Chromosomal abnormalities and FMR1 gene premutation in Chinese women with premature menopause

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Key words:
Fragile X syndrome; Menopause, premature; Ovarian failure, premature; X chromosome

Objective. To study the prevalence of chromosomal abnormalities and FMR1 gene premutation in Chinese women with premature menopause in Hong Kong.

Design. Retrospective study.

Setting. Clinical Genetic Service, Hong Kong.


Main outcome measures. Chromosomal abnormalities, FMR1 gene premutation.

Results. Chromosomal abnormalities were present in 15.6% of Chinese women who suffered premature menopause. X-chromosome abnormality was involved in over 80% of cases. FMR1 gene premutation was present in 0.86% of 116 cases screened for this abnormality. The predominance of X-chromosome abnormality accounted for the shorter stature, younger menopausal age, and higher prevalence of dysmorphic features among the cytogenetically abnormal patients. However, on logistic regression, no clinical feature was significantly correlated with cytogenetic abnormality.

Conclusions. The prevalence of chromosomal abnormalities among Hong Kong Chinese women who suffer premature menopause was comparable with that of Caucasian and Chinese populations elsewhere. Because clinical features are poor predictors of cytogenetic abnormality, a pragmatic approach to screening is advocated. The carrier rate of fragile X premutation in these women appeared lower than that of Caucasians. Nevertheless, a search for FMR1 gene premutation, in addition to conventional chromosomal study, has important implication for prenatal diagnosis and fertility management for the extended family.

目的：研究更年期過早的香港華裔女性出現染色體異常和FMR1基因先期突變的情況。

設計：回顧研究。

參與者：1983年1月至2003年11月期間，因更年期過早獲轉介接受細胞遺傳學檢查的華裔女性。

主要結果測量：染色體異常和FMR1基因先期突變。

結論：在患有更年期過早的華裔女性當中，15.6%出現染色體異常，當中超過80%患有X染色體異常。在116宗病例當中，0.86%出現FMR1基因先期突變。在細胞遺傳異常的病人中，X染色體異常是造成身形矮小、更年期較早，以及畸形情況較為普遍的主因。不過，對數迴歸分析則顯示，沒有任何臨床症狀是和細胞遺傳異常顯著相關。

結論：在更年期過早的香港華裔女性病人中，染色體異常的普遍程度與白
Introduction

Premature menopause affects 1% to 3% of the female population and refers to cessation of ovarian activity before 40 years of age. Although it has long been recognised that certain cases are associated with chromosomal and genetic disorders, local data have not been reported.

This local study represents the largest epidemiological study ever conducted for cytogenetics of premature menopause. It is the first time that cytogenetics for Chinese women suffering premature menopause have been reported in the English literature. It is also the first report of screening for \textit{FMR1} gene premutation in the Chinese population.

Methods

This retrospective study was carried out, following ethical approval, at the Clinical Genetic Service, Department of Health, Hong Kong. An initial computer search on ‘secondary amenorrhoea and premature menopause’ was made on the clinical information system and patient records retrieved for the period 1 January 1983 to 30 November 2003.

Patients were included in the study if they were Chinese, had secondary amenorrhoea for at least 1 year with an onset of less than 40 years, and had menopausal state confirmed by one of the following: confirmed diagnosis by referring gynaecologist, menopausal symptoms, or elevated follicle-stimulating hormone (FSH) level. Follicle-stimulating hormone level was regarded as elevated if it was higher than 20 IU/L or described as ‘elevated’ or ‘in the postmenopausal range’ by the referring gynaecologist.

Table 1. Diagnosis of premature menopause

<table>
<thead>
<tr>
<th>Diagnostic feature in addition to amenorrhoea (&gt;1 year)</th>
<th>Cases, n=295</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH* level &gt;20 IU/L</td>
<td>142 (48.1)</td>
</tr>
<tr>
<td>FSH level not available</td>
<td>80 (27.1)</td>
</tr>
<tr>
<td>‘Elevated FSH’ or ‘FSH in menopausal range’ on gynaecologist’s referral letter</td>
<td>72 (24.4)</td>
</tr>
<tr>
<td>‘Premature ovarian failure’ or ‘premature menopause’ on gynaecologist’s referral letter</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

* FSH follicle-stimulating hormone

Statistical analysis was performed using the Statistical Package for the Social Sciences (Windows version 10.0; SPSS Inc, Chicago [IL], United States). Systematic cross-checking of computer spreadsheet data with original case records was made to ensure accuracy of data entry. The statistical significance level was set at 0.05 by two-tailed analysis.

