Number of CGG repeats in the *FMR1* gene of Japanese patients with primary ovarian insufficiency

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Objective: To define the number of CGG repeats in the *FMR1* gene of Japanese patients with primary ovarian insufficiency (POI) and normal controls.
Design: Retrospective, controlled cohort study.
Setting: Outpatient department of an academic tertiary center.
Patient(s): One hundred twenty-eight consecutive Japanese patients with sporadic, nonsyndromic POI and 98 controls with normal menstruation.
Intervention(s): Deoxyribonucleic acid was obtained from the plasma of each subject.
Main Outcome Measure(s): Differences in the distribution of CGG repeat numbers between patients with POI and controls.
Result(s): Six alleles in the intermediate range and two in the premutation range were found in five and two patients with POI, respectively, but none were identified in normal controls. The prevalence of *FMR1* premutation among Japanese POI patients was 1.56% (2 of 128). The prevalence of having >36 CGG repeats in the *FMR1* gene was significantly higher in patients with POI than in controls, and age at the onset of amenorrhea was significantly lower in patients with >38 repeats.
Conclusion(s): More than 36 CGG repeats in the *FMR1* might intensify the etiology of POI, at least up to the premutation range. (Fertil Steril® 2011;96:1170–4. ©2011 by American Society for Reproductive Medicine.)

Key Words: Primary ovarian insufficiency, *FMR1*, CGG repeat, premutation allele

Primary ovarian insufficiency (POI) refers to the development of hypergonadotropic hypogonadism before the age of 40 years (1). The reported prevalence of POI among women is 1% (2), and the etiology of the spontaneous type is essentially unknown.

The fragile X mental retardation 1 (*FMR1*) gene, which contains a polymorphic CGG trinucleotide repeat in its 5′ untranslated region, is associated with POI. The fully expanded form, which contains >200 CGG repeats, causes the loss of the RNA-binding FMR1 protein and results in the fragile X syndrome phenotype (3, 4). Premutation alleles that expand to >200 repeats over several generations have been defined in families with FMR syndrome. The premutation range is defined as being between 50 and 199 repeats. Premutation carriers have an increased prevalence of POI (5–7), which ranges from 13% to 26% (6, 8). Recent reports indicate that, in addition to POI, the distribution of age at menopause onset among premutation carriers is shifted approximately 5 years earlier compared with that of noncarriers (8–11).

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Intermediate alleles are alleles with fewer repeats than the premutation range, but they have the potential to become unstable when transmitted from generation to generation. They have been defined by different groups as containing from 41 to 60 (8), 45 to 54 (12), and 46 to 60 (13) repeats.

Recent studies have indicated that infertile women tend to have more *FMR1* CGG repeats than normal controls, even if they are considered to be within the normal range (14). Bretherick et al. (15) and Bodega et al. (16) reported that increased risk of POI is not restricted to the premature range of CGG repeats but also to the high ends of the normal and intermediate ranges. Some studies have suggested that a deviation in CGG repeat number from the narrow normal range also causes occult ovarian insufficiency and early ovarian senescence (14). Studies of infertile patients have indicated that the presence of >30 triplet CGG repeats confers increased risk and severity of occult ovarian insufficiency in parallel with increasing expansion (14, 17–19). With regard to ovarian reserve, Gleicher et al. (17) indicated that the normal range of CGG repeats is 26–34 with a median of 30 (18, 20), which encompasses the distribution peaks described herein and by Chen et al. (21). These findings suggest that the range of CGG repeats associated with ovarian reserve differs from that associated with neuro/psychiatric risk (22, 23) and that a repeat number of >30 is associated with decreased ovarian reserve.

The number of CGG repeats in Asians has a characteristic secondary peak of 34–36 repeats in addition to the most frequent peak of 29 to 30 (9). Studies have indicated numbers of <40 CGG repeats in normal Japanese populations, with a minor population showing a peak at 36 repeats in addition to peaks at 29 and 30 repeats (24, 25). However, the distribution of CGG repeats in *FMR1* among

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TABLE 1

Characteristics of patients with POI (n = 128) and control women (n = 98).

Characteristic	Patients		Controls	
	Mean ± SE	Range	Mean ± SE	Range
Age (y)				
At examination	37.82 ± 0.50	20–54	39.12 ± 1.09	21–59
At onset of amenorrhea	29.5 ± 0.61	12–39		
CGG count				
Short allele	28.88 ± 0.23	12–43	28.90 ± 0.23	20–36
Long allele	32.91 ± 0.51	26–68	31.90 ± 0.33	23–40

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East Asian POI patients, including Japanese, has never been reported. Furthermore, the association between CGG repeat size and age at the onset of amenorrhea, which might reflect disease severity in patients with POI, has never been examined. The present study compares the number of CGG repeats in the *FMR1* gene between Japanese patients with POI and normal controls and investigates the relationship between the CGG repeat numbers and the age at onset of amenorrhea in these patients.

