more robust model including observations from the four sets: *equivalent* group – $F_{(6,\ 300)}=1.8278$; $p=0.0934$: Score 2 = -238.3+87.4 × ln(score 1) and *dissimilar* group – $F_{(6,\ 392)}=1.0679$; $p=0.3812$: Score 2=573.9–88.4 × ln(score 1).

FMR1 allelic scores and age at amenorrhea association

To understand the impact of *FMR1 allelic score* on the age at amenorrhea, normal-sized (allele 1) and PM (allele 2)alleles from set 2 samples were analyzed separately ($n=58$). No significant correlation was observed between A1 *allelic score* and age at amenorrhea ($p>0.05$) (Supplementary Fig. 3a, c, e and g). The same was true when PM *allelic score* was used ($p>0.05$) (Supplementary Fig. 3b, d, f, and h). A nearly significant trend ($p=0.058$) is apparent between the A1 *allelic score* and age at amenorrhea in samples showing an *allelic score* between 206 and 234 (Supplementary Fig. 3a) and 16–68 (Supplementary Fig. 3e) (quadrants 1 and 3, respectively). Two distinct behaviors were observed: age at amenorrhea rise with increasing *allelic score* (above 200, quadrant 1, mean age at amenorrhea 40±8.5 years, Supplementary Fig. 3a), and age at amenorrhea decrease with increasing *allelic score* (below 70, quadrant 3, mean age at amenorrhea 38±8 years, Supplementary Fig. 3e). The majority of these samples have alleles with less than 26 CGGs (78.6%, $n=11$), with one or no AGG interspersions (71.4%, $n=10$, 28.6%, $n=4$, respectively), whereas those with higher *allelic scores* have alleles ranging from 29 to 32 CGGs, with two AGG interspersions.

Age of amenorrhea assessment by *allelic scores combination*

PM alleles within the range 70–100 CGGs are known to have increased risk of developing FXPOI [6, 7, 22], however not all carriers develop disease and there is lack of knowledge on the underlying mechanisms. This led us to speculate if FXPOI development could be associated with a combined effect of *FMR1* allelic complexity. To analyze the joint effect of A1 and PM *allelic scores* in the age at amenorrhea, a contour plot was generated. Overall, different trends were observed: menopause age approaches normal (mean 51 years, range 40 to 60 years) when the *allelic score* of both alleles increases or decreases, showing that balanced *allelic scores* have minimal impact on early amenorrhea. Deeper analysis of samples with mean age at amenorrhea below 40 years and PM *allelic score* between 70 and 123 show that age decreases with increasing A1 *allelic score* (Fig. 2, A1 *allelic score* between 50 and 55).

Discussion

In our study, aiming to validate the previously published mathematical model ascertained in normal and intermediate alleles, we compared three distinct datasets with premutation carriers and subsequently explored the relationship between allelic complexity of the *FMR1* gene and age at amenorrhea – a clinical manifestation associated with development of FXPOI.

A comparative analysis of the CGG/AGG substructure across the sets revealed that the normal-sized allele with 30 repeats was the most frequent with the more prevalent AGG interspersion pattern being $(CGG)_{10}AGG(CGG)_9AGG(CGG)_9$ in sets 1 and 3, consistent with findings in other populations [14, 23, 24]. A notably rare pattern, $(CGG)_{11}AGG(CGG)_{10}AGG(CGG)_7$, emerged as the most prevalent in set 2, which can be attributed to intrinsic characteristics of this subpopulation such as complexity and heterogeneity of genetic traits. Expectedly, approximately half of the PM alleles showed one or two AGG interruptions with an overall average repeat length of 90.3 (S.D. = 20.8), since PM alleles with longer CGG lengths tend to demonstrate a lower incidence of AGG interruptions [11]. Notably, many different AGG interspersion patterns were found in PM alleles, most probably due to the inherent instability of these alleles [8].

