



Full length article

Fragile x-associated premature ovarian failure in a large Turkish cohort: Findings of Hacettepe Fragile X Registry



Gülen Eda Utine^{a,*}, Pelin Özlem Şimşek-Kiper^a, Özlem Akgün-Doğan^a, Gizem Ürel-Demir^a, Yasemin Alanay^{a,b}, Dilek Aktaş^a, Koray Boduroğlu^a, Ergül Tunçbilek^a, Mehmet Alikaşifoğlu^a

^a Hacettepe University, Faculty of Medicine, Department of Pediatrics, Department of Pediatric Genetics, Ankara, Turkey

^b Acibadem University, School of Medicine, Department of Pediatrics, Pediatric Genetics Unit, Istanbul, Turkey

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ABSTRACT

Objective: To determine frequency of fragile X associated premature ovarian insufficiency (FXPOI) among Turkish premutation carriers.

Study design: FMR1 premutation is the single most common genetic cause of POI (FXPOI). Fragile X Registry at Hacettepe University has been reviewed for the frequency of FXPOI among female premutation carriers. Since 1991 when FMR1 testing was available, 760 individuals from 243 families have been registered. Actual data on menstrual status of female premutation carriers were gathered and analysed.

Results: Among 314 premutation-bearing females in the cohort, 268 could be reached for an update of their menstrual history; 107 adults were 40 or younger and 156 were older than 40 years of age, whereas the remaining 5 patients were prepubertal. Among 263 postpubertal females with premutations, 90 women stopped menstruating before or at 40 years of age (premature ovarian failure – POF), constituting 34.2% of our cohort. Additionally, one carrier of a gray zone allele experienced FXPOI. History of twinning was present once in 18 women (5.7%) and twice in two women (0.6%), one of the latter interestingly bearing a full-mutation.

Conclusions: FXPOI rates in the present cohort are higher than those reported in other populations. Higher FXPOI rates in Turkish premutation carriers might be a reflection of younger mean menopause age and higher POI rates in otherwise healthy Turkish women. Since POI is much more frequent among premutation carriers than in general population, testing for CGG repeat expansions in FMR1 should be included in the work-up.

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Introduction

Menopause is permanent cessation of menstrual cycles and the end of fertility. The median age at menopause is 50 years in most of the industrialized countries [1]. Premature ovarian insufficiency (POI) is considered in women younger than 40 years in whom amenorrhea of at least 4 months is associated with two serum FSH levels, obtained at least 1 month apart, in the menopausal range [2]. POI affects 1% of women below 40 (3) and 0.1% of those below 30 years of age [2,3].

POI is a broad definition which includes primary amenorrhea due to ovarian dysgenesis, as well as secondary amenorrhea due to

early depletion of the ovarian reserve. Clinically, decreased fertility is the main feature of POI. The POI spectrum is further classified as 1) occult, if decreased fertility is the sole finding, 2) biochemical, when gonadotropin levels increase, or 3) overt, when menstrual cycles become irregular [4]. Premature ovarian failure (POF) refers to the irreversible last stage where the follicular reserve is completely depleted and menstrual cycles stop permanently [4,5].

POI is clinically and molecularly heterogeneous. Genetically, it is a multifactorial condition [6]. Underlying etiology may include chromosomal, genetic, infectious, iatrogenic, autoimmune, metabolic and toxic causes, and a large group remains idiopathic [6]. Families with multiple members affected by idiopathic POI suggest strong genetic component in etiology, and several genes have been detected in various families [6,7]. Furthermore, some well-delineated genetic disorders lead to autoimmune, hormonal,

* Corresponding author.

E-mail addresses: geutine@hacettepe.edu.tr, geutine@gmail.com (G.E. Utine).

metabolic or toxic mechanisms of premature loss of ovarian reserve.

