Gene 521 (2013) 145–149

Contents lists available at SciVerse ScienceDirect

Gene

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

Study of FMR1 gene association with ovarian dysfunction in a sample from the Basque Country

Maitane Barasoain [a], Gorka Barrenetxea [b], Iratxe Huerta [a], Mercedes Télez [a], Amaia Carrillo [b], Cristina Pérez [b], Begoña Criado [c], Isabel Arrieta [a,*]

[a] Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country, Bilbao, Spain
[b] Center for Reproductive Medicine and Infertility Quirón Bilbao, Bilbao, Spain
[c] Cooperativa de Ensino Superior Politecnico e Universitario (CESPU), Porto, Portugal

ARTICLE INFO

Article history:
Accepted 8 March 2013
Available online 26 March 2013

Keywords:
FMR1 gene
Ovarian dysfunction
Premutation
Intermediate alleles
Basque Country

ABSTRACT

Premature ovarian failure (POF) is defined as cessation of menses before the age of 40. The most significant single gene associated with POF is the Fragile X Mental Retardation 1 gene (FMR1). In the present work we screened women with fertility problems from the Basque Country in order to determine, whether in these women, FMR1 CGG repeat size in the intermediate and premutation range was associated with their pathology, and whether intermediate and premutation carriers had endocrine signs of diminished ovarian function, using the most established measure of ovarian reserve, the gonadotropin FSH. A patient sample of 41 women with ovarian insufficiency and a control sample of 32 women with no fertility problems from the Basque Country were examined. The patient sample was classified into three categories according to the results of the retrospective assessment of their ovarian function. In group 2 of patients, women with irregular cycles, reduced fecundity and FSH levels ≥ 10 IU/l, there is a significant increase in the number of intermediate and premutation FMR1 alleles (35–54 CGG repeats). In group 3 of patients, women with amenorrhea for at least four consecutive months and FSH levels ≥ 10 IU/l, a significant increase in the number of intermediate FMR1 alleles (35–54 CGG repeats) was found in patients compared with controls. In this group all the patients had a serum concentration >40 IU/l. The results suggest that in the analysed Basque sample the FMR1 gene has a role in the aetiology of POF. However, elevated FSH levels are more related to the menstrual cycle pattern than to the CGG repeat size.

1. Introduction

Premature ovarian failure (POF; #311360), defined as cessation of menses before the age of 40 in the presence of an elevated FSH level, affects 1% of females of reproductive-age and its aetiology is still unknown in most cases.

A genetic basis for POF has been suggested by familial cases with affected individuals in more than one generation [for review, see (Jin et al., 2012; Murray et al., 2000)]. The most significant single gene associated with POF is the Fragile X Mental Retardation 1 gene (FMR1) at Xq27.3 (Gleicher et al., 2009a; Goswami and Conway, 2007; Sherman et al., 2007).

The FMR1 gene (*309550) (Fu et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991; Yu et al., 1991) contains a highly polymorphic CGG repeat in the 5′ untranslated region of the exon 1. Four allelic forms have been defined with respect to CGG repeat length and instability during transmission. The first allelic form, the normal range, consists of 6 to 54 repeats that are usually transmitted from parent to offspring in a stable manner. The term intermediate or grey zone has been used to define alleles with sizes at high range of normal ones (from 35 to 54 repeats) (Bretherick et al., 2005). These alleles can be unstable upon transmission, leading to a full mutation in several generations, being more instable when transmitted through males (Sullivan et al., 2002). However, some authors reported an expansion of an intermediate allele to a full mutation only in two generations (Fernandez-Carvajal et al., 2009; Terracciano et al., 2004; Zuñiga et al., 2005). Alleles with repeat sizes from 55 to 200 repeats are classified as premutation alleles and are more instable when transmitted by females (Sullivan et al., 2002). The full mutation form consists of over 200 repeats, which induces hypermethylation of the FMR1 gene's promoter and the subsequent silencing of the gene, associated with Fragile X Syndrome (FXS; #300264) (Wang et al., 2012).