Results

A total of 370 cases of ‘secondary amenorrhoea and premature menopause’ between 1 January 1983 and 30 November 2003 were identified on the clinical information system. All case notes were successfully retrieved through the record office. A total of 295 cases fulfilled the inclusion criteria: all had a karyotyping on G-banding and 116 (39.3%) additionally had molecular test of the \textit{FMR1} gene.

Diagnosis of premature menopause

All patients reported secondary amenorrhoea of 1 year’s duration with an onset before 40 years of age. Their menopausal state was additionally confirmed as shown in Table 1.

Chromosomal abnormalities

A total of 46 (15.6%) women had an abnormal cytogenetic study (Table 2). More than 80% of abnormalities involved at least some part of the X chromosome, almost half due to monosomy X and its variants. In addition, autosomes were not entirely silent in terms of ovarian function: they were the only chromosome at fault in 13.0% of cases.

Over the past 20 years, the number of referrals to

人及其他地方的華裔女性相若。由於臨床症狀不能有效預測細胞遺傳異常，病人應接受篩查。雖然香港華裔女性脆性 X 染色體前期突變的帶因率比白人為低，不過，傳統的染色體研究，加上 \textit{FMR1} 基因前期突變測試，對病人下一代的產前診斷和生育管理都是非常重要的。
Chromosomal abnormalities and FMR1 gene premutation

Correlation with clinical features

Cytogenetically normal versus abnormal

The mean age at menopause for those with chromosomal abnormalities was 28.2 years, significantly younger than those without (mean, 31.0 years; P<0.01). Those with an abnormality were also shorter in stature (mean, 151.1 cm vs 157.1 cm; P<0.01). However, there was no statistically significant difference between the two groups in terms of mean FSH level at presentation and family history of mental retardation or premature menopause.

Table 2. Distribution of cytogenetic abnormalities

<table>
<thead>
<tr>
<th>Category</th>
<th>Cytogenetic abnormality</th>
<th>No. of cases</th>
<th>Subtotal, n=46 No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomy X and its variants</td>
<td>Pure monosomy X (45,X)</td>
<td>5</td>
<td>22 (47.8)</td>
</tr>
<tr>
<td></td>
<td>Mosaic monosomy X and X-chromosome structural abnormalities</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mosaic monosomy X (45,X/46,XX)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mosaic monosomy X and trisomy</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>X-chromosome structural abnormalities only</td>
<td>Isochromosome Xq</td>
<td>1</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td></td>
<td>Deletions on Xq</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>X-autosome translocation</td>
<td>X-chromosome 22 translocation</td>
<td>1</td>
<td>2 (4.3)</td>
</tr>
<tr>
<td></td>
<td>X-chromosome 14 translocation</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Excessive No. of X chromosomes</td>
<td>Mosaic trisomy X (46,XX/47,XXX)</td>
<td>2</td>
<td>6 (13.0)</td>
</tr>
<tr>
<td></td>
<td>Pure trisomy X (47,XXX)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell lines mosaic for variable copies of X chromosomes 46,XX (96%)/48,XXXXX (2%)/49,XXXXX (2%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Y chromosome</td>
<td>Mosaic cell lines one of which contains Y chromosome 45,X (10%)/46,XX (20%)/46,XY (70%)</td>
<td>1</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Autosomal*</td>
<td>Chromosome 18 deletion</td>
<td>1</td>
<td>6 (13.0)</td>
</tr>
<tr>
<td></td>
<td>Mosaic marker chromosome of undetermined origin</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chromosome 22 deletion</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extra length on q arm of chromosome 1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Balanced translocation between chromosome 1 and 8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extra heterochromatin on q arm of chromosome 9</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Two cases with 46,XX,inv(9)(p11q13) were excluded because they are known normal variant

