



## ASSOCIATION STUDY OF PREMATURE OVARIAN INSUFFICIENCY (POI) WITH CHROMOSOME ABNORMALITIES AND PREMUTATION OF FMR1 GENE IN WOMEN IN THE NORTHWEST OF IRAN

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### Abstract:

**Objective:** premature ovarian insufficiency (POI) is defined as the loss of ovarian function before the age of 40 and affects approximately 1 percent of women. the disorder is clinically manifested by increased levels of follicle-stimulating hormone(FSH) (>40 IU/I), decreased levels of estradiol E2 (<20 IU/I) and secondary amenorrhea lasting for at least four months in women under 40 years of age. Numerous factors are involved in recurrent miscarriages, the most important of which are genetic factors like as chromosomal abnormalities and premutation in FMR1 gene in women. The present study aimed at investigating the relationship between the women s chromosomal abnormalities and premutation of FMR1 gene in women with premature ovarian insufficiency in the northwest of Iran.

**Materials and methods:** in the present applied research,50 women referring for POI were subjected to cytogenetic experiments via using the high resolution of GTG banding. Analysis of fragile x premutation were also conducted on the women based on PCR method.

**Results:** fifty patients were included, with a median age at menopause of 29 years. 15 out of 50 studied women (30 percent) were diagnosed with chromosomal abnormalities. 6 of these abnormalities were numerical one and nine of them were structural one. Out of 50 women subjected to FMR1 gene premutation, none of them show premutation.

**Conclusion:** the rate of chromosome abnormalities in our sample was higher than in other populations while, the prevalence of FMR1 premutation was lower than in other populations.

### Introduction:

Premature ovarian insufficiency (POI) is defined as the loss of ovarian function before the age of 40 and affects approximately 1 percent of women (1). The disorder is clinically manifested by increased

levels of follicle-stimulating hormone(FSH)( $>40$  IU/I), decreased levels of estradiol E2( $<20$  IU/I) and secondary amenorrhea lasting for at least four months in women under 40 years of age (2). The etiology of POI remains unknown and several mechanisms may be involved such as prior cancer treatments with radiotherapy and/or chemotherapy, iatrogenic and pelvic surgery, autoimmune disease, metabolic disorders, and environmental factors (3). Genetic contribution has been suggested as a significant component of POI pathogenesis (4,5). Numerous karyotypic abnormalities have been reported, ranging from x-chromosome deletions, x- autosome translocations or x- isochromosomes to numerical defects (6,7). X-monosomy, both with and without mosaicism has been associated with an accelerated follicular atresia (6).

Previous studies have reported that 47,xxx patients are also at risk for POI, with a prevalence varying between 1.5 percent and 3.8 percent (8). In 1973, Sarto et al defined an x-chromosome critical region from xq13-xq21 to xq23-xq27 (9). The implication of this region in translocations or deletions was associated with POI (8) Multiple studies have corroborated this finding (6). The fragile mental retardation 1 (FMR1) gene is the strongest genetic association with POI (10). Carriers of permuted alleles, with 55-200 CGG repeats, are known to have a risk of developing POI as high as 34 percent (11,12). X-chromosome and autosomal mutations are the most common genetic associations with POI (3). Concerning defects of the X-chromosome, Turner syndrome 45,x with complete loss of one X-chromosome represents the major cause found in syndromic POI patients (13), probably due to haplo-insufficiency of x- linked genes that escape inactivation (14,15).

Fragile x mental retardation 1(FMR1) gene permutation is also a significant predisposing factor (16). Fragile x syndrome (FMR1, OMIM no.309550) is the most common cause of inherited mental retardation in males (3). The mental retardation is due to a full mutation characterized by an amplification of CGG trinucleotide that exceeds 200 repeats, in the 5 prime untranslated region (UTR)(3). Hyper methylation of full mutations results in the alteration of FMR1 transcription (3). The absence of detection of the FMR1 protein (FMRP) reveals a complete silencing of the FMR1 gene (17). Women who carry the permutation(CGG repeats between 55 and 200) have an increased risk of developing fragile x-associated primary ovarian insufficiency (FXPOI) (18,19). The resulting permutation alleles are unstable upon transmission with a risk to pass on a full mutation in one generation (3). Permuted alleles are methylated and lead to increased FMR1 transcription and decreased but detectable levels of FMRP (20). Normal alleles range are  $<45$  repeats. Intermediate alleles ranging from 45 to 54 CGG (grey zone) , may show some instability upon transmission, including very rare expansion to a permutation and exceptional expansion to full mutation upon transmission in two generations (21).

Carriers of FMR1-PM are not at risk of developing POI but also have an increased risk of fragile- x-associated tremor/ataxia syndrome(FXTAS) (1,22). This is a late onset neurodegenerative disorder, characterized by gait ataxia, dementia and intention tremor, which occurs in male carriers of FMR1-PM (8). The penetrance of symptoms increases with age , affecting more than one third of patients over 50 years of age and exceeding 50 percent for men aged 70-90 years (8). Females are also affected although to lesser extent (22). Another reason to test for FMR1-PM is the increased risk of expanding to the full length mutation( over 200 CGG repeats) in the offspring , leading to the fragile x syndrome(FXS) (8).

