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Research Article

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The Frequency of the Fragile X Syndrome Intermediate Allele and Premutation in Women with Diminished Oocyte Reserve (Dor) vs Normal Oocyte Reserve (NOR)

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ABSTRACT

Fragile X is a genetic disorder that causes developmental and behavioral issues. It is well known that patients who are carriers of the Fragile X gene have a higher frequency of diminished ovarian reserve (DOR). The purpose of this study was to determine the frequency of the Fragile X intermediate allele and premutation in women with DOR versus NOR. A Beacon Carrier Expanded Gene 436 sequencing test with deletion and duplication analysis was performed on 350 consecutive females with a history of infertility. Patients found to have the Fragile X Intermediate Allele or a Fragile X premutation were identified. DOR was defined as having a serum antimullerian hormone (AMH) level of less than 1 ng/mL, compared to those women with NOR with a serum AMH > 1 ng/mL. Finally, the portion of women with the Fragile X intermediate allele or premutation who also had DOR were evaluated. For those with DOR who had carrier screening for the first time, the frequency of either Fragile X intermediate alleles or the Fragile X premutations was 7 of 142 (4.3%). Six of those individuals had the intermediate alleles, while one had the premutation. For those with NOR on the first screening, there were 207 women. 3 (1.5%) were positive for the Fragile X intermediate alleles, and none had the premutation. Thus, of 9 women with the Fragile X intermediate alleles, 6 (66.7%) had DOR. 96% of cases of DOR were not associated with the Fragile X intermediate allele or premutation. Women with the Fragile X premutation are known to be at risk for gonadotoxicity and DOR. The converse is not true, women with DOR do not have a high frequency of Fragile X premutation or even the less clinically important intermediate allele. Therefore, in patients attempting to conceive, routine Fragile X carrier screening may not be necessary in women with DOR any more than in those with NOR.

Keywords: Fragile X Syndrome, Diminished Ovarian Reserve, Intermediate Alleles, Permutations

Introduction

Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and the leading single-gene defect associated with autism, affecting 1/4000 males and 1/8000 females [1]. The disorder results from a CGG trinucleotide repeat expansion in the 5' untranslated region (UTR) of the Fragile X mental retardation 1 (FMR1) gene on the X chromosome, encoding the fragile X mental retardation protein (FMRP). FMRP plays a critical role in brain development and has an emerging role in the regulation of ovarian function and reproductive aging [2,3].

The clinical manifestations of FMR1 alleles differ according to CGG repeat length. Alleles are classified by repeat number. Alleles with >200 CGG repeats are termed a full mutation (FM). A FM results in hypermethylation of the FMR1 gene, which leads to decreased or absent FMRP. This leads to features of FXS including intellectual disability and autism spectrum symptoms [1,4].

Premutations (PM) contain 55-200 CGG repeats and display features distinct from FM, such as predisposing carriers to Fragile-X-Associated Premature Ovarian Insufficiency (FXPOI) [1,2,5-7]. FXPOI is defined as the loss of normal ovarian function before the age of 40, characterized by oligomenorrhea or amenorrhea and elevated follicle-stimulating hormone (FSH) levels (>40 mIU/ml) [8].

PM alleles also carry a risk of expansion to a FM in subsequent generations, meaning both FM and PM may lead to inheritance

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of a FM [8]. Approximately 1/250 females and 1/800 males carry PM alleles in the range of 55-200 repeats [9].

Alleles with 45-54 CGG repeats are classified as intermediates (IM) and have some level of CGG repeat instability with a small risk of expansion to a premutations. An allele that is not associated with abnormal phenotype has less than 45 CGG repeats.

Ovarian reserve reflects the quantity and to a lesser degree quality of remaining oocytes and is a major predictor of response to ovarian stimulation and reproductive potential [10].

A recent increase in studies on the association between FMR1 PM and DOR demonstrates a higher prevalence of PM in DOR populations. The studies support a relationship between CGG repeats in FMR1 and ovarian aging and suggest a potential for exploring permutations as a cause of infertility in DOR populations and a consideration in fertility planning [6,8-10].