Fig 1. Case referrals for cytogenetic study over the past 20 years

the genetic service for premature menopause has increased (Fig 1). Although the detection rate of cytogenetic abnormalities decreased initially, it is now stable at 10% to 15%.
Dysmorphic features were identified in 16% of the referred women prior to cytogenetic study (Fig 2). The prevalence significantly increased to 34% for the group with confirmed cytogenetic abnormality, compared with 13% among those with a negative cytogenetic study (P<0.05). Of all the referrals, 5.4% had more than one dysmorphic feature, and 3% more than two. Likelihood of cytogenetic abnormality increased linearly with the number of dysmorphic features (linear-by-linear association, 12.5; P<0.01). For those with three or more dysmorphic features, 50% had cytogenetic abnormalities. Nonetheless, analysis of individual dysmorphic features revealed that none of them by itself was significantly more prevalent when cytogenetic abnormality was present.

Logistic regression was used to determine whether clinical information collected at the first encounter could predict cytogenetic abnormality. Such information included the age at menopause, body stature, presence of mental retardation, dysmorphic features, and family history of premature menopause or mental retardation. None was significantly associated with cytogenetic abnormality.

**Sex chromosome versus pure autosome abnormalities**

The clinical features of 40 cases of sex chromosome abnormalities were compared with six cases of pure autosome defects: those with normal cytogenetic study served as controls. Analysis of variance test was employed for data on ratio scale. The mean age at menopause was 28.0, 29.7, and 31.0 years, with mean body height of 150.1 cm, 155.9 cm, and 157.2 cm, and prevalence of dysmorphic features of 37%, 17%, and 13%, respectively for the three groups. Significant differences were detected only between those with sex chromosome abnormalities and the control (P=0.03 for age, P<0.01 for height, and P=0.01 for prevalence of dysmorphic features). The FSH level was similar among the three groups.

These results signified that the younger menopausal age, shorter stature, and the higher prevalence of dysmorphic features among the cytogenetically abnormal patients were solely contributed by the high proportion of sex chromosomal abnormalities within this group.

**The X chromosome: numerical versus structural abnormalities**

Sixteen patients with monosomy X (45,X or 45,X/46,XX) were compared with nine patients with X-chromosome structural defects and six patients with excessive copies of X chromosomes. Those with nor-
Chromosomal abnormalities and FMR1 gene premutation

Table 3. Deletions on q arm of X chromosome

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Point of deletion</th>
<th>Age at menopause (years)</th>
<th>Height (cm)</th>
<th>Dysmorphic feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9838</td>
<td>46,X,del(X)(q27.2)</td>
<td>30</td>
<td>151</td>
<td>-</td>
</tr>
<tr>
<td>C3060</td>
<td>46,X,del(X)(q26)</td>
<td>28</td>
<td>147</td>
<td>Epicantus fold</td>
</tr>
<tr>
<td>C7624</td>
<td>46,X,del(X)(q24)</td>
<td>29</td>
<td>-</td>
<td>Cubitus valgus</td>
</tr>
<tr>
<td>C5290</td>
<td>46,X,del(X)(q23)</td>
<td>18</td>
<td>150</td>
<td>Cubitus valgus, bilateral small and low-set ears, anteverted nostrils</td>
</tr>
<tr>
<td>C1570</td>
<td>46,X,del(X)(q22)</td>
<td>13</td>
<td>134</td>
<td>-</td>
</tr>
</tbody>
</table>

ormal cytogenetic study served as controls. Analysis of variance test was used for data on ratio scale. There was no difference in the age at menopause or mean FSH level across the four groups.

Patients with monosomy X and X-chromosome structural defects had similar body stature (147.5 cm vs 146.1 cm; P=0.98). Those with excessive copies of X chromosomes were also similar in height to the control group (165.3 cm vs 157.2 cm; P=0.14). When compared with the former two entities, the latter two were nonetheless significantly taller (P<0.01).

The prevalence of dysmorphic features among the four groups was 13% for the controls, 17% for those with excessive copies of X chromosomes, 31% for those with monosomy X, and 56% for those with X-chromosome structural defects. A difference was detected only between the last group and the controls (P<0.01).