This risk is directly associated with the number of the premutation carrier CGG repeats , increasing significantly with more than 65-70 repeats (23). These figures highlight the importance of the genetic characterization of these patients , both at the chromosomal and molecular level (8). This will contribute to a better understanding of the biological mechanisms associated with POI (8). Moreover this knowledge will allow for an evaluation of their family risk of developing POI or having a fragile x or FXTAS descendent, identifying family member's candidates of genetic assessment, genetic counseling or prenatal diagnosis (8). In this regard, a multidisciplinary approach involving gynecologists, obstetricians, geneticists and neurologists is paramount in correctly counseling these patients (8). It is known that population characteristics, such as ethnicity, may affect POI prevalence and its genetic contribution (1). Therefore, we aimed to describe both cytogenetic abnormalities and the prevalence of FMR1 gene permutation in a northwest Iran population with POI.

### Materials and methods:

The present research examined the chromosomal abnormalities of 50 POI women during 2022-2023 in the Genoteb laboratory. The average ages of the women were 29 years. The disorder is clinically manifested by increased levels of follicle-stimulating hormone (FSH)( $>40$  IU/I), decreased levels of estradiol E2( $<20$  IU/I), and secondary amenorrhea lasting for at least four months in women under 40 years of age. Exclusion criteria of POI patient: several mechanisms may be involved such as prior cancer treatments with radiotherapy and/or chemotherapy, iatrogenic and pelvic surgery, autoimmune disease, metabolic disorders, and environmental factors. In this study, the Ethics code was, IR.TABRIZIU.REC.1402.133

### cytogenetic analysis:

Peripheral blood ( 5 ml for every individual) was collected using syringes containing heparin. 12 blood droplets were grown for every individual on a culture medium containing 9 ml RPMI1640 and the high-resolution GTG banding of the metaphase chromosomes was performed based on standard methods.

### Polymerase chain reaction:

The gene permutation of FMR1 was examined based on the usual PCR by kit of Keysar company. In this process, 2 Landa of extracted DNA and 14 landa of kit were added. The results of PCR were read by a genetic analyzer sequencer and analyzed by gene marker software.

### Results:

#### Cytogenetic:

All 50 women were subjected to the experiments. The POI women average were 29 respectively. All the 50 women were subjected to chromosomal analysis . 15 out of 50 analyzed subjects were diagnosed with chromosomal abnormality (30 percent) (table 1).

**Table 1: spectrum of chromosomal abnormalities detected from the chromosome analysis of 50 POI women for the investigation of POI etiology.**

Percent in 50 women	Numerical abnormalities	Structural abnormalities	No .of cases
4	-	22PS+	2
2	-	13P-	1
8	-	9poly+	4
2	-	Inversion9	1
2	-	21ps+	1
2	47XXX	-	1
4	45XO/46XX	-	2
2	45XO	-	1
2	46XY/46XX	-	1
2	46XY*	-	1

\* this case has a bone marrow transplant from her brother. Production of this karyotype in the blood of this woman who had blood cancer affected her sexual hormones and leading to POI disorder.

### Molecular section:

In the analysis results no permutation was found in our cases and only one carrier of full mutation ( $>200$  repeats) and two carriers of intermediate repeats (23/45 repeats and 45/35 repeats) were observed and the rest of the cases were healthy. Thus, it was concluded that prevalence of FMR1 gene permutation is rare in northwest of Iran.

**Discussion:**

Chromosomal abnormalities were observed in 30 percent of the women referring for POI. Based on previous studies, the women’s chromosomal abnormalities are responsible for POI . The comparison between the results of previous studies and those of the present study is provided in Table 2. All the conducted studies demonstrated the accentuated role the women chromosomal abnormalities in POI. As shown, the obtained results in studies and percentage differences are due to the differences in the size and type of study sample volume.

**Table2. comparison of the results of previous studies with those of the present study.**

Study	Year	Ethnicity	Case	Karyotype anomaly%	Chromosomal anomaly%	Polymorphic variant%
present study	2023	Northwest of Iran	50	30	16	14
Marta Rajkiewicz	2011	Polish	40	5	5	-
Nouha Bouali	2015	Tunisian	100	11.81	11.81	-
Ana Raquel Neves	2020	Portuguese	94	16.5	16.5	-

According to the obtained results out of the 50 women with POI no women had premutation. The results of various studies are described as follows:

A ) Nouha Bouali : 2015: in Tunisian the prevalence of FMR1 premutation was 5 percent in nouha cohort of 100 women affected of idiopathic POI. Genetic counseling revealed that all of these 5 patients had sporadic POI. This FMR1 premutation screening represents to our knowledge the first study on Tunisian POI population. It confirms that FMR1 premutation are a significant etiology of POI before the age of 40 in this population.

B ) Marta Rajkiewicz:2011 in polish : the frequency of premutations in the FMR1 gene in this study group was 7.9 percent. It confirms that FMR1 premutation are a significant etiology of POI before the age of 40 in this population.

C) Ana Raquel Neves:2020: in Portuguese: this is the first study describing the clinical characteristics and both cytogenetic and FMR1 testing in a portuguese population with POI. The prevalence of FMR1-PM in our sample was 6.7% , similar to what has been previously described in non – Asian populations.

In earlier studies of other populations, FMR1 permutations were not observed in patients with POI. In the Asian population, the prevalence seems to be lower (0.5%-1.5%). In a group of female Chinese patients (0.86%) incidence of FMR1 gene permutations were low, but a high percentage of chromosome abnormalities (16%), were reported. The rate of chromosome abnormalities in our study was higher than in other studies while the prevalence of FMR1 premutation was lower than European population and like as Asian population.

**Conclusion :**

The present study evaluated chromosomal abnormalities in women diagnosed with POI and obtained results were consistent with the findings of prior research. women's chromosomal abnormalities play a direct role in POI. As for the FMR1 gene premutation no premutation observed in our study. considering the results of the present study and those of previous studies in Asia, the FMR1 gene premutation is rare at least in Asia and almost in Iran based on zero prevalence.

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