Gene 676 (2018) 189–194

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Premutations of FMR1 CGG repeats are not related to idiopathic premature ovarian failure in Iranian patients: A case control study

Rezvaneh Asadi[a], Mir Davood Omrani[b], Hamid Ghaedi[b], Reza Mirfakhraie[b], Eznollah Azargashb[c], Mohsen Habibi[b], Farkhondeh Pouresmaeili[b,d,*]

[a] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[b] Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Department of Social Medicine, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Infertility and Reproductive Health Research Centre (IRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Premature ovarian failure (POF)
Idiopathic POF
CGG repeat
FMR1
Biomarker

ABSTRACT

Premature ovarian failure (POF) is a reproductive disease which affects 1 in 100 under 40 years women. FMR1 premutation carriers of CGG repeats are supposed to be at increased risk for POF. We have examined the 5′UTR region of the gene to find any association between the repeat size and the disease etiology in Iranian population. 30 women with early idiopathic POF and 30 fertile control women were selected. We used triplet repeat primed PCR (TP PCR) assay and gene-specific primers to amplify the CpG Island of FMR1 gene promoter region. The amplification results were analyzed by capillary electrophoresis and Gene Marker software. Among 30 patients, two had intermediate repeat size, one had premutation and the rest had CGG repeat of the normal range. Two of controls had intermediate repeats and none had a premutation. Two groups had significant differences in the repeat number average ($p = 0.007$) and in the average length of the smallest allele ($p < 0.001$), but had no promising difference in average length of the longest allele ($p = 0.453$).

Although the two groups showed a significant difference in the length of alleles, their length was within normal range. Perhaps the polymorphism, in connection with the genome's entire network, has been involved in the development of the disease, or there has been a fundamentally different mechanism for the disease in Iranian population. A larger number of Iranian POF patients should be investigated for any probable relationship between the CGG triplet repeat length and the etiology of the disease.

1. Introduction

The loss of ovarian follicular property, which results in the breakdown of normal cycles of ovarian function, is called premature ovarian failure (POF) (Welt, 2008; Bricaire et al., 2013). POF is a common disease, with a frequency of about 1% of general women population before the age of 40 and 10–28% of women with primary amenorrhea (Del Mastro et al., 2014). It is a heterogeneous disorder with common symptoms like hypoestrogenism and hypergonadotropism (Kalantaridou et al., 1998), hot flashes, vaginal dryness, sleep disorders and eventual mood disorders (Fassnacht et al., 2006). The main cause of this disease is unknown, but genetic factors (Falorni et al., 2014; Uhlenhaut and Treier, 2006; Depmann et al., 2016; Kenny and Kell, 2017; Davies et al., 2015; Hundscheid et al., 2000), drugs, viral infections (Ernst et al., 2013) and autoimmune disease are reported as POF causes (Coulam et al., 1983; Silva et al., 2014). In some patients, the return of normal ovarian function and gestational effects are rare and occur spontaneously with a low probability of future pregnancy (Somers et al., 2005). In addition, Iatrogenic impairments (Ernst et al., 2013; Coulam et al., 1983; Goswami and Conway, 2007; Deligeoroglou, 1997), lifestyle factors and premutations of fragile X are proposed to contribute to POF (Mattison et al., 1984; Chapman et al., 2015a; Rychlik, 2000; Murray et al., 2014).

One of the most prominent genes in the etiology of the disease is X-linked *fragile X mental retardation 1* (FMR1) (OMIM #300624) located on Xq27.3, prone to the promoter CGG-triplet repeat hyperexpansions and subsequent hypermethylation which increases the risk of fragile X-related disorders like the premature ovarian failure (Guo et al., 2014; Qin et al., 2015; Wang et al., 2014). The mean age of menopause seems to be lower in premutation carriers compared to the women with

Abbreviations: POF, premature ovarian failure; FMR1, fragile X mental retardation 1; dNTP, deoxyribonucleotide triphosphate

* Corresponding author at: Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail address: pouresfar@gmail.com (F. Pouresmaeili).

https://doi.org/10.1016/j.gene.2018.07.034
Received 21 April 2018; Received in revised form 2 July 2018; Accepted 11 July 2018
Available online 18 July 2018
0378-1119/ © 2018 Elsevier B.V. All rights reserved.

normal range repeats (Wang et al., 2014; Yu et al., 2015; Barasoain et al., 2016; Peprah, 2014; Sherman et al., 2014; Persani et al., 2010; Rosario et al., 2016; Pastore et al., 2017). Based on the number of FMR1 CGG repeats, four allelic groups have been identified. Normal alleles carry 5 to 44 replicates, intermediate alleles (44 to 54 repetitions), premutation alleles (55 to 200 replicates), and full mutations (over 200 replicates) (Chen et al., 2017a). The 50–200 repeats are known as risk factors for POF in premutation carriers (Saul and Tarleton, 1993; Gersak and Veble, 2011; Pouresmaeili and Fazeli, 2014). The POF1 region on chromosome X contains several genes, from which FMR1 is involved in ovarian function (Jiao et al., 2017). It seems that the CpG islands in the premutation status are not methylated and the allele codes for the normal level of FMRP. However, the CGG repeats are unstable in premutation alleles and may increase in size when they are transmitted to the next generations (Ayed et al., 2014).

