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Fragile-X premature ovarian insufficiency: a lesser known cause of infertility among Asian women

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Abstract: This study discusses the prevalence of the Fragile X premature ovarian insufficiency (FXPOI) and Fragile X mental retardation 1 genes in Asian women and highlights it as an important cause of infertility in this demographic. A literature search of PubMed and Google Scholar was performed for articles related to studies on FXPOI conducted in the Asian population. Nine studies were selected which focused on phenotypically normal Asian women at high risk of carrying premutation and Asian women who had ovarian insufficiency and premutation. Prevalence of premutation was higher in women with FXPOI vs. phenotypically normal premutation carriers (1.24% vs. 0.235%). Of the women in this study 7.94% had POI related to premutation. However, this percentage reflects a lower prevalence of FXPOI compared to women comprising the western population, where about 13.8% cases of POF are related to premutation. We posit two possibilities: Either there is underreporting of FXPOI due to misdiagnoses, or the reported low prevalence is the true prevalence. Our study shows a higher prevalence of premutations in Asian women with POF versus phenotypically normal Asian carriers who may be at risk of subsequently developing FXPOI, underscoring that FXPOI is an important genetic cause of ovarian insufficiency.

Key words: CGG, Premutation, Asia, FXPOI, Prevalence

Introduction

The *FMR1 (Fragile X mental retardation 1)* gene contains trinucleotide CGG repeats in its 5' untranslated region (UTR) [1]. The gene codes for FMRP (*Fragile X mental retardation protein*). There are four variants or alleles of the FMR1 gene based on the length of the CGG repeats in the 5'UTR region: normal, gray-zone/intermediate, premutation and mutation [2]. The associated length of CGG repeats in the variants is not unanimously agreed upon across the literature. Therefore, in this article we chose the normal CGG repeat range to be between 5 to 45 repeats in length [3]. A range between 55–199 CGG repeats meets the classification for the premutation range. The intermediate length of CGG repeats has been differently defined in the literature as 41–60 [4], 41–54 [5], 45–54 [6] and 46–60 [7]. Greater than 200 CGG repeats is called full-blown mutation. The 5'UTR region is considered unstable because there is a risk of length expansion in successive generations when the allele is transferred from the mother to her offspring [8]. With increasing repeat length, FRMP levels have been shown to gradually decrease. This may occur because of inadequate translation of the transcribed FMR1 mRNA as some studies have found increased FMR1 mRNA with decreased FMR protein levels in cells [8, 9]. However decreased FMRP can also be attributed to transcriptional silencing as other studies have pointed out that an FMR1 gene with a greater than 200 CGG repeat-length is abnormally hyper-methylated which inactivates the gene [2]. Lack of FMRP is associated with an intellectual and developmental disability known as Fragile X syndrome (FXS) [10]. It was thought that only the full-blown mutation was responsible for the clinically relevant disease, but accumulating data supports the interpretation that both premutation as well as intermediate range mutations lead to distinct clinical manifestations differing from Fragile X syndrome, namely, Fragile X tremor ataxia syndrome (FXTAS) which can occur in both genders (usually

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more in men), and Fragile X premature ovarian insufficiency (FXPOI) which occurs exclusively in women[11]. Both disorders demonstrate incomplete penetrance [8].

There is a striking disparity in the prevalence of the full-blown mutation between the Asian and western (Caucasian) populations. Studies demonstrating the prevalence of FXS as a cause of mental retardation (MR) in the Caucasian population have reported ranges from 2.6–8.7% [12, 13] compared to 0.8–2.4% [14, 15] in the Asian population. Since the premutation range accounts for a different clinical disease it is pertinent to know if this disparity in prevalence of the full-blown mutation is reflected in the premutation range between these populations. Indeed, the number of reported cases for FXTAS and FXPOI is less in the Asian population than in western countries [16]. The estimated prevalence of the Fragile X mental retardation 1 (FMR1) premutation in a US population-based sample from 2012 ranges between 1/209–1/151 in women and 1/468–1/430 in men [17]. Individual studies of Asian populations that have reported clinical manifestations of the FMR1 premutation range report a low prevalence, however these studies were mostly limited by sample size. To our knowledge, this review is the first effort to bring together the available data on the prevalence of FXPOI syndrome in the Asian population.