FMR1 premutation is the single most common genetic cause of POI [4,7–9] and it is one of the oldest known causes [10]. It is responsible from 1.6 to 3.2% of sporadic POI and 11.5–16% of familial POI [4,8,11]. On the other hand, one in 130–250 of women are carriers of premutation and POI affects 15–24% of premutation carriers [4,8,10–12], corresponding to a high prevalence of *FMR1*-associated POI in general population.

Hacettepe University Children's Hospital is one of the largest tertiary centers in central Anatolia, with approximately hundred thousand yearly admission. Since the discovery of *FMR1* in 1991 as the causative gene of fragile X syndrome (FXS), molecular analysis for CGG repeats has been performed at Hacettepe University, and families have been registered in the Fragile X Registry. For the purposes of this study, premutation bearing females registered since then have been reviewed for FXPOI.

Materials and methods

Primarily functioning as a Pediatric Genetics department of a Children's Hospital, our Fragile X Registry mainly consists of individuals and families with FXS diagnosed in the pediatric age group. Thus, patients in the registry were mostly assigned either 1) on detection of full mutations causing FXS, 2) on detection of premutation alleles in cascade screening of available family members who were likely carriers. Less commonly, adult patients with suspected fragile X-associated tremor ataxia syndrome (FXTAS) or fragile X-associated premature ovarian insufficiency (FXPOI), without an index with FXS in the family, were tested for CGG repeat expansions.

Majority of the families in the registry are under periodic follow-up, mainly for FXS related morbidities. For purposes of the present study, female premutation carriers in the cohort were reviewed for the frequency of FXPOI and the age at POF. All families in the registry were contacted to survey more recent FXPOI events.

Diagnosis of POI in our cohort depended mainly on self-reporting by women and was not confirmed by two FSH levels above menopausal range. Considering that recall bias would decrease reliability of the data and also for the sake of simplicity and standardization, age at POF rather than the age at POI onset was surveyed, and age at cessation of menses was noted. Menstrual irregularity was also questioned, but was not included in the statistics.

Fragile X mutation analysis was performed by polymerase chain reaction and standard Southern blot testing [13]. Methylation status and CGG repeat numbers were determined in a subset of patients using methylation status kit, according to the manufacturer's instructions [14].

The study was approved by Hacettepe University Non-interventional Clinical Research Ethics Board (Approval no: GO 17/322-20).

Results

Hacettepe Fragile X Registry includes 760 individuals from 243 families; 422 (55.5%) females and 338 (45.5%) males (Fig. 1). Among females, 33 were included in the registry without molecular testing but based on pedigree only; either as intellectually disabled daughters of carrier females or as obligate carrier relatives of affected males. For the purposes of this study, they were left out of the cohort, since mutation status was unknown. The remaining 389 females were all molecularly tested; 74 (19%) had full mutations, 309 (79.4%) had premutations, 4 (1%) had mosaicism of full mutation and premutation, one (0.3%) was a mosaic of normal and premutation alleles, and one (0.3%) had a normal and a gray zone (intermediate) allele; the latter was assigned due to decreased fertility and irregular menses starting at 19 years of age (Fig. 1).

Since the registry was formed over a period of 26 years, some of the families were lost to follow-up and could not be reached by phone. Among 74 full mutation bearing females 64 were available for the survey; 8 were prepubertal and 56 were postpubertal (42 were 40 years old or younger, 14 were above 40). Of postpubertal