PM alleles of FMR1 are associated with an RNA gain-of-function mechanism characterized by increased FMR1 messenger RNA (mRNA) levels. Clinically, PM carriers have increased risk of FXPOI and DOR PM. The role of IM remains less clear - some reports suggest possible associations with reduced fertility, whereas others find IM to have an insignificant increase in prevalence among women with DOR [10].

Current guidelines by the American Association for Reproductive Medicine recommend FMR1 screening for women with a family history of Fragile X-related disorders or primary ovarian insufficiency, but do not endorse testing for all women with DOR. Despite growing evidence for the linkage between FMR1 PM and DOR, no prior study has compared the frequency of FMR1 intermediate alleles and PM in DOR populations to those with normal ovarian reserve (NOR) using AMH-based criteria. The study addresses this gap by evaluating the frequency of FMR1 alleles in DOR and NOR populations, with the goal of clarifying whether DOR alone should prompt targeted Fragile X carrier screening beyond existing guidelines. The objective of this study was to determine the frequency of the Fragile X intermediate allele and premutation in women with DOR versus NOR.

Methods

Data from women at our clinic was collected retrospectively from April 2020 to April 2025. All women underwent molecular genetic testing via Biochemistry and Molecular Genetic Services. They used a Beacon Carrier Expanded Gene which is a pan-ethnic screen DNA test for hundreds of recessives and X-linked conditions. This included 436 sequencing tests with deletion and duplication analysis which was performed on 350 consecutive females with a history of infertility, allowing for randomization and creation of a control group which was patients with NOR. A peripheral venous blood sample was sent to LabCorps. The sample cells were lysed to isolate the genomic DNA. The FMR1 gene region CGG was amplified using a triplet-primed polymerase chain reaction (TP-PCR) in order to help determine the PM and IA.

Patients were determined to have FXSPM if they had between 55 and 200 CGG repeats, and FXS IA included 45-54 CGG repeats, while those below 44 were considered normal. DOR was defined as having a serum anti-mullerian hormone (AMH) level of less than 1 ng/mL, compared to those women with NOR (control group) with a serum AMH > 1 ng/mL. Furthermore, patients found to have the FXS Intermediate Allele or a FXS premutation were identified. Finally, the portion of women with the Fragile X IA or PM who also had DOR vs NOR were evaluated. Continuous data are expressed as the mean, and categorical data as proportions. Groups were compared using Student's t-test and a p-value < 0.05 was considered statistically significant.

Results

Table 1 shows the clinical characteristics of a total of 349/350 patients which were included in the final analysis. One of the women with the FMR1 gene PM was excluded from the analysis. She had known from prior testing that she had the PM and came to the IVF center for Preimplantation Genetic Testing for Monogenic Disorder (PGT-M) despite marked DOR. Overall, this cohort with NOR exhibited a younger age with the average of 34.1 vs 39.3 years old. The study group was then divided between DOR (n=149) and NOR (n=199) patients. The DOR group showed a decreased AMH level (0.443 ng/mL +/- 0.324) compared to the NOR group (4.283 ng/mL +/- 4.818).

Table 1: Clinical characteristics of the study population

PATIENT GROUP	N	AGE	AMH (ng/mL)
DOR	149	39.3 +/- 4.9	0.443 +/- 0.324
NOR	200	34.1 +/- 4.8	4.283 +/- 4.818
TOTAL	349	36.3	2.65

Table 2. represents the data of the frequency of FMR1 Repeat Sizes of patients with DOR and NOR. For patients with DOR who had carrier screening for the first time, the frequency of either FMR1 gene IA or PM and Fragile X 1-17 deletion was 8 of 142 (4.3%). Six of those individuals had the IA, while one had the FXS 1-17 deletion. For those with NOR on the first screening, there were 207 women. 3 (1.5%) were positive for the Fragile X IA, and one had the PM. Thus, of 9 women with the Fragile X intermediate alleles, 6 (66.7%) had DOR.