Abbreviations: FMR1, Fragile X Mental Retardation 1; FXS, Fragile X Syndrome; POF, Premature Ovarian Failure; FSH, Follicle Stimulating Hormone.

* Corresponding author at: Department of Genetics, Physical Anthropology and Animal physiology, Faculty of Science and Technology, University of the Basque Country, Apdo. 644-48080, Bilbao, Spain. Tel.: +34 946012605; fax: +34 946013145.
E-mail address: mariaisabel.arrieta@ehu.es (I. Arrieta).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.03.032

Several studies reported an increased incidence of POF among carriers of FXS (Cronister and Schreiner, 1991; Partington et al., 1996; Schwartz et al., 1994; Vianna-Morgante et al., 1996). However, other authors suggested that full mutation carriers have not altered ovarian function (Streuli et al., 2009).

Premutation alleles have been principally associated with two phenotypes, fragile X tremor ataxia syndrome (FXTAS) (Hagerman et al., 2001) and premature ovarian failure (Cronister and Schreiner, 1991). POF is presented in approximately 16% of women carrying premutation alleles (Allingham-Hawkins et al., 1999; Hundscheid et al., 2003; Mallolas et al., 2001; Murray et al., 2000; Streuli et al., 2009; Sullivan et al., 2005; Vianna-Morgante et al., 1999; Wittenberger et al., 2007) whereas it occurs in only 1% of the general population. Moreover, FMR1 premutation size alleles are found in approximately 3.2% of women with sporadic POF and in 11.5% of those with a family history of ovarian failure [for review, see Gleicher et al., 2009a].

Some studies have shown that intermediate or grey zone alleles could also be related to the development of POF (Bodega et al., 2006; Bretherick et al., 2005; Streuli et al., 2009; Sullivan et al., 2005). Bretherick et al. (2005) found that 14.2% of alleles among women with premature ovarian failure were between and including 35 and 54 CGG repeats.

Concerning the molecular level, a non-linear association between premutation repeat size and risk for ovarian failure has been found (Allen et al., 2007; Ennis et al., 2006; Sullivan et al., 2005), having premutation sizes of ~80–100 repeats a higher risk for POF and an earlier age at menopause. Women with premutation in the midsize range seem to have an increased prevalence of POF, a 7-year reduction in mean age at menopause and increased rates of menstrual dysfunction, infertility and dizygotic twinning (Allen et al., 2007).

Additionally, some authors have shown that premutation carriers without signs of ovarian dysfunction have a reduction in the average age at menopause comparing with non-carriers (Hundscheid et al., 2000; Murray et al., 2000; Van Esch et al., 2009). Sullivan et al. (2005) established this reduction in five years. In addition, higher levels of follicle stimulating hormone (FSH), an indicator of reduced ovarian function, were found in premutation carriers who are still cycling (Hundscheid et al., 2001; Murray et al., 1999; Welt et al., 2004). A predictive value of the FSH of 79% in predicting ovarian response has also been reported (Creus et al., 2000).

Different terms have been used in medical literature to describe the spectrum of clinical signs associated with ovarian function decline (Ahmed Ebbiary et al., 1994; Beckers et al., 2002; Buckler et al., 1991; Cameron et al., 1988; Coulam et al., 1986; De Moraes-Ruehsen and Jones, 1967; Farhi et al., 1997; Healy, 1994; Hunter et al., 2008; Kalantaridou et al., 2007; Nelson, 2005; Welt, 2008), but it is not even clear which terminology better describes ovarian dysfunction, remaining a subject of continued discussion (De Caro et al., 2008). In the present work we used the term POF, describing in each case the phenotypic characteristics of the ovarian condition.

