Significance of ovarian histology in the management of patients presenting a premature ovarian failure

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BACKGROUND: Premature ovarian failure (POF) is a heterogeneous syndrome, possibly due to mutations of genes involved in the normal development of the ovary and/or follicles. Based essentially on animal models, these mutations are associated with various ovarian phenotypes, from a complete absence of follicles to a partial folliculat

Introduction

Premature ovarian failure (POF) is defined as the complete cessation of menses before the age of 40 years (Coulam et al., 1986). In the absence of surgical oophorectomy, chemotherapy and pelvic radiation, POF encompasses a heterogeneous spectrum of conditions, from ovarian dysgenesis to gonadotrophin resistance (Anasti, 1998). In molecular terms, most of the data were obtained from animal models with various ovarian phenotypes—from the complete absence of follicles to impaired follicle maturation—depending on the gene defect (Elvin and Matzuk, 1998; Burns and Matzuk, 2002). We set up a retrospective study to analyse women presenting a POF syndrome associated with a normal karyotype. Of these, three patients presenting different FSHR gene mutations have already been described, leading to a partial or almost complete loss of function of FSHR (Beau et al., 1998; Touraine et al., 1999; Meduri et al., 2003). However, this cause of POF is rare (Aittomaki et al., 1995; Doherty et al., 2002; Allen et al., 2003) and, in most cases, the aetiology of POF remains unknown. Therefore, a diagnostic strategy must be defined to orientate the search for the genetic causes of POF. The absence or the presence of follicles may lead to the distinction of two different situations: while the absence of follicles is likely due to defects of genes involved in oogenesis, an impaired follicle growth is rather due to defects of genes involved in follicular maturation (Anasti, 1998). To date, the presence of follicles is most often assessed by pelvic ultrasonography. However, ultrasonography is unable to detect follicles <2 mm. The aim of our study was to determine whether ovarian histology, when compared to pelvic ultrasonography,
would be helpful in identifying which patients display an impaired follicular reserve and/or growth and in orienting the search for POF aetiology. Consequently, 61 patients underwent both ovarian biopsies and pelvic ultrasonography. Results obtained by the two methods were analysed. In our opinion, the ovarian biopsy with the description of either the presence or the absence of follicles appears a helpful procedure to orient the search for candidate gene mutations.

Subjects and methods

Patients
We present here 61 patients aged between 15 and 39 years (median age at diagnosis: 26 years) who were referred to the Departments of Endocrinology—Adults and Pediatrics—at Necker Hospital, Paris, France between 1995 and 2003 for POF and clinical, hormonal, pelvic ultrasonography and histological investigations. None had a history of chemotherapy and/or pelvic radiotherapy, and all patients had a normal karyotype. Most patients presented with normal pubertal development (n = 56; 92%) and secondary amenorrhea (n = 45; 74%). A familial history of POF was suggested in nine cases with the description of either a mother or a sister with amenorrhea before age 40 years. However, no hormonal confirmation was obtained from these families. Finally, five patients suffered from an autoimmune disease that had been diagnosed before the POF syndrome. Of these, two patients presented a Hashimoto thyroiditis associated with POF.

All patients gave written informed consent for participating in the present study. On the first day of the investigation, blood samples for hormonal measurements and a pelvic ultrasonography were performed. The following day, patients underwent a surgical laparoscopy with an ovarian biopsy.

Hormone measurements
Plasma levels of FSH and LH were measured by conventional radioimmunoassay (Immunotech Beckman, France). Those of estradiol (E₂) were determined (DiaSorin, Italy) after previous plasma extraction. Normal range was based on hormonal results obtained from women during a normal menstrual cycle and given by the laboratory of hormonal investigations from Necker Hospital. The intra-assay coefficient varied from 2.6 to 6.7% whereas the inter-assay coefficient varied from 3.7 to 6.3%. The inhibin B concentration was measured in duplicate in serum samples using a solid phase sandwich enzyme-linked immunosorbent assay. The inhibin B assay used a capture monoclonal antibody raised against a sequence from the inhibin B subunit of inhibin coupled to alkaline phosphatase. The assay detection limit was 5 pg/ml.