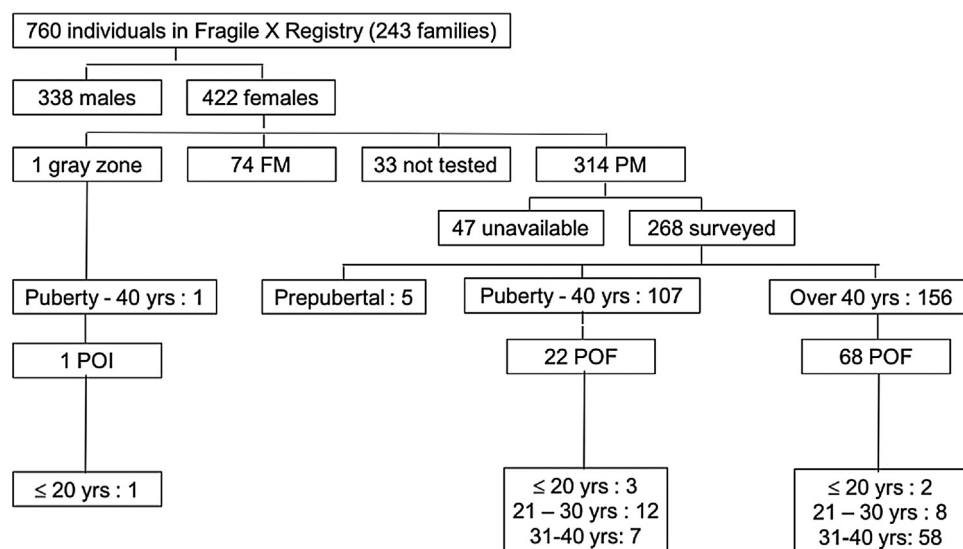


Fig. 1. Summary of the results in the present cohort. Boxes show numbers of female patients in each of the mutation groups, age distribution in relevant groups, and numbers of patients affected by FXPOI. Boxes at the bottom show numbers of FXPOI patients, distributed in three groups of reported age at ovarian failure (≤ 20 years, 21–30 years, and 31–40 years).

FM: full mutation, PM: premutation, POI: premature ovarian insufficiency, POF: premature ovarian failure, yrs: years.

females with full mutations, only one had POF before 40 years and only one subject still younger than 40 years reported irregularity of menses of unknown cause.

Except this group of females bearing pure full mutations, the other groups collectively were included in the study as pre-mutation carriers, considering that the toxic gain of RNA function might affect ovarian functions in all. Apart from the patient with a gray zone allele along with a normal allele, these constituted altogether 314 females; among which only 268 were available for the survey (Fig. 1). Among these, 5 were still prepubertal and were not included in statistical analysis. Of postpubertal females with premutations, 107 were 40 years of age or younger than this, and 156 were above 40 (Fig. 1). In the former group of 107 females, 22 had POF (20.6%) and another 11 reported irregular menses, but were excluded from statistical analysis since POI was not confirmed by FSH testing. Among 156 females above 40 years who were bearing premutations, 68 had POF before age 40 (43.6%). Another 11 in this group had menopause between 41 and 45 years, and four of these reported irregular menses before age 40, but were not included in the statistical analysis since they were not tested then for FSH levels.

In summary, among 263 postpubertal pre-mutation carriers included in our cohort, 90 (34.2%) had cessation of menses before 40 years of age (POF); 22 patients below or at 40 years of age plus 68 patients above 40 years. Additionally, another patient carrying a gray zone allele along with a normal allele also had decreased fertility and irregular menses before 20 years of age. Those who reported menstrual cycle irregularities without FSH testing were excluded from statistical analysis. POF was reported to take place before 20 years of age in 5, between 21 and 30 years in 20 and between 31 and 40 years in 65 (Fig. 1).

Eight of the 243 families were ascertained on detection of pre-mutation carrier females during work-up of ovarian failure, in the absence of an index patient with FXS. History of twin pregnancies, considered a sign of POI, was present at least once in 18 women (one being the gray zone allele carrier), and twice in 2 women (one with a full-mutation). Only 2 women in our cohort reported spontaneous pregnancies after clinically overt POF.

Comments

FXPOI is the most common manifestation of the *FMR1* pre-mutation. Carrier frequency of *FMR1* pre-mutation is high among females with POI in many populations [8,11,12]. Analysis of data from the Hacettepe Fragile X Registry revealed that POF may be manifest in up to 34.2% of pre-mutation carriers. FXPOI may also affect carriers of intermediate alleles. Age at POF in our cohort was also younger than previously reported in other studies.

