Correlation between *FMR1* CGG Repeat Sizes in the High Normal Range and Idiopathic Premature Ovarian Failure in Hong Kong Chinese Women

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Objective: The aim of this study was to examine the relationship between *FMR1* gene CGG repeat sizes in the high normal range and risk of premature ovarian failure in a Hong Kong Chinese population.
Methods: In this retrospective study, the *FMR1* gene CGG repeat sizes of women with idiopathic premature ovarian failure and control participants were determined. The control participants were healthy mothers of children with de-novo genetic conditions. Correlation study analysis was performed to test for the relationship between *FMR1* gene CGG repeat sizes in the high normal range and idiopathic premature ovarian failure.
Results: A total of 196 and 204 women were included in the idiopathic premature ovarian failure and control groups, respectively. The mean ages of menopause in the women with all causes of premature ovarian failure and women with idiopathic premature ovarian failure were the same at 27.7 years. Correlation study analysis showed no correlation between *FMR1* gene CGG repeat sizes in the high normal range and idiopathic premature ovarian failure in this population.
Conclusion: This study showed no correlation between *FMR1* gene CGG repeat sizes in the high normal range and idiopathic premature ovarian failure in this population. The results confirm the characteristic bimodal distribution of CGG repeat sizes shown in previous studies in Chinese populations.
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Introduction

Premature ovarian failure (POF) is defined as the occurrence of menopause before the age of 40 years. POF affects approximately 1% of women in the general population. No cause can be identified in 90% of patients with POF. Less common causes of POF include Turner's syndrome and its variants, autoimmune diseases, and iatrogenic causes (e.g. after chemotherapy or radiotherapy).

Fragile X syndrome is the most frequent cause of inherited mental retardation syndrome and autism. In 1994, Schwartz et al[1] reported increased risk of POF among fragile X gene carriers. Bodega et al[2] found a significant association between intermediate CGG expansions (41–58 CGG repeats) and POF. However, recent studies examining the relationship between *FMR1* CGG repeat sizes in the high normal range (35–54 CGG repeats) and POF yielded conflicting results[3,4].

In view of this controversy over the relationship between *FMR1* gene CGG repeat sizes in the high normal range and risk of POF, we performed this study to examine the association between *FMR1* gene CGG repeat sizes in the high normal range and the risk of POF in the Chinese population of Hong Kong. Identification of the relationship between *FMR1* gene and POF would give possible insight into the underlying molecular mechanism of POF.

Methods

This retrospective study was performed at the Clinical Genetic Service (CGS), Department of Health, Hong Kong. The CGS received referrals of patients with POF for karyotyping and *FMR1* analysis. A search was

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performed in the computer database of the CGS for patients with the diagnosis of POF or secondary amenorrhoea. The medical records and laboratory results of patients referred during the period 2000 to 2011 were retrieved and reviewed.

Premature Ovarian Failure Group

POF was defined as cessation of menstruation for at least 1 year before the age of 40 years. Women were included in the study if they had an elevated follicle-stimulating hormone level in the postmenopausal range or they were diagnosed to have POF by the referring gynaecologist. Women were excluded if they were of non-Chinese ethnicity; they had identifiable causes for POF, chromosomal abnormalities (including Turner's syndrome, XXX syndrome), or other causes for amenorrhoea (e.g. polycystic ovarian syndrome); or they did not have an *FMR1* test performed in the unit. Written consent was obtained for the *FMR1* test to be performed. *FMR1* test results were traced and reviewed. For women whose *FMR1* reports were not available, *FMR1* test was repeated on the stored DNA samples.

Control Group

The control group comprised healthy mothers of children with de-novo genetic conditions, including neurofibromatosis type 1, Noonan syndrome, achondroplasia/hypochondroplasia, and Angelman/Prader-Willi syndrome. Written consent for inclusion in the control group was obtained when they agreed to the molecular test for the above syndromes. *FMR1* test was performed on the stored DNA samples.

