**Short Report**

**Cytogenetic analyses of premature ovarian failure using karyotyping and interphase fluorescence in situ hybridization (FISH) in a group of 1000 patients**


To evaluate the implication of chromosome abnormalities in the etiology of premature ovarian failure (POF), 1000 patients with POF recruited at the Department of Cytogenetics of Farhat Hached Hospital (Sousse, Tunisia) between January 1996 and December 2008. Chromosome analyses were performed by using karyotyping and interphase fluorescent in situ hybridisation (FISH) using a centromeric probe of the chromosome X to look for low-level mosaicism of X-chromosome monosomy. Hundred and eight chromosomal abnormalities (10.8%) were found using karyotype analysis. Anomalies were detected in 61 cases out of 432 primary amenorrhea patients (14.12%) and 47 cases out of 568 secondary amenorrhea patients (8.27%). In 23 POF patients among 200 (11.5%) with 46,XX normal karyotype and explored using interphase FISH analysis, the percentage of cells with X-chromosome monosomy was significantly higher as compared with controls in the same age. The cytogenetic study of POF patients showed a high prevalence of chromosome anomalies either in primary or in secondary amenorrhea. Mosaic X-chromosomes aneuploidy was the most frequent abnormality and some patients with POF may be attributable to low-level 45,X/46,XX mosaicism detectable using FISH analysis.

Premature ovarian failure (POF; OMIM 311360) is defined as a primary ovarian defect characterized by absence of menarche (primary amenorrhea) or premature depletion of ovarian follicles before the age of 40 (secondary amenorrhea) (1). It affects 1–3% of females and represents a major source of female sterility (2). A wide spectrum of pathogenic mechanisms may lead to the development of POF including genetic, autoimmune, metabolic, infectious, and iatrogenic causes. Among genetic causes of POF, chromosome abnormalities are the most common, and POF has been frequently linked to X-chromosome abnormalities (2). However, apart from the Turner syndrome phenotype characterized by X-chromosome monosomy, the implication of mosaic X-chromosome monosomy has been reported but remains controversial particularly in cases with low-level mosaicism 45,X/46,XX.
and/or 47,XXX and the precise impact of this low-level sex chromosome mosaicism in ovarian function is unknown (3, 4). Moreover, conventional cytogenetic analysis involves routine scanning of no more than 20 metaphases. Thus, a low mosaicism cannot be properly estimated using this technique. Interphase fluorescence in situ hybridization (FISH) using centromeric probes is the best means to scan a large number of cells to evaluate more precisely numerical chromosomes mosaicism (5).

The aim of this study is to evaluate the prevalence of chromosome abnormalities in a large series of 1000 Tunisian females affected by POF and to evaluate the implication of 45,X low-level mosaicism detected using Interphase FISH in POF patients with normal karyotype.

Materials and methods

The study protocol was approved by the institutional review board at the Farhat Hached University Teaching Hospital.

Patients

A total of 1000 patients presenting with POF were recruited by the Department of Cytogenetics and Reproductive Biology, Farhat Hached University Teaching Hospital, (Sousse, Tunisia) between January 1996 and December 2008.

Four hundred and thirty-two patients had primary amenorrhoea (43.20%) and 568 had secondary amenorrhoea (56.80%). Also, the mean age of patients was 25 years (range: 16–38 years).

In our study, all of the patients had a cessation of ovarian function for longer than 6 months before the age of 40, associated with a high-FSH level detected on two occasions. Patients with a clinically evident Turner syndrome or presenting a known POF-inducing condition (ovarian surgery, chemo- or radio-therapy, autoimmune diseases, or known genetic disorders) were excluded. All of the patients gave their consent to a check of their medical history and cytogenetic studies.

Conventional cytogenetics

Chromosomal analysis was performed by standard cytogenetic techniques from peripheral blood lymphocyte culture. In all patients, karyotype analysis using R-banding was performed on 20 metaphases. Chromosomal abnormalities have been reported in accordance with the current international standard nomenclature (6).