CGG repeat expansions at the 5'UTR of *FMR1* gene were discovered in 1991 as the genetic cause of FXS [15]. Healthy people have CGG repeats between 5 and 44, the mode being 30 [8]. Repeats between 45–54 and 55–200 are referred to as gray zone (intermediate) and premutations, respectively. Premutations are subject to expansion, as in other trinucleotide repeat disorders. Repeats above 200 lead to the well-recognized intellectual disability syndrome, FXS, where the transcription is abolished and no FMRP protein is produced.

Apart from the predisposition to expand into full mutations during gametogenesis, pre-mutation alleles may cause clinical conditions as well. These alleles undergo transcription normally, however, producing FMRP even in less than the normal amount requires an increased rate of transcription. Overproduced mRNA in those cells expressing the gene lead to formation of inclusion bodies over time [8]. Accumulations of inclusion bodies disrupt normal functioning of these cells; inclusions in neurons lead to FXTAS, and inclusions in oocytes and granulosa cells lead to FXPOI [8].

Female fetuses develop approximately 7 million primordial follicles at intrauterine 5th month [16]. Newborns have approximately one million follicles, of which 300–400 thousand reach puberty. Until menopause, about 400 are ovulated and the rest goes under follicular atresia. Therefore, 3 mechanisms overall are responsible from early depletion of follicular reserve; (i) Smaller than normal follicular reserve at the beginning, (ii) Accelerated rate of follicular atresia, and (iii) Defective oocyte recruitment. The mechanisms underlying FXPOI are not fully elucidated yet. Toxic consequences of mRNA are being suspected strongly, however, it is not clear at the moment whether this toxicity affects the size of initial follicular pool, the rate of follicular atresia, oocyte recruitment, hypothalamus-pituitary-gonadal axis, or a combination of these [8,17,18].

For unclear reasons, there is no linear relationship between the repeat number and POI. POI is most frequent in pre-mutation carriers of 80–99 repeats, less frequent in carriers of 55–79 repeats, and least common in carriers of 100–199 repeats [4,12]. Considering the role of FMRP as a regulator of translation, one possible explanation was proposed to be dysregulation in expression of various ovarian genes and that the expression of some ovarian genes may be affected by certain repeat numbers whereas others may be suppressed in different repeat lengths. Moreover, ovarian function may be suppressed at different developmental stages of follicles by different mechanisms [7]. FXPOI was repeatedly observed in carriers of intermediate alleles as well, but this was not always evident in studies [8]. We observed only one such patient in the cohort.

Hacettepe FXS Registry cohort, 34.2% of pre-mutation carriers had POF. This frequency is higher than previously reported ratios in pre-mutation carriers from other populations. Eleven patients younger than 40 reported irregularity of menses. Eleven others had cessation of menses between 40 and 45 years, four of these reported irregular menses before 40. These 22 patients were not tested for postmenopausal FSH levels. Confirmation of POI in these 22 might have increased the overall rate of FXPOI, however, other causes for menstrual irregularity could as well be responsible.

This study being the first in Turkish population, may be suggestive of a higher FXPOI prevalence among Turkish fragile X pre-mutation carriers. Alternatively, this finding may be a reflection of population characteristics of the entire Turkish population, since previous studies have shown that mean age at menopause among Turkish women overall is around 47 years [19–22]. Turkish Demographic and Health Survey [2013], which surveyed 9,746 reproductive age women (aged 15–49), reports that 1.9% of women aged 30–39 are menopausal [23], and this is higher than European populations where POI affects only 1% of women before 40 years of age [3]. These data suggests an earlier onset of menopause in Turkish women compared with European women. Hence, this might have an additive effect on POI susceptibility in Turkish pre-mutation carriers.

