

The association of *FMR1* gene (CGG)_n variation with idiopathic female infertility

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Abstract

Introduction: The *FMR1* gene plays an important role in brain development and in the regulation of ovarian function. The *FMR1* gene contains CGG repeat variation and the expansion of the repeats is associated with various phenotypes e.g. fragile X syndrome, premature ovarian failure, etc. Repeats ranging < 55 CGG are considered normal, however recent studies suggest that high-normal (35–54 CGG) and low-normal (< 26 CGG) alleles may also have an impact on female reproductive function.

Material and methods: We have performed a case-control study to assess the impact of *FMR1* gene CGG repeats on female infertility. The study comprised 161 women with primary and secondary idiopathic infertility and 12 females with diminished ovarian reserve. The control group consisted of 129 healthy women with children. The *FMR1* gene trinucleotide CGG repeat variation was detected using a triplet repeat primed polymerase chain reaction with capillary electrophoresis.

Results: The analysis of CGG repeats revealed that high-normal alleles are statistically significantly more common in the secondary infertility group than in controls (12% vs. 4.3%, $p = 0.03$, OR = 3.1, 95% CI: 1.1–8.3). The distribution of high-normal alleles and genotypes did not differ between patients with primary infertility and controls ($p > 0.05$). In addition, the analysis of low-normal allele and genotype frequencies did not present a difference between primary, secondary infertility and the control group ($p > 0.05$).

Conclusions: In our study, the *FMR1* gene high-normal alleles were associated with secondary infertility. However, to address the controversies related to the role of *FMR1* genes in the development of diminished ovarian reserve, further studies on the subject are required.

Key words: infertility, secondary infertility, diminished ovarian reserve, *FMR1* gene.

Introduction

Infertility is a global problem and undoubtedly not only implicates health problems, but also has negative social and psychological consequences [1]. Infertility is a dynamic disorder and can be caused by a variety of female (e.g. ovulatory disorders, tubal and uterine pathologies, endocrine disorders, etc.) as well as male factors or both of them [2], but the etiology of infertility is still an open issue – approximately 20–30% of pairs have idiopathic infertility [3].

The *FMR1* gene encodes the protein FMRP (fragile X mental retardation protein), which plays an important role in brain development and in the regulation of ovarian function [4, 5]. The *FMR1* gene is located on the X chromosome and contains trinucleotide (CGG) repeats in the 5'-untranslated region, which regulates the expression of FMRP [5]. The region containing more than 200 CGG repeats associated with abnormal methylation and is defined as a full mutation and causes fragile X syndrome [6]. The repeat numbers between 55 and 200 CGG are defined as premutation and can cause premature ovarian insufficiency (POI) and diminished ovarian reserve (DOR) [7, 8]. Moreover, the premutation allele is unstable and can expand to a full mutation in subsequent generations. The number of repeats below 55 CGG is defined as normal length (associated with normal gene expression); however, recent studies have stirred up the discussion as to whether all these repeats are really normal. Some authors reported that just a small number of the repeats (26–34 CGG) are actually normal and have normal FMRP expression and that the “high-normal” (35–54 CGG) and “low-normal” (< 26 CGG) repeat alleles have decreased levels of protein expression [9] and are associated with increased risks of DOR and POI, polycystic ovarian syndrome and therefore infertility [4, 10, 11]. However, the results of many studies on this association with ovarian reserve are contradictory [12] and the clinical utility for the *FMR1* gene CGG repeat testing in patients with idiopathic infertility and the interpretation of “high- and low-normal” alleles are still to be determined.

In this context, we aimed to investigate the impact of CGG variation in the *FMR1* gene on the development of idiopathic DOR, primary and secondary infertility in Latvian women.

Material and methods

Study design and patients

To analyze the impact of the *FMR1* (OMIM#309550, NCBI Gene ID 2332) gene CGG variation (ClinVar allele ID 25011) on the development of idiopathic infertility in women we performed a case-control study. Primary and secondary infertility were defined using the definitions provided by the World Health Organization [1]. The DOR was defined as described before [13]. We enrolled in the study women diagnosed with primary and secondary idiopathic infertility and DOR from the infertility treatment clinics. Women with known causes of infertility were excluded from the study (e.g. patients with endometriosis, congenital adrenal hyperplasia, uterine tube obstruction or known male factor). Testing for the most common hereditary thrombophilias (factor V variation c.1691G>A [rs6025]

and factor II c.*97G>A [rs1799963] variant) was performed for all infertile patients, and in the case of a positive result, the patient was excluded from the subsequent analysis. A total of 161 women with primary and secondary idiopathic infertility, 12 females with DOR were included in our study and 129 healthy women with children and without endocrine disorders who were selected from the Genome Database of the Latvian Population [14] were used as the control group.