FISH analysis

Among patients with normal constitutional karyotypes, FISH analysis of interphase lymphocyte preparations was performed on 200 patients with POF (92 patients with primary amenorrhoea and 108 with secondary amenorrhoea) to detect low-level XXX mosaicism. FISH study was performed on nuclei conserved from chromosomal preparations used for karyotyping. We used a centromeric probe of chromosome X labeled with FITC (green fluorescence) and a control centromeric probe of chromosome 18 labeled with Rhodamine (orange fluorescence). In all cases, 500 nuclei were analyzed.

Hundred control women aged between 17 and 37 years and with normal reproductive history were also studied using interphase FISH to establish the range of normality.

Descriptive statistics and analyses of variance were performed using Statistical Package for the Social Sciences (spss). p < 0.05 was the threshold of significant considered when hypotheses were statistically tested.

Results

We detected 108 chromosomes abnormalities (10.8%) using karyotype analysis. Anomalies were distributed as follows: 61 patients out of 432 primary amenorrhoea (14.12%) and 47 patients out of 568 secondary amenorrhoea (8.27%). There were 62 patients with homogeneous or mosaic X-chromosome aneuploidy (57.41% of all anomalies), 38 patients with X-chromosome structural anomalies, 4 patients with balanced X-autosome translocations, and 4 patients with autosomal anomalies. Karyotype analysis findings are detailed in Table 1.

Among 208 patients with familial history of POF, chromosome anomalies were detected only in 9 patients (4.33%). However, the same chromosomal rearrangement was observed only in one case of Xq deletion detected in a patient and her or his mother, both are affected by secondary amenorrhoea.

FISH analysis on interphase lymphocyte preparations performed on the control group, detect X-chromosome monosomy in 0.80–3.85% of cells (mean ± 2SD, 2.33 ± 1.34). The percentage of X-chromosome monosomy increases considerably according to the age of studied women (Fig. 1). Interestingly, in the group of patients, the percentage of cells with X-chromosome monosomy varied from 0.64% to 10.23% without any correlation with the age of patients. Twenty-three patients...
Cytogenetic studies of premature ovarian failure

Table 1. Detailed list of chromosomal aberrations detected in 1000 patients with POF

<table>
<thead>
<tr>
<th>Type of amenorrhea</th>
<th>Numerical X-chromosome abnormalities</th>
<th>X-chromosome X-autosome rearrangements</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>28</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>Secondary</td>
<td>34</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>6</td>
<td>68</td>
</tr>
</tbody>
</table>

Xq: isochromosome for the short arm of the chromosome X; Xp: isochromosome for the long arm of the chromosome X.

Fig. 1. Graph showing the percentage of cells showing one X-chromosome signal detected by FISH analysis of interphase cells of the control group. There is a linear relationship between the age and the percentage of X-chromosome monosomy.

Fig. 2. Graph showing the percentage of cells displaying a single X-chromosome in the group of 200 POF patients. In 177 patients, mosaicism is located in the gray area corresponding to a physiological low-level mosaicism, and 23 cases (highlighted with dot) indicate a percentage of mosaicism above the normal range.

(11.5%) have a significantly higher mosaicism compared with controls in the same age (Fig. 2).