Pelvic ultrasonography
Pelvic ultrasonography was performed in all patients, using a Siemens Sonoline Elegra sonograph and a 6.5 MHz probe. The surface area of the ovaries was calculated as: S = L (length) × W (width) × 0.8. The normal surface area of the ovary is between 2 and 6 cm² (Halligan et al., 2000).

Morphological analysis of ovarian biopsies from POF patients
After informed consent, the 61 patients underwent ovarian biopsies per laparoscopy. For each patient, two biopsies of 3–5 mm, one on each ovary, were sampled and fixed in neutral formalin. After embedding in paraffin, biopsies were serially sectioned at 5 μm, then stained with haematoxylin/eosin/safran. One section out of every 20 was observed and the number of resting and growing follicles was counted. The sections were carefully examined to detect fragments of large follicles that could be present on the edges of the biopsy as well as atretic follicles in the ultimate stages of atresia and degenerated corpora lutea.

Inhibin B (pg/ml) 5 5–105 60–200
Estradiol (pmol/l) 18.5 18.5–555 70–1100
FSH (IU/l) 67 13–155 3–9
LH (IU/l) 29.5 5.9–63 1–5

Table I. Hormonal evaluation in the 61 patients presenting with premature ovarian failure

Morphological analysis of ovarian samples from normo-ovulating women
In an attempt to test the reliability of small-sized biopsies sampled at random, to reflect accurately the presence of follicles in a given ovary, follicular counts were performed on ovaries from 20 normo-ovulating patients (A. Gougeon). These patients were operated on between 1973 and 1975 for tubal ligation, tubal plasty, explorative laparotomy, hysteroplasty, fibromas, breast carcinoma and small benign teratoma. All these patients were either fertile, having between one and three children, or presented with apparently normal folliculogenesis, as proven by the presence of a preovulatory follicle. The age of these patients ranged from 17 to 31 years (26.4 ± 0.9 years). The volume of the ovarian biopsies varied from 26 to 3200 mm³ (654 ± 147 mm³). Some patients underwent several biopsies on both their ovaries, and the whole ovaries were sliced for pathological studies. The tissues were fixed in Bouin’s fluid, embedded in paraffin, and serially sectioned at 10 μm, then stained with haematoxylin Masson Blue.

Sections were observed at ×2.5 magnification. In one section out of every 100, a surface area of 10 mm² (5.5 mm long × 1.8 mm deep) was chosen at random in the cortical part of the sample. The surface area studied was equivalent to the mean size of sections in biopsies from POF patients. All non-growing and growing follicles, whether healthy or atretic, as well as follicular or luteal fragments, were counted.

Statistical analysis
Analyses were processed with Statview version 4.5 (Abacus Concepts, USA). Descriptive statistics were performed for each variable; quantitative results are presented as median (range); qualitative results are presented as a distribution of a number of patients. Hormonal and ovarian parameters were compared using the Mann–Whitney test. Proportions for the two groups were compared using the χ²-test. The concordance between histology and ultrasonography was analysed with the Kappa test. P < 0.05 was accepted as significant.

Results

Hormonal evaluation
The main hormonal parameters are presented in Table I. Plasma FSH and LH levels were high and plasma E₂ and inhibin B levels were low. No significant difference was observed between FSH, inhibin B and E₂ levels of patients presenting with either primary or secondary amenorrhea (data not shown).

Table I. Hormonal evaluation in the 61 patients presenting with premature ovarian failure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median Range</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/l)</td>
<td>67</td>
<td>13–155</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>29.5</td>
<td>5.9–63</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>18.5</td>
<td>18.5–555</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>5</td>
<td>5–105</td>
</tr>
</tbody>
</table>
Pelvic ultrasonography

The surface area of the ovaries was 1.6 cm² (0.6–5.6). Ovaries were considered to have either a normal surface area (≥2 cm²), which was the case in 22 patients (36%), or a small surface area (<2 cm²), this being the case for the 39 other patients (64%). Images suggesting the presence of follicles of ≥2 mm were observed in 32 patients. This clearly correlates with the size of the ovaries (P = 0.008), since images of follicles were observed in 17 of the 22 patients with ovaries of normal surface area (77%) (Figure 1).

Ovarian histology in POF patients

The results of ovarian histological examination led to classification of the patients into two categories: (i) ovaries with the presence of follicles, either non-growing or with evidence of follicular growth consisting of the presence of follicles at various stages of maturation/involution and/or corpora lutea at various stages of their involution (n = 28, Figure 2A); and (ii) ovaries showing no follicle (n = 33, Figure 2B).

