Introduction

Premature ovarian failure (POF) is defined as menopause before the age of 40 years and occurs in 1% of women. Causes of POF are of genetic, autoimmune, iatrogenic and environmental origin. Genetic causes of POF probably comprise about one-third to one-half of all cases (Bione and Toniolo, 2000; Santoro, 2001), and include large chromosomal X defects, mutations or breakpoints in POF-1 and POF-2 regions, fragile X (FRAXA), and polymorphisms in the FSH and estrogen receptors. In addition to as yet undiscovered genetic factors, the epidemiological contribution of recognized genes in unselected idiopathic cases of POF still remains to be investigated.

A possible association between POF and fragile X syndrome carriers was first investigated by Cronister et al. (1991), who found eight women with POF among 61 normal FRAXA heterozygotes. The FRAXA fragile site is located in the untranslated exon 1 of the fragile X mental retardation 1 (FMR1) gene on Xq27.3 (Kremer et al., 1991; Oberle et al., 1991; Verkerk et al., 1991). Normally, 6–54 trinucleotide CGG repeats occur in this exon and they are usually stably inherited (Fu et al., 1991). The full mutation is defined as >200 repeats. It is associated with methylation of the FMR1 promoter and silencing of gene transcription resulting in mental handicaps in the male, and may affect females to a lesser degree. Carriers have 60–200 CGG repeats, which are termed the premutation.

Several reports have detected women with POF among FRAXA carriers (Schwartz et al., 1994; Turner et al., 1994) or FRAXA premutations among women with familial POF (Conway et al., 1995; Vianna-Morgante et al., 1996; Murray et al., 1998). Using the combined information from women interviewed at the age of ≥40 years, the estimated rate of POF among carriers is 21% [95% confidence interval (CI) 15–27] (Allingham-Hawkins et al., 1999; Sherman, 2000a). The frequency of premutation carriers among women with sporadic form of POF has been found to range between 1.6 and 3.3% (Kenneson et al., 1997; Conway et al., 1998; Murray et al., 1998; Uzielli et al., 1999; Marozzi et al., 2000). While the cellular and molecular mechanisms of the POF phenotype in FRAXA premutation carriers remain largely unknown, recent findings indicate the increased FMR1 transcription and diminished levels of FMR protein (FMRP) in premutation carriers (Tassone et al., 2000; Kenneson et al., 2001). According to our previous results (Gersak et al., 1999), the aim of the present study was to evaluate the contribution of FRAXA premutation in the women with sporadic idiopathic POF in the Slovene population.

Materials and methods

A total of 83 consecutive women with sporadic idiopathic POF were included in the study. They were treated at the Department of Obstetrics and Gynaecology, University Medical Centre, Ljubljana, between 1991 and 2001. The diagnosis of POF was based on the criteria of at least 6 months of amenorrhoea or the age of menopause <40 years, and two
consecutive determinations of serum FSH >40 IU/l. There was no family history of mental retardation in any of the patients. They were phenotypically normal and had normal female karyotype (46,XX), without a past history of pelvic surgery, chemotherapy or autoimmune diseases. The study was approved by the National Medical Ethics Committee (no. 97/05/01).

DNA was extracted from white blood cells. The FRAXA premutation was determined using PCR with primers F/517R (Erster et al., 1992). The length of premutation alleles was confirmed with Southern blot. After EcoRI restriction, the genome DNA was blotted on a nylon membrane and hybridized with the digoxygenin-labelled StB12.3 probe (Rousseau et al., 1991).

After screening, all first-degree relatives of the premutation carriers were also invited to participate in the study. They were interviewed either personally or via mail.

The frequency of FRAXA premutation carriers was compared with the expected prevalence in the female Caucasian population (1 in 317) (Crawford et al., 1999) using Fisher’s exact test.

**Results**

At menopause, the mean age (± SD) of 83 women with sporadic idiopathic POF was 29.5 ± 5.2 years (range 15–39). The premutation in FRAXA locus was found in four of the 83 women screened (4.8%; 95% CI 1.9–11.7). Our results showed a higher prevalence of the FRAXA premutation in women with idiopathic sporadic POF than expected in the female Caucasian population (P < 0.001). No full mutations were identified.