FMR1 gene CGG repeat sizes were determined in the POF group and the control group. The number of CGG repeats in the *FMR1* gene was initially determined by polymerase chain reaction (PCR) amplification, then by size fractionation by capillary gel electrophoresis and fluorescence detection[5]. CGG repeat size was determined by GeneScan Analysis Software using the ABI3130x1 Genetic Analyzer (Applied Biosystems by Life Technologies; Foster City [CA], USA). There was a limitation with this method in that large expansions were refractory to PCR amplifications; therefore, sizing of CGG repeats was inaccurate in a premutation range of up to 100 to 120 repeats. Women with only one normal-sized allele were subject to triplet-primed PCR to test for expanded alleles of *FMR1*[6]. Statistical analysis was performed using the Statistical Package for the Social Sciences (Windows version 18; SPSS, Inc, Chicago [IL], USA) using the Chi-square test.

Results

A total of 273 women were referred to the CGS for POF from 2000 to 2011; 44 women were excluded from the study as they had other causes of POF, including 34 with Turner's syndrome, Turner variants, or XXX syndrome; five with other chromosomal abnormalities; four with a history of immunosuppressive therapy for malignancy or after organ transplant, and one with Perrault syndrome (gonadal dysgenesis with sensorineural hearing impairment). Three women with polycystic ovary syndrome instead of POF were also excluded. A total of 20 women did not have *FMR1* test, including two who defaulted follow-up and did not have the test, and 18 others had not made arrangements for the *FMR1* test for unknown reasons. Additionally, the *FMR1* retest failed in 10 women due to the poor quality of aged DNA samples. Hence, 77 women in the POF group were excluded from the study. A total of 196 women were included in the idiopathic POF group and 204 women were included in the control group.

Mean Age of Menopause

The mean age of menopause, including in women with all causes of POF, was 27.7 years. The mean ages for menopause based on different causes were similar: 27.7 years for idiopathic POF; 28.8 years for Turner's syndrome, Turner variants, and XXX syndrome; and 29.3 years for other causes of POF (Table 1).

Distribution of* FMR1 *CGG Repeat Sizes

The most common repeat size in both groups was 29, followed by repeat sizes of 30 and 28. There was a smaller secondary peak at repeat sizes of 35 and 36 in both groups. Seven alleles (3.57%) in the idiopathic POF group had CGG repeat sizes in the premutation range. None of the women in the control group had premutation CGG repeat sizes (Figure).

Table 1. Mean (range) age at menopause according to the cause of premature ovarian failure (POF)

Cause of POF	No.	Mean (range) age at menopause (years)
All POF	240	27.7 (11-39)
Idiopathic POF	196	27.7 (11-39)
Turner's syndrome/Turner variants/XXX syndrome	34	28.8 (15-38)
Other causes of POF*	10	29.3 (25-37)

* Other causes of POF include chromosomal abnormalities other than Turner's syndrome and variants / XXX syndrome, history of immunosuppressive therapy, and Perrault syndrome