E₂, inhibin B, FSH and LH levels were compared in patients with histological evidence of the presence of follicles and those without follicles present (Table II). Inhibin B and E₂ levels were significantly higher in patients for whom histology showed the presence of follicular structures. However, the overlap between the two groups appears considerable (Figure 3). No difference was seen for gonadotrophin levels between the two groups.

Follicular counts from normally ovulating patients

The observations are summarized in Table III. Follicular counts in different parts of the same ovary displayed strong variations. Even in samples containing high numbers of resting follicles, some areas contained either no or very low numbers of resting (e.g. patients G2 and N). Although all these patients were either fertile or regularly ovulating, 214/372 sections (57.5%) exhibited only resting and growing follicles <2 mm. In these sections no follicle larger than 2 mm, detectable at US, were observed. In 158/372 sections (42.5%), resting and growing follicles, including those larger than 2 mm, were observed and 46/372 sections (12.4%) exhibited resting follicles, without evidence of follicular/luteal activity, either recent or old. In 2/372 sections (0.5%), neither resting follicles nor follicular/luteal activity was observed.

Comparison of ultrasonography and histological data

The follicular pattern at pelvic ultrasonography was compared to that observed in the corresponding ovarian biopsy. Results for the 61 patients are presented in Figure 4. In 32 patients, images suggesting the presence of follicles were observed at ultrasonography. Among them, only 18 had follicles observed at histology, whereas 14 did not. In 29 patients, no follicle was found at ultrasonography. Among them, 19 were actually devoid of follicles at histology, whereas 10 exhibited the presence of follicles. Therefore, ultrasonography appears to be a poor marker for predicting the presence of resting follicles at ovarian histology (predictive value of 56%).

Discussion

The POF aetiology in women with normal karyotype remains poorly understood (Anasti, 1998). However, an increasing number of genes that control normal ovarian development and function have recently been identified and, according to data obtained in animal models (Greenhouse et al., 1998; Burns and Matzuk, 2002), these genes are likely to be involved in POF disease. A retrospective study was performed to evaluate whether the ovarian biopsy, when

Figure 1. Correlation between the presence of follicles and ovarian size at ultrasonography. Patients with a surface area ≥2 cm² (n = 22) had images of follicles in most cases (n = 17, 77%), whereas patients with ovarian surface area <2 cm² (n = 39) had images of follicles in only 38% of cases (n = 15) (P = 0.008).

Figure 2. Histological aspect of the ovary of 61 POF patients. (A) A sample of an ovarian section with partial follicular maturation (seen in 28 cases): from primordial follicles (white arrow) up to the early antral follicle stage (black arrow). (B) A sample of ovarian cortex deprived of follicles (seen in 33 cases).
compared with data from pelvic ultrasonography, could be a reliable tool for identifying patients with an ovarian phenotype suggestive of particular gene mutations, therefore implying the usefulness of laparoscopy and ovarian histology in orientating the search for the possible genetic aetiologies of the POF syndrome.

The population of 61 women studied in the present report, recruited either in our Department of Endocrinology for adult patients or in the Pediatric Gynecology Unit of Necker Hospital, showed a typical pattern of ovarian insufficiency with high FSH and low E2 plasma levels. Inhibin B levels were also low, confirming previous data (Kalantaridou et al., 1998; Petraglia et al., 1998).

In this study, only 56% of patients with normal-sized ovaries and a presence of follicles >2 mm suggested at ultrasonography displayed follicles when histological examination of an ovarian biopsy was performed. Nevertheless, a parallel can be drawn between this proportion of 56% and that of 57.5% corresponding to histological sections in which no follicle theoretically detectable at US were seen. When women had no image of a follicle at ultrasonography, histological examination did not detect any follicle in 19 out of 29 patients, whereas follicles were observed in the 10 other patients. Consequently, it can be assumed that ultrasonography is not predictive of the presence of follicular structures within the ovary. We also wished to determine whether hormonal levels were sufficient to indicate the presence or absence of ovarian activity. Although E2 and inhibin B median levels were significantly higher in patients with the presence of follicles at histology, the overlap between the two groups appears considerable, pointing to the poor sensitivity of the hormonal markers to predict the presence or absence of a follicular activity; specifically, the predictive value of the presence of follicles appears very weak for low plasma levels of either estradiol or inhibin B.