Discussion

In addition to screening for genes mutations, cytogenetic analysis should be recommended in investigations of POF because chromosome anomalies have been detected in several cases with different forms of POF (7). However, to the best of our knowledge, few if any data exist regarding the prevalence of chromosome abnormalities in a large series of POF patients. Our study confirms the implication of chromosome anomalies as an essential etiology of POF with a prevalence around 10.8%. This prevalence is significantly higher in patients with primary amenorrhea compared with cases of secondary amenorrhea (14.12% vs 8.27%; p < 0.01).
45,X/46,XX and/or 47,XXX mosaicism was the most frequently detected anomaly. X-chromosome monosomy is classically associated with Turner syndrome, but mosaicism with 46,XX cells seems to attenuate phenotype and causes the installation of POF at variable ages without Turner syndrome stigmata (3). Nevertheless, in our series, we could not detect any correlation between the importance of 45,X mosaicism and the age of the installation of POF, probably because mosaicism was different from lymphocytes and ovarian tissues (8). In some previously reported cases of POF with normal karyotype, FISH studies detected a low-level mosaicism with 45,X cells (3). Based on this, we decided to study a large group of POF with normal karyotypes using interphase FISH to evaluate the implication of 45,X low-level mosaicism in the installation of POF. Mosaicism was also searched for in a control group of 100 non-affected females to establish the boundaries of 'physiological' mosaicism. In this control group, the percentage of 45,X cells was between 0.80% and 3.85% and the average of this physiological mosaicism shows a significant evolution with age. The range of normality was then established according to the age of controls (Fig. 1).

Among the 200 POF patients explored using interphasic FISH, 23 (11.5%) had a significantly higher mosaicism compared with controls of the same age (Fig. 2). There were 9 patients among 92 primary amenorrhea (9.78%) and 14 patients among 108 secondary amenorrhea (12.96%). We suggest that mosaicism in these patients was pathological and linked to POF.

X-chromosome deletions, particularly those involving the long arm of X-chromosome, are the most frequent structural rearrangements associated with POF, as reported previously (9). However, deleted segments were shared throughout the X-chromosome and no common (shared) critical deleted region could be identified. In our series, X-chromosome deletions involved various chromosomal segments (Fig. 3) and supported the findings that the totality of the X-chromosome is required region for normal ovarian function (10). Interestingly, in our series, Xq27.1–Xq27.3 region was implicated in 10 among the 12 Xq deletions. This region is located in the POF1 region previously suggested as a critical region in the installation of POF.

Also, cytogenetic analysis using conventional karyotyping is limited by the resolution of this technique and it is not easy to detect deletions smaller than 10 megabases. Identification of X-chromosome microdeletions using array comparative genomic hybridisation (array CGH) will be a great help in the identification of genes involved in POF.

Balanced X-autosome translocations were the most studied and reported anomalies with the aim of identifying regions and genes implicated in POF using breakpoint mapping methods. Unfortunately, no recurrent breakpoint has been detected thus far and a minimum of 100 different breakpoints located in the long and the short arms of X-chromosome are known (11). In our series, four balanced X-autosome translocations were detected in four cases of primary amenorrhea. In these cases, breakpoints were located in Xp22, q22, q24, and q27.

Contrary to male spermatogenesis defects (12), very few cases of autosomal anomalies associated with POF have been reported and the most frequent anomalies are robertsonian translocations (13, 14). Gametogenesis failure was not induced by genomic imbalances but by a mechanical chromosome difficulty during meiosis and this difficulty is probably more important in spermatogenesis as compared to oocytogenesis. Therefore, a correlation between robertsonian translocations and POF was suggested in many previous studies (13). However, robertsonian translocations are the most frequent translocations reported in human and the presence of two cases of 14;21 robertsonian translocation in 1000 POF in our series...
could be a coincidence. On the other hand, the detection of two unrelated cases of chromosome 12 inversion in this series and in a previously reported case of male azoospermia suggests the possibility of a chromosome 12-linked gene implicated in gametogenesis (12). In these two cases, breakpoints are located in 12p12 and 12q12.

In conclusion, cytogenetic study of POF patients showed a high prevalence of chromosome anomalies either in primary or in secondary amenorrhoea. X-chromosome numerical anomalies were the most frequent and particularly in the mosaic form. Because routine chromosomal analysis involves generally the study of a limited number of metaphases, a low-level mosaicism cannot be excluded on this basis. Therefore, we suggest that interphasic FISH analysis must be systematically used to search for low-level X-chromosome aneuploidies in patients with idiopathic POF and normal karyotype. Moreover, new molecular cytogenetic techniques and particularly microdeletions detection using high resolution X-chromosome array CGH, can give new information about chromosomal anomalies associated with POF.

References