Given the available data, we aimed to assess the prevalence of FXPOI in Asian women and quantify it. Since FXPOI shows incomplete penetrance, we also collated the data showing the prevalence of CGG repeats in the premutation range and intermediate range in Asian women who did not develop symptomatic FXPOI. A concise table showing the relevant findings of the different studies reviewed for this paper has been included in the results section and it is followed by an objective assessment of the reviewed literature. This is followed by a discussion of the possibility that the FMR1 gene may lead to POF. Finally, we touch upon some factors that might be responsible for the lower premutation rates observed in the Asian population.

Materials and Methods

A literature of PubMed and Google Scholar was performed for articles related to studies on FXPOI conducted in the Asian population. The search yielded many articles including case reports and cross-sectional studies from 1990 to 2017. The total number of search results available on Google Scholar for our keywords was 4689. The total number of search results for the same keywords on PubMed was 53. For ease of data collection these articles were divided into 3 groups. Group 1 comprised phenotypically normal premenopausal Asian women who had reached puberty. These women had been tested for the FMR1 gene because they had a strong family history of Fragile X syndrome or unexplained premature ovarian insufficiency. Group 2 consisted of Asian women who were undergoing tests to explain the cause of their premature ovarian insufficiency. Group 3 consisted of studies of general Asian populations including men and women that performed for genetic testing for the FMR1 gene. Only English articles were selected. Finally, two study populations were defined: 1) Phenotypically (symptomless) normal Asian women who were found to be carriers of abnormal CGG repeats and 2) Asian women who presented with premature ovarian failure and had been tested for abnormal CGG repeat lengths. Articles pertaining to both populations were chosen. There were many articles that used the key word “Asia” in their title and there were others that used words such as ‘China’, ‘Japan’, ‘Taiwan’, ‘Singapore’, ‘Indonesia’ and ‘Hong Kong’. All the articles that named individual countries that generally have an Asian population were chosen. The key words used were “CGG premutation Asia” (which yielded 503 results in Google Scholar and 24 results in PubMed), “FXPOI Asia” (27 results in Google Scholar and 1 result in PubMed), “FXPOI prevalence Asia” (70 results in Google Scholar and 0 results in PubMed), “CGG premutation China” (1,500 results in Google Scholar and 16 results in PubMed), “CGG premutation Japan” (1,750 results in Google Scholar and 8 results in PubMed), “CGG premutation Korea” (563 results in Google Scholar and 2 results in PubMed), “CGG premutation Malaysia” (104 results in Google Scholar and 0 results in PubMed), “CGG premutation Indonesia” (172 results in Google Scholar and 2 results in PubMed).

A total of 9 articles were selected for this study, and these focused on Asian populations. Four studies were from Mainland China, 1 study was from Japan, 2 studies were of the Chinese population in Hong Kong, and 2 studies were from Korea. Of these, only the studies from Korea had investigated phenotypically normal women who were either pregnant or preconceptual and had been tested for CGG repeats. The rest of the studies were conducted on women who had premature ovarian failure who had been tested for CGG repeats to determine the cause of POF. The number of women who were reported to have CGG premutation was noted. The number of women who had POF due to CGG premutation was also noted. These numbers were used to calculate the overall prevalence of CGG premutation and symptomatic POF caused by the premutation.

Results

The salient features of each study are shown in (Table 1, 2). Data that was not available in some studies has been denoted with a hyphen sign. The total number of women from all the studies included in our review who had idiopathic POF was calculated as the sum-total of the individual sample size of each study, i.e. 1,133. Review of our data shows that among the total of 1,133 woman (from different Asian countries) who were diagnosed cases of idiopathic/non-syndromic premature ovarian failure when tested for CGG repeat length, 90/1,133 (7.94%) women had POI that was related to the Fragile X gene disorder (FXPOI) and 1,043/1,133 (92.1% correct to 3 significant figures) of the women in our review data had POF due to causes other than FXPOI. Furthermore 14/1,133 (1.24%) women had CGG repeats in the premutation range, and 76/1,133 (6.71%) women had CGG repeats in the intermediate range in their FMR1 gene (Table 3). Two studies on the Korean Population [24, 25] (n1=1,408 and n2=5,829) which investigated a large sample of phenotypically normal women, when combined (n=7,237) show that 0.235% (17/7,237) of reproductively normal women had premutation and 0.677% (49/7,237) of women had intermediate range CGG repeats. The Korean study recognized the higher prevalence of premutation found in their study compared to other Asian populations [24, 25]. The total percentage of women with CGG repeats out of the normal range was 0.912% (66/7,237) (Table 4**).** The prevalence of the number of women with premutation and intermediate range of CGG repeats from the entire data reviewed, irrespective of whether the women had clinical manifestation of premature ovarian failure, was calculated to be 31/8,370 (0.0037 or 0.370%) and 125/8,370 (0.0149 or 1.49%), respectively (Table 5).