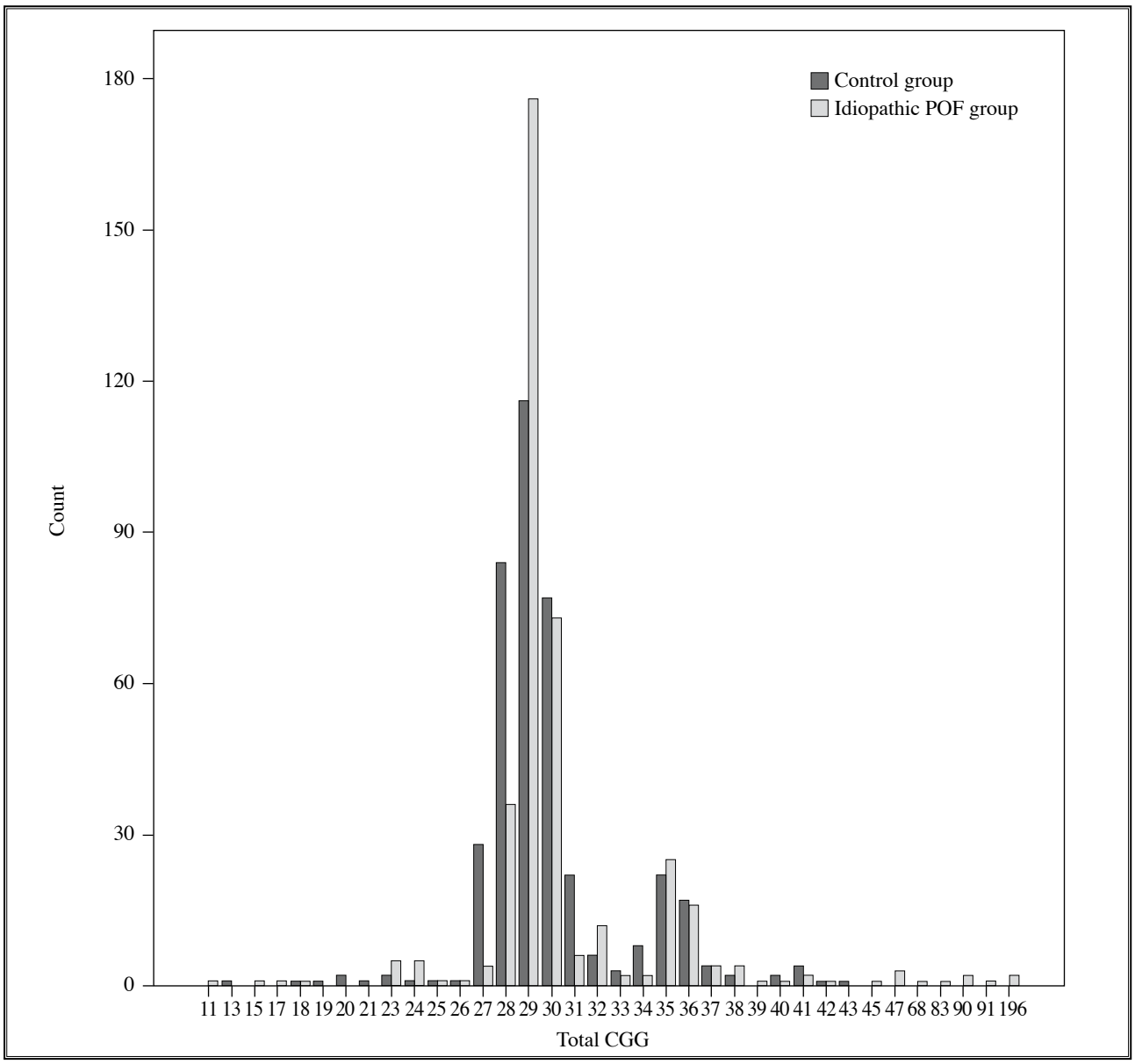

Figure. Frequency distribution of FMR1 *gene CGG repeat sizes in the idiopathic premature ovarian failure (POF) and control groups*

FMR1 ***Repeat Sizes in the High Normal Range***

FMR1 gene CGG repeat sizes in the range of 35 to 54 were defined as belonging to the high normal range according to a previous study[4]. Repeat size in the high normal range was more common among women with idiopathic POF (n=58; 14.8%) compared with women in the control group (n=53; 13.0%) [p=0.46]. A similar trend was observed in repeat sizes of 35–36, 37–54 and ≥35 (Table 2). The difference did not reach statistical significance.

Discussion

Fragile X syndrome is an important inheritable cause of autism and mental retardation. In 1991, Verkerk et al[7] identified the *FMR1* (fragile X mental retardation 1) gene, which was located on the X chromosome at Xq27.3. The gene product, FMRP (fragile X mental retardation protein), is expressed at high levels in the brain. It has been suggested that FMRP acts as a translational suppressor. Lack of FMRP may result in overexpression of multiple mRNA and neurotoxicity that accounts for the fragile X syndrome phenotype[8].