No statistically significant difference was observed among the *allelic score* of PM alleles, but was found for the *allelic score* of normal-sized alleles. A similar result was observed when comparing the total CGG repeat length of normal alleles, revealing great variability in the complexity of the CGG repetitive region of normal-sized alleles. The combination of *allelic scores* revealed the emergence of two groups with distinct characteristics: *equivalent* and *dissimilar*, both exhibiting significant correlations. Similar outcomes had been reported by Rodrigues et al. [14]. The validation of our mathematical model in females with *FMR1* expansions showed that this model can be applied in populations that exhibit varied

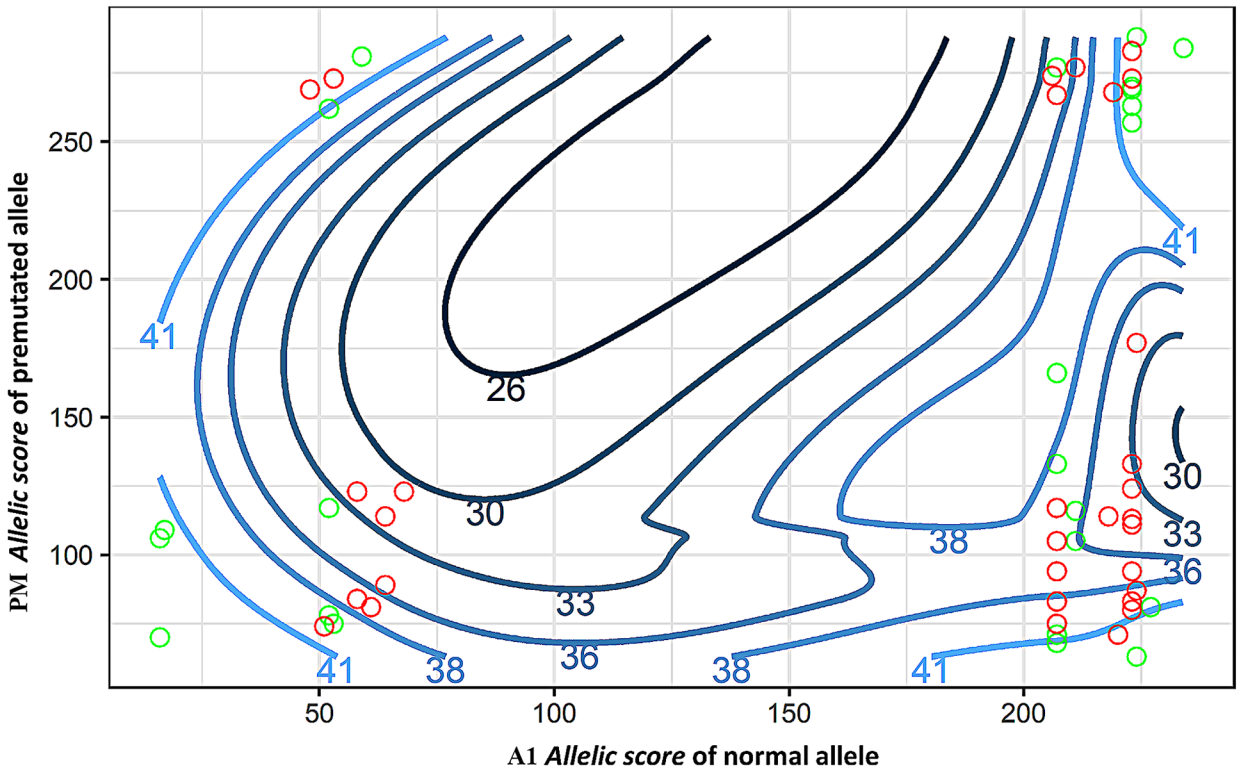

Fig. 2 Contour plot evaluating the interaction between three variables: *allelic score* of both alleles (independent variables – x, y) and age of amenorrhea (dependent variable – z). Samples in witch combination of allelic complexity is associated with amenorrhea before age 40 years and after age 40 years are represented by red and green circles, respectively. The different colors of the contour lines indicate the ages at amenorrhea as a function of the *allelic score* of both alleles

genotypic characteristics, namely expanded alleles such as premutations.