MATERIALS AND METHODS

We investigated 128 Japanese female patients aged 37.8 ± 0.50 (mean ± SE; range, 20–54) years with normo-karyotypic (46,XX), sporadic, and nonsyndromic POI, who were aged 29.5 ± 0.61 (range, 12–39) years at the onset of amenorrhea (Table 1). The patients provided written, informed consent to participate in the study, which was approved by the Ethics Committee for Human Genome/Gene Research at St. Marianna University School of Medicine. They were also treated at the Center of Reproductive Medicine, Department of Obstetrics and Gynecology at the same institution. The diagnosis of POI was based on criteria comprising ≥3 months of amenorrhea, <40 years of age, and having serum FSH levels of ≥40 IU/L on two consecutive occasions. None of the patients had a family history of early menopause or mental retardation. They were phenotypically normal and had no history of pelvic surgery or chemo/radiotherapy. We also analyzed blood samples from 98 normal Japanese women aged 39.1 ± 1.09 (range, 21–59) years with proven fertility and normal menstruation or who had undergone normal menopause. These samples were obtained from the Japanese Collection of Research Bioresources.

White blood cells were isolated from these blood samples by density-gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare), and genomic DNA extracted from the cells using a PUREGENE DNA isolation kit (Gentra Systems) served as polymerase chain reaction templates. The sense and antisense sequences of the primer set for *FMR1* were 5′-gctcagctccgtttcggtttcacttccggt-3′ and 5′-agccccgcacttccaccaccagctcctcca-3′, respectively. Partial sequences containing CGG repeats of the *FMR1* gene were specifically amplified as described by Fu et al. (26). Polymerase chain reaction products were sequenced using sense or antisense primers and an ABI 3100 Avant sequencer (Applied Biosystems).

Correlations between *FMR1* CGG repeat size and age at the onset of amenorrhea in the patients were analyzed by linear regression. Other data were statistically analyzed using the Mann-Whitney *U* and Fisher exact tests. A *P* value of .05 was considered to indicate statistical significance.

RESULTS

We estimated the numbers of CGG repeats in the *FMR1* gene of 128 Japanese patients with POI and in 98 controls. Table 1 shows the characteristics of the patients and controls.

Figure 1 shows the distribution of long and short alleles (Fig. 1A), long alleles (Fig. 1B), and short alleles (Fig. 1C) in the patients and controls. The number of repeats in all alleles was ≤40, except for six in the intermediate range defined by Sullivan et al. (8) from five patients, and two in the premutation range from two patients (Table 2). We found common peaks at 29 and 30 repeats (Fig. 1), as well as at 36 repeats in approximately 10% of all alleles and in approximately 20% of long alleles in both the patients and controls (Fig. 1).

Alleles with >40 CGG repeats (the lower limit of the intermediate zone of reported definitions) (8) were identified only in the POI patients (Table 2), and the prevalence of alleles with >36 repeats (secondary modal frequency) was significantly higher in the POI patients than in the controls (Table 2).

The onset of amenorrhea occurred significantly earlier in the patients with >38 CGG repeats than in the patients with ≤38 CGG repeats (29.9 ± 0.62 vs. 25.1 ± 2.50 years, $P<.05$; Fig. 2).

DISCUSSION

The present study demonstrated that the distribution of CGG repeats in the *FMR1* gene of Japanese patients with POI was identical to that of normal controls, except for five and two patients with repeat numbers in the intermediate (8) and premutation ranges, respectively. The distribution of CGG repeats in our study participants was similar to that reported for Japanese in studies of 370 unrelated, non–mentally retarded males (24) and 576 normal males and 370 normal females (25), all of whom had a primary modal peak at 29 to 30 or 28 to 29 repeats (24, 25) and a secondary modal peak at 36 or 33 to 34 (24, 25). These secondary peaks were not identified in studies of Western populations (9, 24–26). The secondary peak found in the present study was at 36 repeats but was reported at 33 and 34 repeats in the studies of Arinami et al. (24) and Otsuka et al. (25), respectively. These differences might be associated with the automated sequencer methodology and whether AGG interruptions in the sequence were included in the count. Chen et al. (9) reported that the most common pattern of triplets is $(CGG)_9$ AGG $(CGG)_9$ AGG $(CGG)_9$. The modal peaks in Japanese females described by Otsuka et al. (25) were at 27 and 33 or 34 repeats, suggesting that the difference in peaks is due to the inclusion or exclusion of the triplet AGG within the sequence. Chen et al. (9) also showed that the minor modal frequency with 36 repeats among Asian populations typically has the sequence $(CGG)_9$ AGG $(CGG)_9$ AGG $(CGG)_6$ AGG $(CGG)_9$. We demonstrated here that 20% of long alleles and 10% of total alleles showed a peak at 36 repeats in both patients and normal controls, suggesting that this ratio of Japanese women have the same pattern of triplets described by Chen et al. (9).