In healthy individuals, the *FMR1* gene comprises up to 49 CGG repeats. The clinical phenotype largely depends on the number of trinucleotide repeats. The size of the trinucleotide repeats is classified into normal (<50 CGG repeats), high normal (35–54 CGG repeats), premutation (59–200 CGG repeats), and full mutation (>200 CGG repeats)[9]. Expanded CGG repeat is associated with meiotic instability, especially in the female germ line, and results in

Table 2. *FMR1* CGG repeat sizes in the high normal range in the idiopathic premature ovarian failure (POF) and control groups

	No. (%)		Odds ratio (95% confidence interval)	p Value
	Control group	POF group		
No. of alleles	408	392	–	–
35–54	53 (13.0)	58 (14.8)	0.86 (0.58 - 1.28)	0.46
35–36	39 (9.6)	41 (10.5)	0.90 (0.57 - 1.44)	0.671
37–54	14 (3.4)	17 (4.3)	0.78 (0.38 - 1.61)	0.507
≥35	53 (13.0)	65 (16.6)	0.75 (0.51 - 1.11)	0.152

further expansion in the offspring. Risk of expansion into the full mutation range among the offspring depends on the size of the CGG repeats carried by the mother. Individuals with full mutation would have fragile X syndrome.

In the United States, the prevalence of male and female premutation carriers is estimated to be 1 in 400 and 1 in 178, respectively. The overall prevalence of the full mutation is calculated to be 1 in 3335[10]. The majority of premutation carriers are asymptomatic, but a proportion of premutation carriers will develop fragile X-associated tremor/ataxia syndrome or late-onset neurological disorders in middle age[11]. Offspring of premutation carriers are also at risk for developing a full mutation due to expansion of the CCG repeat. It has been noted that fragile X carriers have high risk for early menopause[1].

A non-linear association between CGG repeat sizes and age at menopause in premutation carriers has been noted. Studies have revealed that women with a premutation in the mid-size range (approximately 80–100 CGG repeats) are at higher risk for POF. Women with larger CGG repeat sizes in the premutation range are at lower risk for POF[12,13]. Allen et al[14] hypothesised that ovarian insufficiency in premutation carriers was related to a diminished initial oocyte pool. Other hypotheses include accelerated atresia of follicles or impaired follicular function[8].

Bretherick et al[4] tested the risk for POF in women with *FMR1* CGG repeat size in the high normal range (35–54 CGG repeats). Such a high normal range was considered the grey zone between the normal and premutation ranges. It was shown that *FMR1* CGG repeat size in the high normal range was more common among women with POF. A study in 2010 by Bennett et al[3], however, did not support such an association.

These results showed a higher prevalence of repeat sizes in the high normal range in the POF group than in the control group, but the difference did not attain statistical significance. A bimodal pattern of distribution of *FMR1* CGG repeat sizes was observed in this study. The most common CGG repeat size was 29, followed by 30 and 28. The second peak occurs at 35 to 36 repeats. These findings are consistent with previous studies among Chinese populations in Hong Kong[15] and Taiwan[16]. However, there has been no such observation in western populations. Brown et al[17] demonstrated a multimodal distribution of CGG repeat sizes in Caucasian populations. The highest peak was at 29 to 31 repeats and there was a minor peak between 20 and 23 repeats[17]. CGG repeat sizes of 35 and 36 are not common among the western population. As CGG repeat sizes of 35 and 36 are defined as within the high normal range, the characteristic pattern of CGG-repeat distribution in the Chinese population may complicate this analysis. In order to reduce the influence of bimodal distribution on the analysis, the high normal range was divided into subgroups of 35 to 36 and 37 to 54. However, correlation study in the subgroup analysis also did not reveal statistically significant results.

The difference in frequency distribution of *FMR1* CGG repeats between the Chinese and western populations implies that results from previous studies of the *FMR1* gene and POF in the western population cannot be simply extrapolated to the Chinese population. There has been no previous study investigating the association between fragile X premutation and POF in the Chinese population. It is possible that fragile X is not contributory to the pathogenesis of POF in this population. In this case, no association would be found between *FMR1* CGG repeat sizes in the high normal range and POF.

For sample size calculation, alpha and power values of 0.05 and 0.8, respectively, were used. In one study, it was reported that 5% of the population had POF and 20%

of premutation carriers of fragile X syndrome had POF[18]. The sample size calculated was 90. Therefore, this study was not underpowered.

Conclusion

This study shows no correlation between *FMR1* gene CGG repeat sizes in the high normal range and idiopathic POF in this Chinese population. The results also confirm the findings of previous studies on the characteristic bimodal distribution of CGG repeat sizes in the Chinese population.

Declaration

No conflicts of interest were declared by the authors.

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