Table II. Hormonal evaluation of patients presenting with premature ovarian failure, depending on the presence or the absence of follicles at histology

<table>
<thead>
<tr>
<th>Presence of follicles at biopsy</th>
<th>Absence of follicles at biopsy</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/l)</td>
<td>61 (13–141)</td>
<td>70 (26–160)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>32.5 (5.9–63)</td>
<td>25 (9.9–60)</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>77 (18.5–555)</td>
<td>18.5 (18.5–166.5)</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>5 (5–105)</td>
<td>5 (5–15)</td>
</tr>
</tbody>
</table>

Values are median (range).

* Mann–Whitney U-test.

Figure 3. Plasma estradiol (pmol/l) and inhibin B (pg/ml) levels in patients with the presence or the absence of follicles at ovarian biopsy.

Table III. Reliability of ovarian biopsies to detect follicles in normally ovulating patients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (years)</th>
<th>Volume of the biopsy (mm³)</th>
<th>Density of R (mean ± SEM)</th>
<th>Range</th>
<th>Number of area observed</th>
<th>R + Ga</th>
<th>R + Gb &gt;2mm</th>
<th>O + G</th>
<th>O + O</th>
<th>R + O</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17</td>
<td>194</td>
<td>71.0 ± 17.0</td>
<td>27–110</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>B1</td>
<td>19</td>
<td>whole ovary</td>
<td>12.3 ± 1.2</td>
<td>2–23</td>
<td>25</td>
<td>20</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>B2</td>
<td>19</td>
<td>whole ovary</td>
<td>8.0 ± 2.8</td>
<td>0–23</td>
<td>26</td>
<td>24</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>48</td>
<td>14.0 ± 5.0</td>
<td>9–19</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>21</td>
<td>930</td>
<td>18.4 ± 10.0</td>
<td>6–58</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>24</td>
<td>264</td>
<td>22.8 ± 6.3</td>
<td>10–40</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>25</td>
<td>whole ovary</td>
<td>27.3 ± 8.8</td>
<td>1–68</td>
<td>33</td>
<td>29</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>G1</td>
<td>25</td>
<td>whole ovary</td>
<td>4.0 ± 0.9</td>
<td>0–33</td>
<td>42</td>
<td>27</td>
<td>15</td>
<td>8</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>G2</td>
<td>25</td>
<td>whole ovary</td>
<td>5.0 ± 1.8</td>
<td>0–89</td>
<td>36</td>
<td>32</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>H</td>
<td>25</td>
<td>26</td>
<td>21.5 ± 3.5</td>
<td>18–25</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>26</td>
<td>656</td>
<td>22.5 ± 2.8</td>
<td>10–42</td>
<td>13</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>28</td>
<td>1235</td>
<td>30.7 ± 5.1</td>
<td>16–46</td>
<td>6</td>
<td>6</td>
<td>4</td>
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<td>0</td>
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<tr>
<td>K</td>
<td>29</td>
<td>120</td>
<td>81.0 ± 15.3</td>
<td>14–151</td>
<td>9</td>
<td>7</td>
<td>3</td>
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</tr>
<tr>
<td>L</td>
<td>29</td>
<td>580</td>
<td>23.5 ± 4.4</td>
<td>14–42</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>29</td>
<td>2850</td>
<td>15.7 ± 2.7</td>
<td>3–39</td>
<td>18</td>
<td>18</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>29</td>
<td>1220</td>
<td>16.2 ± 4.2</td>
<td>0–56</td>
<td>14</td>
<td>13</td>
<td>7*</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O</td>
<td>30</td>
<td>3180</td>
<td>2.9 ± 0.6</td>
<td>0–12</td>
<td>21</td>
<td>17</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P</td>
<td>30</td>
<td>1140</td>
<td>1.8 ± 0.8</td>
<td>0–5</td>
<td>6</td>
<td>2</td>
<td>0*</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Q</td>
<td>30</td>
<td>2110</td>
<td>9.3 ± 1.0</td>
<td>4–13</td>
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<td>7</td>
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<td>0</td>
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<tr>
<td>R</td>
<td>30</td>
<td>193</td>
<td>6.0 ± 1.4</td>
<td>1–10</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>S</td>
<td>31</td>
<td>780</td>
<td>3.1 ± 1.2</td>
<td>0–10</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>T1</td>
<td>31</td>
<td>whole ovary</td>
<td>9.4 ± 1.0</td>
<td>0–30</td>
<td>39</td>
<td>31</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>T2</td>
<td>31</td>
<td>whole ovary</td>
<td>8.8 ± 1.5</td>
<td>1–63</td>
<td>41</td>
<td>32</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