Therefore, while X-chromosome structural defects contributed significantly to the higher prevalence of dysmorphic features among sex chromosome abnormalities, structural and numerical X-chromosome defects played a role in determining short stature. Other sex chromosome abnormalities might however account for the younger age at menopause for sex chromosome abnormalities.

**Xq deletions: extent of lesion and the clinical effect**

Although patient numbers were too small to allow definitive conclusions, it would appear that the extent of lesion on the q arm of X chromosome had affected menopausal age, other clinical effects being less obvious (Table 3).

**FMR1 gene premutation**

Of the 116 cases who presented after 2000, one (0.86%) had fragile X premutation. The number of CGG repeats was 92 (normal: <54, full mutation: >200). She had a positive family history of mental retardation but not of premature menopause. Unfortunately, she defaulted from further follow-up and the family were not available for further testing.

**Discussion**

To date, a total of nine studies have examined the prevalence of cytogenetic abnormalities among women with premature menopause (Table 4). This study is the largest one and the only one in the English literature to report the cytogenetics of premature menopause in Chinese women. Over the past 20 years, the number of referrals has increased. The increased readiness to refer is reflected by a corresponding drop in detection rates of cytogenetic abnormalities. The current abnormal karyotype rate of 15.6% is in line with western data and the Chinese premature menopausal population elsewhere.

Consistent with previous study findings, X-chromosome abnormality is the main contributor to cytogenetic abnormality among women undergoing premature menopause. It accounts for many of the clinical features in the cytogenetically abnormal group, including younger age at menopause, shorter stature, and higher prevalence of dysmorphic features. The deficiency of genetic material on the X chromosome, be it numerical (Turner variant) or structural, has a stronger impact on clinical presentation than redundancies (trisomy X and variants). On the contrary, autosomal abnormalities among this population are relatively silent in terms of their effect on clinical features.

Although no clinical feature is significantly associated with the presence of cytogenetic abnormality, patients who are cytogenetically abnormal are more likely to have shorter stature, younger menopausal age, and identifiable dysmorphism. When more than three dysmorphic features are identified, there is a 50% chance of cytogenetic abnormality. The FSH level and family history are not helpful.

Turner variants are the most common sex chromosome abnormality in women with premature menopause and account for almost 50% of the cyto-
genetic diagnoses. There are evidences showing that low level of 45,X/46,XX mosaics that can be detected by fluorescent in situ hybridisation (FISH) but missed on routine cytogenetic analysis may account for even more cases of premature menopause.\textsuperscript{11,12} Mosaic 45,X/47,XXX are considered Turner variants because they are phenotypically similar to the 45,X/46,XX.\textsuperscript{13} The current view is that in monosomy X, the pathogenesis of germ cell failure is increased atresia secondary to meiotic pairing errors, not deficient germ cell formation.\textsuperscript{14} Isochromosome Xq also behaves like monosomy X. Duplication of Xq does not compensate for deficient Xp.\textsuperscript{13} Patients are universally short because the gene for short stature (\textit{SHOX}) is on Xq.

About 70\% of terminal deletions of the X chromosome lead to premature menopause. Both incidence and severity increase proportionally with the increasing loss of DNA from the two far ends of the X chromosome to a maximum at Xq13, more or less at the centre of the chromosome.\textsuperscript{15} There are two regions on Xq that are hypothesised to contain putative genes related to premature ovarian failure (POF): the POF1 region at Xq26-28 and a more recently designated region of Xq22 now termed POF2.\textsuperscript{16} Because the presentation is variable, it is highly likely that there are other genes on Xq involved in POF. The actual mechanism is much more complicated.

Balanced X-autosome translocations very often lead to POF.\textsuperscript{15} Two cases of X-autosome translocation with premature menopause were identified in this study. Another study showed that most X-autosome translocations associated with POF do not interrupt X-linked genes. Instead, they cause premature menopause by a chromosomal effect, such as inhibition of meiotic pairing or altered X inactivation.\textsuperscript{17}

The current study is the first to report the results of fragile X premutation screening among Chinese women with premature menopause. This study identified one premutation carrier among 116 consecutive cases screened. The estimate of premutation carriers is 13.8\% and 2.1\%, respectively for familial and sporadic cases of POF in western populations.\textsuperscript{18,19} The reported rate of 0.86\% in this study is much lower.