Ethics

All patients signed a written informed consent form. The study was approved by the Central Medical Ethics Committee of the Republic of Latvia. The patients were referred for genetic counseling to a medical geneticist where necessary.

Molecular analysis

The patients' DNA was extracted from the peripheral venous blood samples using the innuPREP blood DNA kit (Analytik Jena, Germany) to establish the CGG repeat alleles triplet repeat primed polymerase chain reaction (TP-PCR) with capillary electrophoresis, adapted from the literature [15]. This method allows detection of carriers of expanded alleles (> 100 CGG). The testing and interpretation were confirmed by the external quality assessment scheme for normal range alleles with Sanger sequencing.

Statistical analysis

To assess the impact of high- and low- normal alleles on the development of infertility, following *FMR1* gene CGG repeats testing, the patients with the premutation allele were excluded from further statistical analysis. The *FMR1* CGG alleles and genotypes were stratified as described previously [16]. For the analysis of the allelic mode of inheritance, CGG repeats were stratified as low-normal (< 26 CGG), high-normal (35–54 CGG) and normal (26–34 CGG) alleles. The association with genotypes was evaluated in dominant and genotypic modes of inheritance (recessive mode of inheritance was not analyzed because of the low frequency of homozygous genotypes). The statistical analysis was performed using χ^2 and Fisher's exact tests using the SPSS v24.0 software.

Results

Study group

Out of 173 included patients with idiopathic infertility, 66 had primary and 30 had secondary infertility, 12 were suffering from infertility and DOR, but for 65 patients the type of infertility was not specified.

Frequency of premutation

The analysis of *FMR1* CGG repeats revealed that one infertile patient is a carrier of a 61 CGG repeat allele. She is suffering from secondary infertility. The frequency of premutation carriers in the infertile female group is 1/173 (0.6%), but in the secondary infertility group it is 1/30 (3.3%). No patient with DOR had a premutation allele.

To our surprise, one control group patient also carried a premutation allele (63 CGG). The frequency of premutation among the healthy Latvian female population is 1/129 (7.8%).

Association with infertility

The distribution of the *FMR1* gene alleles in infertility, DOR and control groups is shown in Table I. The frequency of low-normal and high-normal alleles is similar in all three analyzed groups ($p > 0.05$). The different *FMR1* genotypes were not statistically significantly associated ($p > 0.05$) with infertility and DOR in the analyzed modes of inheritance (data not shown).

When separately analyzing the association with primary and secondary infertility (Table I) we found that the frequency of high-normal alleles is statistically significantly more common in the secondary infertility group than in the control group (12% vs. 4.3%, $p = 0.03$, OR = 3.1, 95% CI: 1.1–8.3). The analysis of the role of *FMR1* genotypes revealed that the patients with secondary infertility have more than three times higher odds of having a genotype with a high-normal allele ($p = 0.02$, OR = 3.3, 95% CI: 1.5–9.3) in the dominant mode of inheritance. The distribution of high-normal alleles and genotypes did not differ between patients with primary infertility and controls ($p > 0.05$). Low-normal allele and genotype frequencies did not differ between the primary and secondary infertility groups and the control group ($p > 0.05$).

Discussion

There is still an ongoing extensive debate on how the *FMR1* gene, mRNA and FMRP influence

the ovarian function and cause premature ovarian insufficiency. It is known that patients with *FMR1* low-normal and high-normal alleles have altered protein expression levels [9]. FMRP regulates translation and RNA transport in cells [17]. FMRP is expressed in granulosa cells and plays an important role in the signaling pathway in oocyte maturation [9, 18, 19]. Previous studies suggest that FMRP participates in the regulation of follicular development and the changes in the FMRP expression levels are associated with increased follicle atresia and the initial decrease of ovarian reserve [20]. In addition, FMRP is expressed in the brain and there are speculations that this protein can influence the hypothalamic-pituitary-gonadal axis and critical regions of hormonal regulation [20]. The carriers of premutation and high-normal alleles also have higher *FMR1*-mRNA levels which could lead to the RNA-toxicity effects and to increased follicle atresia or a reduced number of growing follicles [21, 22].