In previous investigations, our group analysed the prevalence of FXS among mentally retarded individuals of Basque and non-Basque origin from the Basque Country. We found that the frequency of the full mutation in individuals of non-Basque origin was in the range of other Spanish populations while in the Basque sample the repeat size was in the lower normal range (Arrieta et al., 1999a). In other previous works, we extended the study to a normal Basque sample and we found a low frequency of large alleles. Also, the prevalence of premutation and intermediate/grey zone alleles in normal Basque sample was lower than that reported in Caucasian populations (Arrieta et al., 1999b, 2003a, 2008a, 2008b; Barasoain et al., 2012; Peñagarikano et al., 2004).

In the present work we screened women with fertility problems for the most informative genetic marker, FMR1 gene, in order to determine the significance of this marker in this sample of the Basque Country population. We also analysed FSH levels to determine whether the sample showed endocrine signs of premature ovarian dysfunction.

2. Materials and methods

2.1. Study sample

We studied a sample of 41 unrelated women with diminished ovarian function from the Basque Country. They were recruited at Ginecology, Obtetrics and Assisted Reproduction Services of Centres from the Basque Country. The sample constitutes a solid proportion of women with diminished ovarian function and Basque origin from the Biscay province. The mean age of this group was 36.24 ± 3.17. Complete information related with the reproductive condition of each patient was obtained from the infertility specialist. All the patients included in this study were phenotypically and cytogenetically normal and considered idiopathic because they did not show any condition related with ovarian dysfunction (ovarian surgery, previous chemo or radiotherapy, autoimmune diseases, metabolic disorders such as galactosaemia or genetic disorders). Chromosome analysis determined with previously used procedures (Arrieta et al., 1993, 1996a, 1996b, 2002, 2004; Télez et al., 2010) did not reveal any structural or numerical anomalies and the cytogenetic expression of the FRAXA fragile site was negative in all cases.

Following Streuli et al. (2009) and De Caro et al. (2008), the sample was classified into three categories according to the results of the retrospective assessment of their ovarian function: group 1, women with irregular cycles, reduced fecundity and FSH levels < 10 IU/l; group 2, women with irregular cycles reduced fecundity and elevated FSH levels (≥10 IU/l) and group 3, women with amenorrhea for at least 4 consecutive months and elevated FSH levels (≥10 IU/l). We also included a control group of 32 women (group 4) with no infertility problems and in which menopause occurred as a natural process. The mean age of this group was 53.45 ± 12.93.

All protocols were approved by the Ethical Committee of the University of the Basque Country and all subjects gave their informed consent. Participants in the patient and control groups provided a venous blood sample for DNA analyses to determine FMR1 CGG repeat size. For FSH analysis, serum was separated from blood sample.

2.2. DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes according to standard procedures (Sambrook et al., 1989). The size of the FMR1 CGG repeat was analysed with the Abbott Fragile X kit (part no: 6L4301. Abbott) used according to the manufacturer's instructions. PCR amplifications were performed in a 20 μl reaction volume containing 13 μl high GC PCR buffer, 0.6 μl gender primer, 0.8 μl FMR1 primers, 1.2 μl TR PCR enzyme mix and 60 ng genomic DNA as a template. The PCR cycling profile was as follows: 15 cycles of 98.5 °C for 10 s, 58 °C for 1 min and 75 °C for 6 min, followed by 15 cycles starting at 98.5 °C for 10 s (increasing by 0.1 °C/cycle), 56 °C for 1 min and 75 °C for 6 min, in the GeneAmp PCR System 9700 (Applied Biosystems). PCR products were visualized on a 2.5% agarose gel. Then, PCR products were purified using CleanUp Enzyme Mix (Abbott) and alleles were sized by capillary electrophoresis in an automated sequencer ABI 3130XL Genetic Analyzer (Applied Biosystems). The results were analysed using the Genescan 3.1 software (Applied Biosystems).

2.3. Hormone analysis

Patients who were still menstruating were asked to provide a blood sample for hormone analysis on days 3–5 of their cycle.

We analysed the FSH endocrine profiles to determine whether the sample showed endocrine signs of premature ovarian dysfunction. Serum FSH concentration was measured using the random access analyser AxSym (Abbott) which is an immunology automated analyser based on microparticle enzyme immunoassay (MEIA) technology.