Studies have identified differences between Chi-

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample size</th>
<th>No. of cytogenetic abnormality</th>
<th>Monosomy X</th>
<th>X deletion</th>
<th>Isochromosome X</th>
<th>X inversion</th>
<th>Dicentric X</th>
<th>Y-fragment</th>
<th>X-autosome translocation</th>
<th>Fragile X premutation</th>
<th>Trisomy X</th>
<th>Autosomal abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chongqing, China</td>
<td>104</td>
<td>13 (12.5%)</td>
<td>3 (25.7%)</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NT\textsuperscript{†}</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Shanghai, China</td>
<td>Not available</td>
<td>9 (22.0%)</td>
<td>1 (14.3%)</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NT\textsuperscript{†}</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Shanghai, China</td>
<td>41</td>
<td>2 (32.0%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NT\textsuperscript{†}</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Chile</td>
<td>47</td>
<td>1 (13.3%)</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NT\textsuperscript{†}</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Japan</td>
<td>14</td>
<td>4 (12.5%)</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NT\textsuperscript{†}</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>62</td>
<td>1 (13.3%)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT\textsuperscript{†}</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>United States</td>
<td>30</td>
<td>1 (13.3%)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT\textsuperscript{†}</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>United States</td>
<td>8</td>
<td>1 (13.3%)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT\textsuperscript{†}</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ohio, United States</td>
<td>45</td>
<td>1 (15.9%)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT\textsuperscript{†}</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>295</td>
<td>1 (15.9%)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT\textsuperscript{†}</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{†} Patients were younger than 30 years at menopause
\textsuperscript{NT} Not tested

Table 4. Earlier studies on the cytogenetics of premature menopause

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nese and Caucasian women in the structure of the CGG repeat on the FMR1 gene. No differences have been found in the repeated pattern in subjects from four Chinese cities (Hong Kong included), suggesting no geographical differences among Chinese populations. Our reported rate of 0.86% may therefore be applicable to Chinese women elsewhere.

The causes of POF in premutation carriers remain unidentified. It has been postulated that factors related to genomic imprinting or the higher incidence of dizygotic twinning in female carriers of premutation may be implicated. Alternatively, the mRNA with the large repeat may affect ovarian development or function or interfere with X inactivation. When a woman is found to be a premutation carrier, the expansion of trinucleotide repeats to full mutation during female meiosis may result in mentally retarded offspring. Prenatal screening should be offered. The extended family should also be screened and women who may have premature menopause identified and appropriately counselled.

Chromosomal defects related to premature menopause have been reported over various autosomes. It is unclear if they reflect disruption of autosomal loci integral to ovarian preservation and oogenesis. That no chromosome is consistently involved suggests non-specific meiotic perturbation.

In addition to chromosomal abnormalities and FMR1 gene premutation, many other genetic mechanisms pertain to the aetiology of premature menopause. The cytogenetic diagnosis revealed here may represent just ‘the tip of the iceberg’. Other rare entities are nonetheless less definite and many are still the subject of active research.

In the present review, FISH and molecular study were not used to further delineate the break point in those with structural chromosomal abnormality. The use of FISH or molecular study improves the accuracy and precision in diagnosis. However, it is costly and the additional information thus gained is so subtle that it does not influence management. Molecular and FISH studies are therefore not routinely performed.

Conclusion

The prevalence of cytogenetic abnormalities among Hong Kong Chinese women with premature menopause is similar to that seen in Caucasian and Chinese populations elsewhere. Among all cytogenetic abnormalities, monosomy X and its variants predominate. Although cytogenetically abnormal women are shorter in stature, of younger age at menopause, and higher prevalence of dysmorphism, clinical features are generally poor predictors of cytogenetic abnormality. A pragmatic approach to screening is advocated.

The prevalence of FMR1 gene premutation carrier among Chinese women with premature menopause was around 0.86%. Workup for premature menopause in Chinese women should include testing for FMR1 premutation. It has practical implications for counselling, prenatal diagnosis, and fertility management.

References