Table 2: Frequency of FMR1 Repeat Sizes Among the PM vs. IA Study Groups

	DOR	NOR
Fragile X Premutation	1/142 (0.7%)	0/207 (0.0%)
Intermediate Allele	5/142 (4.2%)	2/207 (1.5%)
Fragile X 1-17 deletions	1/142 (0.7%)	0/207 (0.0%)
Total Participants	142	207

Furthermore, a comparison of AMH levels between IA and non-IA patients in the DOR group was completed. In the total of patients with DOR, there were 5 with IA and 136 with no IA. The ones with IA had a slight increase in AMH levels (0.476 ng/mL) compared to the ones with no IA (0.418 ng/mL) (P=NS).

Discussion

This study showed that an overwhelming majority of cases of

women in their 30's to early 40's with DOR do not have the FMR1 gene IA or PM. No statistically significant differences were identified between the DOR group and NOR group.

The majority of cases within the CGG repeats were associated with the IA, making up about 2.57% of total cases. They had a slight non-significant increase in AMH levels (0.476 ng/mL) compared to the ones with no IA (0.418 ng/mL). We found that there was an insignificant increased prevalence of patients with FMR1 gene IA with DOR (4.93%) compared to ones with NOR (2.11%). FMR1 IA has been increasingly researched in connection with DOR, with many studies concluding that the IM allele should not even be considered a high-risk factor for DOR. A recent meta-analysis of six articles pertaining to evaluations of the PM and IA alleles in patients with DOR found that there was no significant correlation between DOR and the IA. Our results mirrored current literature as they were also non-significant correlations between IA and CGG repeats. The presence of IA was uncommon in women with DOR (only 4.2%) but PM was even more rare (0.7%).

Our results contradict some studies which have suggested that the prevalence of FMR1 gene is increased in women with DOR compared to women with NOR with other causes of infertility. Eslami et al. concluded that the frequency of FMR1 PM is higher in women with DOR and POI compared to patients in the control group [10].

Some research studies suggest the possible explanation for the mechanism of linkage between PM and DOR is associated with an RNA gain-of-function toxicity and increased FMR1 mRNA levels, which are associated with increased risk of FXPOI and DOR [2,5,7]. Another theory is related to the CGG repeat expansion which has been shown to sequestering proteins and transcripts which ultimately interfere with normal follicles in ovarian stromal cells [2,5,7]. Our study showed that there were no patients with NOR who had an FMR1 gene PM, compared to one patient with DOR that had the PM.

This brings up the question of whether completing genetic testing for the FMR1 gene CGG repeat allele is a clinically important test for patients to have if DOR is present, especially pertaining to women who do not have a family history of FXS and/or the ones with mental instability.

Overall, in patients attempting to conceive, routine Fragile X carrier screening may not be necessary in women with DOR any more than in those with NOR. If a given patient is concerned about a 4.2% risk of the presence of an intermediate allele and would thus do carrier screening before attempting conception, then they should also do so for a 1.5% risk if they had NOR since there is no clinical statistical significance. To extrapolate for statistical difference, we would need more power. But even if a difference was found, it wouldn't have great clinical significance. Also based on insurance coverage and finances a woman does not need the screening unless based on the information she would proceed to in vitro fertilization embryo transfer with pre-implantation genetic testing for monogenic disorders.

Current guidelines for screening for FXS include a targeted approach for individuals with sufficient likelihood of premutation or full mutation carrier status. Those include individuals with family history of FXS, males or females with intellectual disability, individuals with late onset intention tremor or ataxia with a history of infertility, FXS or intellectual disability and young females with elevated FSH with a family history of premature ovarian insufficiency. It is well established that there is a relationship between FMR1 gene and POI, however its connection with DOR and its implications remain unknown. Therefore, there are currently no established guidelines in neither European or American assisted human reproductive guidelines for testing in patient with DOR.

Conclusion

- 96% of cases of DOR were not associated with the Fragile X intermediate allele or premutation.
- Women with the Fragile X premutation are known to be at risk for gonadotoxicity and DOR.
- The converse is not true, women with DOR do not have a high frequency of Fragile X premutation or even the less clinically important intermediate allele.
- Therefore, in patients attempting to conceive, routine Fragile X carrier screening may not be necessary in women with DOR any more than in those with NOR.

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