Analyzing the impact of the *FMR1* gene CGG repeats on the development of infertility, we found that the patients who suffer from secondary infertility are more frequently carriers of high-normal alleles. Several studies have also found an association between infertility and high-normal allele [5, 11], but they were not specifically addressing secondary infertility. A recent study suggested that the decline in ovarian reserve could be one of the etiological factors for idiopathic female infertility [23]. We did not find an association between *FMR1* alleles and patients diagnosed with DOR, possibly because of the small number of patients included, but we hypothesize that the high-normal allele increases the risk of secondary infertility by decreasing the ovarian reserve. Unfortunately, our study did not cover the assessment of ovarian reserve in our patients. As the prevalence of secondary infertility increases with age and the patients suffering from secondary infertility are older than the primary infertility patients, the study outcomes could be explained by the accumulation of mRNA with years and subsequent RNA toxicity and diminished ovarian reserve.

Table I. Distribution of *FMR1* gene CGG repeat alleles among study groups

| Allele (number of CGG repeats) | Low-normal (< 26 CGG) | Normal (26–34 CGG) | High-normal (35–54 CGG) |
|-----------------------------------|--------------------------|-----------------------|----------------------------|
| Infertility ($n = 160$)#, % | 18.4 | 74.4 | 7.2 |
| Primary ($n = 66$), % | 16.7 | 75.0 | 5.3 |
| Secondary ($n = 29$), % | 27.6 | 60.3 | 12.1* |
| DOR ($n = 12$), % | 25.0 | 70.8 | 4.2 |
| Controls ($n = 128$), % | 22.7 | 73.0 | 4.3 |

*Statistically significantly more common than in the control group ($p = 0.03$, OR = 3.1, 95% CI: 1.1–8.3). DOR – diminished ovarian reserve. #For 65 patients the type of infertility was not specified.

On the other hand, low-normal alleles were not statistically significantly associated with infertility. Prior studies have reported conflicting results on low-normal alleles – some of them claim that these alleles have the most negative effects on reproduction and ovarian reserve [24, 25], while other studies at the same time did not demonstrate any significant differences between the carriers of low-normal alleles and women with normal range alleles [26, 27].

A considerable number of females are being tested for the *FMR1* gene CGG repeat worldwide, e.g. patients with POI, DOR, infertile patients, asymptomatic oocyte donors and also those subject to newborn screening studies [11, 28]. Approximately 5% and 20% are carriers of high-normal and low-normal alleles, respectively. The knowledge on the impact of these alleles on the ovarian function and reserves could affect the reproductive decision-making in patients carrying the mentioned alleles, e.g. deciding to perform oocyte cryopreservation or to bear a child at an earlier age. The outcomes of our study also suggest an increased risk of secondary infertility. Further prospective cohort studies are necessary to evaluate the *FMR1* gene low-normal and/or high-normal allele carrier reproductive outcomes. Nowadays the *FMR1* premutation testing in clinical practice is recommended only for patients with POI [6, 8]. There is accumulating evidence that this testing could be expanded also for other phenotypes, e.g. the results of our study suggests *FMR1* CGG testing also in patients with secondary infertility in Latvia, because premutation allele carriers were common among this group (3.3%) and premutation allele frequency could be higher among the healthy general population of Latvia than in other general populations (1/128 vs. 1/250–500) [28]. Detection of premutation in these patients could explain the cause of infertility and in case of use of assisted reproductive technologies or if pregnancy is achieved spontaneously, the embryo or fetus should be tested for the expansion of the premutation to full mutation. Also, identification of 1 patient could allow cascade testing of family members, increasing the utility of the test.

This study has several limitations. The main limitation was that the main information we had was that the patients have idiopathic infertility, as mentioned in the methods; however, we lacked other clinical information (e.g. ovarian reserve), and for one third of patients no information was available regarding the specific type of infertility. Another limitation that could affect the outcomes is that the study involved a limited number of patients and controls. The study should be continued with enlarged patient and control groups.

Moreover, infertility is a dynamic process and can develop or resolve at any point of time throughout the reproductive age, and it is hard to define and recruit a control group with excluded infertility for similar types of studies. We have used healthy controls with children, but it is impossible to exclude that they had or will have infertility in their lifetime.

In conclusion, secondary infertility is associated with the *FMR1* gene high-normal CGG repeat allele. It is necessary to undertake further studies to understand the mechanism of high-normal allele association with secondary infertility. According to the outcomes of our study, the frequency of *FMR1* gene premutation allele carriers in Latvia may be higher than in other European populations. The study should be continued with increased patient and control groups.

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Conflict of interest

The authors declare no conflict of interest.

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