All pedigrees of the premutation carriers and their ages at menopause are shown in Figure 1. One premutation was maternally and one paternally inherited; for two carriers we were not able to determine the origin of transmission. In the normal allele the sizes ranged from 22 to 39 trinucleotide repeats. The premutation fragment was detected in the range from 5.36 to 5.45 kbp after EcoRI restriction (an increase in size approximately from 53 to 85 trinucleotide repeats).
In family A (Figure 1A) the transmission of premutation was associated with expansion of trinucleotide repeats within the defined range of premutation (Figure 2). Patient no. 22 (Figure 1A, III2) had only six, irregular, menstrual cycles between menarche at age 14 years and last spontaneous menstrual bleeding at 16 years. Her mother (Figure 1A, II2) had an 8-month period of oligomenorrhoea before the onset of menopause at age 45 years. Her first pregnancy occurred spontaneously after an 18-month period of regular coitus and the second with the assistance of ovulation induction with clomiphene 3 years later.

A 3-year-old male, the second child of patient no. 58 (Figure 1C), had one band of 6.66 kbp (Δp 1460 bp) corresponding to the full mutation. He had a slightly elongated face and large ears. In terms of behaviour, he had a short attention span and hyperactivity. The proband (Figure 1C, II2) did not know her carrier status prior to entering the study. Her father (Figure 1C, I1) did not present obvious clinical symptoms.

Patient nos. 16, 58 and 11 [Figure 1D (II4), C (II2) and B (II1)] had 36-, 22- and 12-month periods of oligomenorrhoea respectively.

**Discussion**

In this study we have confirmed an important association between FRAXA premutation and the pathogenesis of POF. The frequency of premutation carriers in our group of women with sporadic idiopathic POF was 1 in 21 (4.8%), i.e. statistically significantly higher than expected in the general Caucasian population, which is in agreement with two previous observations. Conway et al. (1998) observed the FRAXA premutation in 3 of 106 (3%) English women with sporadic POF, and Marozzi et al. (2000) in 2 of 61 (3%) Italian women.

Hundscheid et al. (2000) hypothesized a paternal genomic imprinting effect when POF occurs in FRAXA premutation carriers. We were able to determine the inheritance pattern of premutation in two patients. There was paternal and maternal transmission of the phenotype.

In addition to achieving the aetiological diagnosis of POF, FRAXA premutation screening has practical implications for the management of patients. The mother of patient no. 22 had a regular menstrual cycle and POF did not occur. However, she had fertility problems and required the assistance of ovulation induction. Unfortunately, we do not have the data on her endocrine profile at that time, suggesting diminished ovarian function. POF and lower spontaneous pregnancy rates may originate from various stages of follicular development and could be related to a decreased primordial oocyte pool, increased follicular atresia, altered recruitment of the dominant follicle or interrupted maturation of the follicle (Sherman, 2000b; Hundscheid et al., 2001). According to an initial decrease in the primordial oocyte pool, the period of irregular menstrual cycle or diminished ovarian function may be associated with the selection of germ cells with expansion of trinucleotide repeats, similar to the selection of germ cells estimated for the progression to the full mutation (Feng et al., 1995; Murray et al., 1999).

On the other hand, the expansion of trinucleotide repeats to full mutations during female meiosis may result in mentally retarded offspring. Genetic counselling and prenatal diagnosis should be offered to all POF patients who are carriers of the FRAXA premutation.

The association between the FRAXA premutation and POF improves the understanding of FRAXA phenotypic spectrum in the range of <200 CGG repeats.

Recently, two groups have demonstrated reduced FMRP levels and the increased FMR1 transcription to be proportionally associated with CGG repeat number in intermediate-length premutation carriers (Tassone et al., 2000; Kenneson et al., 2001).

A defect of FMRP expression might contribute to clarify the molecular mechanisms involved in formation of oocyte pool and follicular development in the FRAXA premutation carriers.

In this study we have confirmed an important association between FRAXA premutation and the pathogenesis of POF. We provide evidence that ~4.8% of women with sporadic idiopathic POF in Slovenia are carriers of the FRAXA premutation. This result has practical implications for genetic counselling and fertility treatment.

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**References**


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