The reported age at POF was before 20 years of age in 5 (5/263; 1.9%), between 21 and 30 years in 20 (20/263; 7.6%), and between 31 and 40 years in 65 (65/263; 24.7%). Thus, the data show a higher frequency of earlier onset of FXPOI in our Turkish cohort. Previous literature reports that 15–24% of pre-mutation carriers experience POF prior to age 40, approximately 3% can expect this to occur younger than 29 years of age and ~1% can expect onset at ≤ 18 years of age [4,24]. These numbers represent a 20-fold increase in the incidence of POF prior to age 40 and a 30-fold increase ≤ 29 years of age compared to the general population [4]. Overall, our data revealed that 34.2% of Turkish pre-mutation carriers experience POF prior to age 40, 9.5% before 30 years of age, while 1.9% can expect POF earlier than 20 years of age. Unavailability of FSH measurements is a limitation in this study.

However, we could speculate that higher rates would likely be reported otherwise.

Eight families in the cohort were registered during evaluation for POF, without an index patient with FXS. Although this number was extracted from a larger group of women who were admitted for ovarian insufficiency and were tested for premutation, it does not represent the real proportion of fragile X premutations among all women with POI. Our department's referring physicians being mainly pediatricians, rather than obstetricians is worth noting. The increasing awareness among obstetricians has increased referrals in the more recent years. Detection of carrier females prior to birth of children affected by FXS is another aspect dependent on obstetrician referrals. Nonetheless, assigning carrier women before birth of an affected child should be one of the goals in elucidating the etiology of premature ovarian failure.

Dizygotic twinning is considered as a sign of diminished ovarian reserve, which was also reported in *FMR1* premutation bearing women [25,26]. In our cohort, there was history of twinning once in 18 of the families, and twice in two of the families. Interestingly, one of the women with twinning history twice had full mutation. Twin pregnancies in women bearing full mutation are not known to occur in higher rates than in normal population [24]. Twin pregnancy was considered coincidental in the subject with one normal and one gray zone allele. Another clinical feature in premature ovarian depletion may be spontaneous fertility taking place after the onset. Fifty percent of women with POI may have intermittent ovarian function and 5–10% may conceive spontaneously [16,27]. In our cohort, there are two patients in whom this was observed. They conceived spontaneously after POF was clinically evident, and afterwards POF ensued.

In our cohort, we have also aimed to compare repeat numbers in premutations, in sisters discordant for FXPOI phenotype. Assigning sister pairs as discordant for this phenotype is not possible to determine definitely before both reach the age of 40. Besides, the molecular diagnosis was previously done by Southern Blot, and only since 2015, the repeat numbers can be accurately determined by using a commercial confirmatory *FMR1* methylation status kit that enables both the repeat number and the corresponding methylation status at the CGG repeat region. Consequently, until today we have only tested 5 discordant sister pairs in whom the CGG repeat numbers were not statistically different. These discordant sister pairs bearing premutations were also tested for presence of accompanying CNVs which could possibly modify the phenotype, but this also yielded negative results.

FMR1 premutation carriers may present with FXTAS and FXPOI, usually after they already bear children, or with subtle clues like anxiety or mood disorders [25]. Prevention of FXS in the family would be better achieved if carrier females were assigned before they have children bearing full mutations. Since our cohort mainly consisted of families ascertained on diagnosis of an index patient with FXS, it is impossible to draw conclusions on an increase in FXS patients in our population. A previous report presented data on Turkish population frequency of FXS among intellectually disabled patients being comparable to other populations [28].

FMR1 testing for CGG repeat expansion is recommended to be a part of the work-up for women with POI [29–31]. Apart from the purpose of preventing FXS recurrence in the family, carrier mothers of FXS patients should also be prudently surveyed for symptoms and signs of POI, since while they face numerous other difficulties in raising their affected children, they may ignore their menopausal health care needs, including optimal management of osteoporosis and cardiovascular risk.

Condensation

Fragile X-associated premature ovarian insufficiency (FXPOI) may be manifest in up to 34.2% of fragile X premutation carriers. Carriers of gray zone alleles may also experience FXPOI. Earlier age at FXPOI-related cessation of menses in our cohort could be a reflection of earlier menopausal age in Turkish women.

Conflicts of interest

None.

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