Discussion

Genetic basis of POF

Premature ovarian failure (POF) is defined as cessation of ovarian function before 40 years and it presents as amenorrhea for at least 4 months, with follicle stimulating hormone (FSH) concentrations exceeding 40 IU/L on at-least 2 occasions 1 month apart, resulting in infertility and other systemic consequences (e.g. cardiovascular disease, osteoporosis) because of estrogen deficiency [26]. Primary ovarian insufficiency (POI) has been proposed to reflect a continuum of altered ovarian function, and if it occurs before the age of 40, it is considered a subset of POF. Premature ovarian failure affects approximately 1% of women by the age of 40 years, 0.1% of women by age 30 and 0.01% by age 20. Although such conditions as autoimmune diseases or diabetes mellitus are also associated with ovarian failure, the cause is unknown in approximately 95% of cases. Various data showing a familial form of POF in 4–30% of subjects suggests a strong genetic predisposition [27]. Among genetic causes, X monosomy as in Turner syndrome, or X gene deletions and translocations have been shown to correlate with POF. The genes involved in ovarian function located on the X chromosome are still unknown. Possible associations between gene polymorphisms and POF/POI have been investigated for X-linked genes like BMP15 and FMR1 and other autosomal genes, but the results remain controversial [28].

Role of FMR1 in POF

Fragile X Mental Retardation Gene 1 (FMR1) is located on the long arm (q) of the X chromosome at a cytogenetic location of 27.3 (Xq27.3) and it encodes a protein called Fragile X Mental Retardation Protein (FRMP) [29]. This RNA binding protein associates itself with polysomes and is involved in translational control [30]. Extensive alternative splicing at the 3' end creates multiple FMRP isoforms that appear in a variety of tissues, e.g. the brain, testicles, ovaries, liver, lungs, kidney, spinal-cord and gastro-intestinal tract. Some of them are present at different quantitative levels [31, 32]. However, the protein is expressed most abundantly in the brain and testis [33]. FMRP roles in neurons has been studied. FMRP performs nucleocytoplasmic shuttling of mRNA, dendritic mRNA localization, and regulation of synaptic protein synthesis, therefore it affects synaptic plasticity [33].

Molecular studies of ovarian dysfunction and Xq abnormalities from the last 10 years show that breakpoints are proximal at Xq13.3–q21.3 and Xq21.3–q22, and distal at Xq26–q28. The most common fragile X syndrome mutation is amplification of CGG repeats in the first exon of the FMR1 gene at Xq27.3. The premutation causes accumulation of toxic FMR1 messenger RNA that may affect a nearby gene (s) for ovarian function, inducing abnormal methylation so that fewer oocytes develop. Although the protein's role in the testis and ovaries is not clear [34], this is a potential explanation for POF in pre-mutated fragile X carriers in which a reduced FMR1 protein level somehow alters the expression of oocyte development related genes. This mechanism may be relevant to associations of intermediate length CGG repeats in FMR1 with POF. However, it is interesting to note that all women who are carriers of the premutation do not develop POF, as is shown in Table 3 in the cohort of Korean women, and the disease may present at CGG lengths other than

Table 1. Premature ovarian insufficiency patients with sporadic/idiopathic non-syndromic POI