Several studies have sought to comprehend the impact of the CGG repetitive region on the development of FXPOI. However, the majority focus on examining the influence of the CGGs and AGGs independently, as exemplified by Friedman-Gohas et al. study [25]. Here, we employed our formula, which integrate the total CGG repeat length, the number of AGGs, and the AGG interspersion pattern. No statistically significant correlations were found between A1 *allelic scores* and age at amenorrhea, nor PM *allelic scores* and age at amenorrhea. The lack of statistical significance might be due to the reduced number of observations in each grouping [26]. Nevertheless, a significant trend was observed with normal *allelic scores* between 206 and 234 and 16–68 and age at amenorrhea. The influence of *FMR1* gene alleles within normal size in ovarian reserve is controversial. Gleicher et al., demonstrated a negative effect in ovarian reserve of alleles with less than 26 CGGs, evidenced by low levels of anti-Müllerian hormone (AMH) [27–29]. Wang et al. demonstrated reduction in *FMR1* mRNA levels in granulosa cells from females carrying alleles with CGGs < 26 and simultaneously a misregulation steroidogenic enzymes and hormone receptors, leading to ovarian dysfunction and ultimately infertility [30]. Rechnitz et al. illustrated a poor response to ovarian stimulation and elevated expression of *FMR1* mRNA in granulosa cells when compared to samples with different *FMR1* gene sub-genotypes [31]. Interestingly, the majority of our samples with alleles < 26 CGGs show one or no AGGs, while alleles with a repeat size between 29 and 32 CGGs show two AGG interruptions. Lekovich et al. demonstrated that premutation with none or one AGG showed poorer ovarian reserve than those with two, suggesting AGG interspersions have a protective effect [32]. It is thus tempting to speculate that by a similar mechanism the absence of AGGs in normal alleles correlates with ovarian dysfunction.

A minimal effect of *FMR1* allelic complexity with age at amenorrhea is observed in balanced *allelic scores*. Moreover, it appears that the age of amenorrhea decreased with increasing A1 *allelic score* when the PM allele had a score between 70 and 123. Despite the absence of statistical significance, a trend towards a correlation with the *allelic score* of the A1 suggests the need for larger-scale investigations to assess the impact of the combined *allelic scores* on the age at amenorrhea. It is likely that the age

at amenorrhea may not provide a comprehensive assessment of FXPOI development. Therefore, it is important to test other clinical parameters, such as AMH levels, to gain a deeper understanding of the impact of combining *allelic scores* on disease development.

Conclusion

This is the first report investigating the combined effect of normal and premutation *allelic scores* on FXPOI development impacted by age at amenorrhea. In our analysis, the presence of the correlation trend indicates the need for further studies and additional samples to explore the complex relationship between *allelic score* combinations and the development of FXPOI. Skewed X chromosome inactivation and hormonal deregulation were not considered and might impact age at amenorrhea. Nevertheless, the use *allelic scores* combination may pave the way to the identification of an ovulatory dysfunction biomarker. This is of major clinical importance to improve fertility in premutation carriers, to make choices about preservation strategies such as oocyte cryopreservation, increasing chances of a successful pregnancy.