Population based studies show a significant difference between carriers of FMR1 pre-mutation of POF and control subjects in India and Italy (Tosh et al., 2014; Bennett et al., 2010; Marozzi et al., 2000). The increased levels of FSH in POF patients are mostly associated with the pattern of menstrual cycles based on the CGG repeat size (Okeke et al., 2013). The importance of FMR1 gene intermediate alleles (35–54 repeats) has been shown in 14.2% of POF patients (Matthews et al., 1989) and in carriers of moderate allele who are at risk for POF (Matthews et al., 1989; Tehrani et al., 2011).

To better understand the causes and the mechanisms of the disease, we examined the relationship between the CGG repeat sizes of FMR1 gene in a population of Iranian women with POF in comparison to control fertile women.

2. Materials and methods

2.1. Sampling

In this study, 30 women with idiopathic POF, stopped menstrual periods for at least 6 past months, elevated serum FSH level (> 40 IU/l), decreased estradiol level (< 50 pmol/l), normal karyotype, with no family history of premature menopause and fragile X syndrome were selected as case group. Besides, 30 menstruating fertile women with normal hormone levels were selected as control subjects for this study. All individuals were under 40 years of age and the groups were matched by age.

Patients who were referred to the fertility center of Shahid Beheshti University of Medical Sciences and their disease was approved by a clinical consultant were enrolled in the study and completed a questionnaire.

2.2. DNA extraction

DNA samples were prepared from peripheral blood by salting out method. A number of samples which were previously prepared at Fertility Clinic of Endocrine and Metabolism Research Center, Shahid Beheshti University of Medical Sciences, were presented for this work.

2.3. PCR

2.3.1. Primer Design

For FMR1 gene amplification, the previously reported primers were used after their sequences were checked by Gene Runner to avoid the formation of secondary structures such as hairpin and dimer (Tassone et al., 2008). Primers were also matched for the lack of mismatch by NCBI database. The primers' sequences are shown in Table 1.

2.4. Amplification of the target sequence by polymerase chain reaction

Amplification of the desired part of the gene was done by modified TP-PCR method (Tassone et al., 2008). For this purpose, reactions including 2.5 mM dNTP, 3 μl of DMSO, 10 pmol of each primer (C and F) with one labeled primer by a fluorochrome stain (6FAM), 200 ng DNA, 3.5 μl of 5× HQ buffer, 3.5 μl of 10× Buffer with Mg2+, 2.5 U/μl Taq polymerase and up to 25 μl H2O were prepared for all of case and control samples. The preparations were amplified by different steps in thermocycler system as followings: An Initial denaturation at 95 °C for 15 s, 35 cycles of denaturation at 98 °C for 30 s, annealing at 60 °C for 1 min, extension at 72 °C for 2 min and a final extension at 72 °C for 10 min. To detect PCR products and to ensure the proper and specific function of PCR, the products were initially electrophoresed on 2% agarose gel. To examine the sensitivity of the analysis method, a positive control was subjected to a second round of PCR with the c and chimeric primers and was analyzed by either 2% agarose gel or capillary system.

Table 1
The sequence of primers used for FMR1 CGG repeats amplification.

Primer	Sequence
c	5′GCTCAGCTCCGTTTCGGTTTCACTTCCGGT3′
f	5′AGCCCCGCACTTCCACCACCAGCTCCTCCA3′
Chimeric	5′AGCGTCTACTGTCTCGGCACTTGCCCGCCGCCGCCG3′

To identify the true homozygotes, all of the samples that show homozygosity by the first primer pairs, were amplified once again by PCR using c and chimeric primers through the following steps: The reaction mixture (2.5 mM dNTP, 10 pmol of each primer, 200 ng DNA, 5× HQ buffer, 10× Buffer with Mg2+, 2.5 U/μl Taq polymerase, and up to 25 μl H2O) was prepared, amplification procedure was done through an initial denaturation at 98 °C for 10 min, 9 cycles of denaturation at 97 °C for 35 s, annealing at 64 °C for 35 s, extension at 68 °C for 4 min, 24 cycles of denaturation at 97 °C for 35 s, annealing at 64 °C for 35 s and 4 min extension at 68 °C followed by one cycle of a final extension step at 68 °C for 10 min, and ultimately capillary analysis was performed.

2.5. Analysis of PCR products

The analysis of PCR products was done by either 2% agarose gel or capillary electrophoresis. Due to the high resolution, short separation time, need to very low sample size and low cost the latest system was selected as the basic analysis tool in the current work (Chen et al., 2010). Therefore, after preparing the PCR products and labeling, they were sent directly to the Kosar and Pishgam companies (Tehran-Iran) for DNA fragmentation analysis at the South Korean Macrogen Company by ABI system (ABI 3730xl machine, Applied Biosystems, 96 well capillary, 50 cm capillary size, standard 55 °C for Annealing). The data were evaluated by Gene Marker software. Based on Gleicher, et al. nomenclature, the allele with a lower number of CGG repeats was introduced as allele1 and the one with a higher number of the repeats was called allele2 (Gleicher et al., 2009).

3. Results

All case and control samples in addition to a positive control demonstrated an approximately 280 bp band on 2% agarose gel after amplification by C and F primers (Fig. 1). The amplification product of the positive control with c and chimeric primers appeared as a smear on the agarose gel (Fig. 2A), while the product became visible as measurable peaks by capillary analysis (Fig. 2B). Real homozygotes (20% of case and 30% of control group) showed one specific peak (Fig. 3A) and the samples with heterozygote nature (80% of case and 70% of control group) revealed two distinct peaks when they were examined by capillary assay (Graph 1 and Fig. 3B).

Download English Version:

https://daneshyari.com/en/article/8644453

Download Persian Version:

https://daneshyari.com/article/8644453

Daneshyari.com