No.	Year of study/ reference	Country	Nos. of POI patients (n)	Onset of amenorrhea (mean age) yr	Nos. of POI patients with pre-mutation	Nos. of Intermedi-ate range patients	Conclusion of study
1	2011/ Ishizuka *et al.* [18]	Japan	128	29.5 +/−0.61	2/128 (1.56%)	5/128 (3.91%)	Similar distribution between patients and controls when the number of CGG repeats was less than or equal to 36. >36 CGG repeats were significantly higher in patients with POI than in controls. Age at onset of amenorrhea was significantly lower in patients with >38 CGG repeats.
2	2013/ Lai *et al.* [19]	Hong Kong	196	27.7	7/196 (3.57%)	58/196 (29.6%)	This study was done to assess if the normal range of CGG repeats was linked to POF. It found no correlation between FMR1 gene CGG repeat sizes in the high normal range (35–54) and idiopathic POF in this Chinese population. Repeat size in the high normal range was more common in women with idiopathic POF (n=58; 14.8%) than in the control group (n=53; 13.0%) [P = 0.46]. The difference was not statistically significant.
3	2013/ Lin *et al.* [20]	China	85	-	0	-	This study found no correlation between FMR1 gene CGG repeat sizes in the premutation range and POF.
4	2014/ Ye *et al.* [21]	China	117	30	1/117 (0.9%)	-	One case with premutation (73 repeats) was identified, but no significant association between CGG repeats in the normal or intermediate range (35–54) and POF in this Chinese population was detected. There was no significant difference in the incidence of intermediate mutations of CGG repeats between patients and controls.
5	2016/ Cui *et al.* [5]	China	122	6.33 +/−4.71	1/122 (0.82%)	2/122 (1.64%)	Two intermediate range cases were found in controls (2/105). This finding questions the contribution of FMR1 repeat sizes BELOW premutation range (<55) to the ovarian aging process, since CGG repeat numbers did not statistically significantly differ between POI cases and controls.
6	2014/ Guo *et al.* [22]	China	379	25.1 +/−5.6	2/379 (0.5%)	11/379 (2.9%)	This study found that FMR1 premutation was not commonly associated with POF in Chinese population. However, having both alleles with CGG repeats outside the normal range might adversely affect ovarian aging. In a comparison of cases of sporadic POF with controls no significant difference in the prevalence of intermediate FMR1 (41–54) (2.9% vs. 1.7%, P = 0.343) was found indicating that even intermediate FMR1 does not contribute to POF in Chinese.
7	2005/ Lo *et al.* [23]	Hong Kong	116	28.2	1/116 (0.86%)	-	The prevalence of the FMR1 gene premutation carrier in Chinese women with premature menopause was approximately 0.86%. The estimates of premutation carriers are 13.8% and 2.1%, respectively, for familial and sporadic cases of POF in western populations. Thus, the carrier rate of fragile X premutation in study population appeared to be lower than that in Caucasians population.

Table 2. Salient features of studies with phenotypically normal women

No.	Year of study/ reference	Country	No. of normal patients (n)	No. of POI pts with premutation	No. of Intermediate range pts	Conclusion of study
1	2016/ Kang *et al*. [24]	Korea	1,408	7/1,408 (0.5%)	9/1,408 (0.6%)	The identified premutation prevalence is higher than that of other Asian populations and lower than that of Caucasian populations. Although this study was limited by size and population bias. A high-risk group was defined as patients with a family history of Fragile X syndrome, mental retardation or gynecological diseases such as POF. This study identified 8 intermediate alleles in the low risk group (1:167, 95% CI 1:130–213), 1 intermediate allele in the high-risk group (1:76, 95% CI 1:11,533), 2 premutation alleles in the low risk group (1:666, 95% CI 1:250–1,776), and 5 premutation alleles in the high-risk group (1:15, 95% CI 1:6–36) were identified.
2	2013/ Kim *et al*. [25]	Korea	5,829	10/5,829 (0.17%)	40/5,829 (0.69%)	This study was performed on 5,829 phenotypically normal women of reproductive age group between 2003–2011 who underwent Fragile X screening. Of the total of 5,829 women, 5,470 were categorized as minimal risk and 359 women were categorized as high-risk based on suspicious family history. Of the total number of alleles (2 × 5,829 = 11,658) 40 alleles were in the intermediate range (40/11,658, 0.343%) and 10 premutation carriers (20/11,658, 0.171%), and 1 woman had a full mutation in the high-risk group but was phenotypically normal.

Table 3. Review of results of women with POF

Total number of women with POI in the review	1,133
Women with POI with CGG repeats above the normal range (5–45 CGG)	90
Women with POI with CGG repeats in the premutation range	14
Women with POI with CGG repeats in the intermediate range	76
Percentage of cases with FXPOI	7.94%
Percentage of premutation cases	1.24%
Percentage of intermediate range CGG repeat cases	6.71%

Table 4. Review of results of phenotypically normal Korean women

Total number of phenotypically normal Korean women	7,237
No. of women with CGG repeats in the premutation range	17
No. of women with CGG repeats in the intermediate range	49
Percentage of reproductively normal Korean women with premutation	0.235%
Percentage of reproductively normal Korean women with intermediate range CGG repeats	0.677%

Table 5. Prevalences and numbers of women with premutation and intermediate range of CGG repeats

Total number of women in the literature reviewed	7,237 + 1,133 = 8,370
Total number of women in the premutation range	17 + 14 = 31
Total number of women in the intermediate range	76 + 49 = 125
Percentage prevalence of women in the premutation range	0.370%
Percentage prevalence of women in the intermediate range	1.49%

premutation length, e.g. the intermediate range or even the normal range [5]. According to one study, 20–28% of the females who are premutation carriers have POF [34]. One may expect that a full mutation carrier would manifest ovarian dysfunction, but only 14% [34] of full-mutation carriers were found to have POF, whereas most of the full mutation carriers were found to have normal ovarian function [35], and 6% of non-carriers were diagnosed with POF.