Abbreviations

AGG	Adenine-Guanine-Guanine
AMH	anti-Müllerian hormone
CGG	Cytosine-Guanine-Guanine
FMR1	*fragile x messenger ribonucleoprotein 1* gene
FSH	Follicle-stimulating hormone
FXPOI	Fragile X-associated Primary Ovarian Insufficiency
FXS	Fragile X Syndrome
FXTAS	Fragile X-associated Tremor/Ataxia Syndrome
PM	Premutation

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

P.J. and A.J.A.N. conceived and designed the study together with B.R. A.J.A.N. performed the statistical analysis with B.R. analysed all data and drafted the manuscript. C.M.Y., F.T., O.V.B., E.G.A., A.G., N.T. and S.L.N. worked on samples and provided the necessary data. C.M.Y., F.T., O.V.B., E.G.A., and V.S. provided critical feedback, and contributed towards the manuscript. All authors discussed the results and critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

Data is contained within the article or supplementary material.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all participants within previously published studies. Participants of set 1 the informed consent approved by the clinical ethical committee of Cruces Hospital was obtained in all cases prior to genetic testing. Protocols and consent forms of participants in set 2 were conducted in concordance with the Belmont Report and approved by the Institutional Review Board at Emory University, and written informed consent was obtained from all subjects (IRB00045808). Participants of the set 3 were recruited through the Fragile X Research and Treatment Center at the MIND Institute – University of California Davis, USA. Whole blood was collected according to protocols approved by the Institutional Review Board at the University of California, Davis, and informed consent was obtained from all patients. This research was approved from the Ethics Committee of the Unidade Local de Saúde de Santo António (2020.119 /097-DEFI/099-CE) as part of B. Rodrigues's Ph.D. studies.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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References