A reduced FMR1 protein level is thought to alter the expression of oocyte development related genes, resulting in POF. Nevertheless, more comparative studies need to be performed to determine why only some women with premutation and intermediate mutation develop the symptomatic disease. Since women are homozygous for the X chromosome it may be possible that a particular allele of FMR1 with a certain length of CGG repeats may be associated with the development of symptoms. Cui-Ling Lu *et al*. used a bi-variance logistic regression method to test the possible role of CGG repeats in different FMR1 alleles in Chinese women with POI [5]. The results show that CGG repeats in the short allele (allele1) were the major contributor to POI occurrence not the long allele (allele2). Even <26 as well as >29 CGG repeats in allele1 were revealed to be a genetic risk in their study.

Other studies have shown that the length of CGG repeats by itself is not the sole factor determining FMR1 instability. The factors that have an impact on the stability of FMR1 gene include: length of the repeat CGG, AGG interruption pattern, length of uninterrupted CGG, and DXS548-FRAXAC1 markers [36]. Normally there are trinucleotide AGG interruptions in CGG triple repeat length occurring once in every nine or ten CGG repeats. Most normal alleles have greater than or equal to 2 AGG interruptions between 5 and 50 CGG repeats, whereas most permutation alleles (50 to 200 repeats) have only a single or no interruption [37]. Hence these might explain why some women with a certain length of CGG repeats develop symptoms whereas others don't. More studies comparing the patterns of AGG interruptions in CGG repeat units between western and Asian populations may provide insights into the role of these trinucleotides in understanding FMR1 gene-related infertility cases.

Length of CGG repeats in the Asian population

Studies in the western population estimate that 13.8% of familial cases of POF are caused by a premutation [38] and 2.1% of POF cases are sporadic [39]. The risk of a woman with premutation manifesting POF is between 3–15% [40]. Our review shows a higher prevalence of FMR1 permutations (1.24%) and intermediate range mutations (6.71%) in Asian women with premature ovarian failure than in Asian women who were phenotypically normal (0.235% premutation, 0.677% intermediate). However, this percentage reflects a lower prevalence than in the western population. The paucity of articles on this topic may suggest that few cases of FXPOI are being reported as FMR1 genetic testing is not being carried out in women who present with POF. This might be masking the real cause of POF, thereby accounting for the low prevalence of premutation and intermediate range alleles of CGG. Increasing awareness of premutated FMR1 gene related premature ovarian failure may help lead clinicians to definitive diagnoses for presumed idiopathic POF cases. Alternatively, perhaps differences in premutation carrier rates existed in the various founding populations, accounting for the low prevalence, as has been suggested by Richards *et al.* [41] and Chakravarti *et al.* [42]. It is also possible that low carrier frequencies were created and maintained after these populations were established owing to spontaneous genetic mutations and environmental factors such as diet, exercise, climate etc. It would be informative to study the Asian population living in non-Asian countries for the CGG repeat length and pattern of AGG interruptions to determine the effects of environment on the premutation carrier frequency.

We recommend that all phenotypically normal Asian women who have a suspicious family history of Fragile X disorders should be offered genetic testing to determine their FMR1 CGG repeat length before conceiving so that adequate interventions and family planning can take place. Perhaps the identification of premutation carriers in phenotypically normal women can help further lower the prevalence of Fragile X Syndrome in the Asian population via identification of unstable length CGG repeats. As mentioned above there is an increased risk of CGG length expansion manifesting as a full mutation in a child via maternal transfer of the allele. Genetic testing would also benefit women who are planning to have children later. Working women may not delay pregnancy if they are at increased risk of premature ovarian failure. In addition, they could monitor their ovarian reserve yearly to decide when to have children, or store their eggs via oocyte retrieval, and access them later for medically assisted fertilization.

In conclusion, our study underscores the low prevalence of FMR1 gene premutation as a cause of POF in the Asian women. We posit two possibilities. Either there is underreporting of FXPOI due to misdiagnoses, in which case we recommend increasing awareness regarding this disorder, or the reported low prevalence is the true prevalence. There is vast scope to understand

infertility as a result of genetic premutation via exploration of the roles of the FMR1 gene. Such studies may provide insight into genes that determine the quality of eggs and could potentially provide solutions of curbing infertility in the western cohort of premutated patients. More data is needed to reach a definitive conclusion.

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