1. Jin P, Warren ST. Understanding the molecular basis of fragile X syndrome. Hum Mol Genet. 2000;9:901–8.
2. Man L, Lekovich J, Rosenwaks Z, Gerhardt J. Fragile X-Associated diminished Ovarian Reserve and primary ovarian insufficiency from Molecular mechanisms to Clinical manifestations. Front Mol Neurosci [Internet]. 2017;10:1–17.
3. Latham GJ, Coppinger J, Hadd AG, Nolin SL. The role of AGG interruptions in fragile X repeat expansions: a twenty-year perspective. Front Genet. 2014;5:1–6.
4. Willemsen R, Levenga J, Oostra B. CGG repeat in the FMR1 gene: size matters. Clin Genet. 2011;80:214–25.
5. Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet - Semin Med Genet. 2000;97:189–94.
6. Mailick MR, Hong J, Greenberg J, Smith L, Sherman S. Curvilinear Association of CGG Repeats and age at menopause in women with FMR1 premutation expansions. Am J Med Genet B Neuropsychiatr Genet. 2014;0:705–11.
7. Ennis S, Ward D, Murray A. Nonlinear association between CGG repeat number and age of menopause in FMR1 premutation carriers. Eur J Hum Genet. 2006;14:253–5.
8. Tabolacci E, Nobile V, Pucci C, Chiurazzi P. Mechanisms of the FMR1 repeat instability: how does the CGG sequence expand? Int J Mol Sci. 2022;23:1–17.
9. Manor E, Gonen R, Sarussi B, Keidar-Friedman D, Kumar J, Tang HT, et al. The role of AGG interruptions in the FMR1 gene stability: a survey in ethnic groups with low and high rate of consanguinity. Mol Genet Genomic Med. 2019;7:1–14.
10. Nolin SL, Glicksman A, Ersalesi N, Dobkin C, Brown WT, Cao R, et al. Fragile X full mutation expansions are inhibited by one or more AGG interruptions in premutation carriers. Genet Med. 2015;17:358–64.
11. Yrigollen CM, Durbin-Johnson B, Gane L, Nelson DL, Hagerman R, Hagerman PJ, et al. AGG interruptions within the maternal FMR1 gene reduce the risk of offspring with fragile X syndrome. Genet Med. 2012;14:729–36.
12. Domniz N, Ries-Levavi L, Cohen Y, Marom-Haham L, Berkenstadt M, Pras E, et al. Absence of AGG interruptions is a risk factor for full mutation expansion among Israeli FMR1 Premutation Carriers. Front Genet. 2018;9:1–8.
13. Napierala M, Michalowski D, de Mezer M, Krzyzosiak WJ. Facile FMR1 mRNA structure regulation by interruptions in CGG repeats. Nucleic Acids Res. 2005;33:451–63.
14. Rodrigues B, Vale-Fernandes E, Maia N, Santos F, Marques I, Santos R, et al. Development and validation of a Mathematical Model to predict the complexity of FMR1 allele combinations. Front Genet. 2020;11:1–8.
15. 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;7:1–6.
16. Allen EG, Glicksman A, Tortora N, Charen K, He W, Amin A, et al. FXPOI: pattern of AGG interruptions does not show an association with age at amenorrhea among women with a premutation. Front Genet. 2018;9:1–7.
17. Yrigollen CM, Martorell L, Durbin-Johnson B, Naudo M, Genoves J, Murgia A et al. AGG interruptions and maternal age affect FMR1 CGG repeat allele stability during transmission. J Neurodev Disord. 2014;6.
18. Zar JH. Bioestatistical Analysis fifth edition. 2010.
19. Team RC. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. 2023.
20. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. 2016. https://ggplot2.tidyverse.org
21. Basuta K, Lozano R, Schneider A, Yrigollen CM, Hessl D, Randi J, Hagerman A, et al. A family with two female compound heterozygous for the FMR1 premutation alleles. Clin Genet. 2014;285:458–63.
22. Allen EG, Charen K, Hipp HS, Shubeck L, Amin A, He W, et al. Refining the risk for fragile X–associated primary ovarian insufficiency (FXPOI) by FMR1 CGG repeat size. Genet Med. 2021;23:1648–55.
23. Yrigollen CM, Sweha S, Durbin-Johnson B, Zhou L, Berry-Kravis E, Fernandez-Carvajal I, et al. Distribution of AGG interruption patterns within nine world populations. Intractable Rare Dis Res. 2014;3:153–61.
24. 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 Part A. 2019;179:1148–56.
25. Friedman-Gohas M, Kirshenbaum M, Michaeli A, Domniz N, Elizur S, Raanani H, et al. Does the presence of AGG interruptions within the CGG repeat tract have a protective effect on the fertility phenotype of female FMR1 premutation carriers? J Assist Reprod Genet. 2020;37:849–54.
26. Bonett DG, Wright TA. Sample size requirements for estimating Pearson, Kendall and Spearman correlations. Psychometrika. 2000;65:23–8.
27. Gleicher N, Yu Y, Himaya E, Barad DH, Weghofer A, Wu Y, et al. Early decline in functional ovarian reserve in young women with low (CGGn < 26) FMR1 gene alleles. Transl Res. 2015;166:502–7.
28. Gleicher N, Weghofer A, Oktay K, Barad DH. Revelance of triple CGG repeats in the FMR1 gene to ovarian reserve. Acta Obstet Gynecol Scand. 2009;88:1024–30.
29. Maslow BSL, Davis S, Engmann L, Nulsen JC, Benadiva CA. Correlation of normal-range FMR1 repeat length or genotypes and reproductive parameters. J Assist Reprod Genet. 2016;33:1149–55.
30. Wang Q, Kushnir VA, Darmon S, Barad DH, Wu Y, Zhang L, et al. Reduced RNA expression of the FMR1 gene in women with low (CGGn < 26) repeats. Fertil Steril. 2017;108:e143.
31. Rehnitz J, Alcoba DD, Brum IS, Dietrich JE, Youness B, Hinderhofer K, et al. FMR1 expression in human granulosa cells increases with exon 1 CGG repeat length depending on ovarian reserve. Reprod Biol Endocrinol. 2018;16:1–9.
32. Lekovich J, Man L, Xu K, Canon C, Lilienthal D, Stewart JD, et al. CGG repeat length and AGG interruptions as indicators of fragile X–associated diminished ovarian reserve. Genet Med. 2018;20:957–64.

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