Presences of follicles is described in various numbers of sections obtained from 23 ovarian samples of 20 patients. In three patients (named B, G and T), the two ovaries were studied. Follicle density is calculated by 10 mm². Resting (R) follicles are distinguished from growing (G) follicles. O + G = only growing follicles; O + R = only resting follicles; O + O = neither resting nor growing follicles.

*An active corpus luteum was present in the biopsy and was visible in each section.

*All resting and growing follicles; +Resting follicles and growing follicles > 2mm.

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To ensure that follicular content in ovarian biopsies from POF patients was representative of the whole ovarian follicular content, counts were randomly performed on ovaries from 20 normal patients, without POF, who were considered controls in this study. The present study provides useful data to determine whether ovarian biopsies sampled at random allow for reliable assessment of follicle presence and activity for a given patient. Ovarian biopsies are effective in discriminating between patients having or not having resting follicles, even if a very small biopsy (less than $5 \times 2 \times 2 \text{mm}$) is less powerful in estimating the actual number of resting follicles. In our opinion, it can be assumed that the error in estimating the presence or absence of resting follicles in a given ovary is only 0.5%. Less accurate but still informative is the reliability of small ovarian biopsies to detect early follicular growth. Therefore, small biopsies appear very instructive for distinguishing ovarian phenotypes marked by the presence of a follicular reserve from ovaries deprived of such resting follicles. On the contrary, the determination of the stage of follicular growth, based on the observation of a few slides from an ovarian biopsy, appears less accurate and should be carefully considered.

The informative aspect of the ovarian biopsy has also been demonstrated in both healthy volunteers and patients with cancer, enabling the selection of women with numerous primordial follicles and for whom an ovarian conservation before chemotherapy may be suggested (Meirow et al., 1999). The follicular density measured after an ovarian sampling obtained by laparoscopy has also been shown to be correlated with age and appears to be a good marker of the ovarian reserve (Lass et al., 1997), even if follicular density may be highly variable within the same ovary (Schmidt et al., 2003). However, a prospective study comparing all methods available (hormonal and morphological) to appreciate the follicular reserve would probably be very interesting in the future for common medical practice.

To date, in our experience, the distinction between two different ovarian phenotypes, i.e. with or without follicles, appears useful in determining a strategy to search for genetic anomalies that could be responsible for the POF syndrome. In the past, this strategy has enabled us to identify patients with FSHR gene mutations, already described by other groups (Aittomaki et al., 1996; Allen et al., 2003). The description of a partial follicular maturation was also given in the initial report of a patient bearing an FSHβ gene mutation (Rabin et al., 1972; Matthews et al., 1993). However, these cases are probably rare and both the absence/presence of follicles and the stage at which follicular growth is impaired should prove helpful in orienting research and screening among other candidate genes. The comparison of the phenotype of POF patients with data obtained from mouse models presenting gene defects related to ovarian development and function suggests that mutations of those genes are possibly associated with POF syndrome. Various sets of genes are now identified and involved in the different steps of ovarian and follicular development. Genes involved in migration of germinal cells and meiosis such as Sporulation 11 (SPO 11), Disrupted Meiotic CDNA1 (DMC1), Mut S Homologue 4 (MSH4), Mat S Homologue 5 (MSHS) (Cotinot et al., 2002) may be associated with defects leading to a complete absence of follicles. On the contrary, in mice bearing defects of genes normally involved in follicular maturation, such as Connexin 37 or GDF9 (Burns and Matzuk, 2002), the ovarian histology confirms the presence of the resting follicles with a blockade of the follicular maturation at later stages. Finally, the recent description of the target disruption of genes encoding for transcription factors such as Fox12 (Schmidt et al., 2004) and Foxo3 (Castrillon et al., 2003) confirms their role in the regulation of the follicular growth. It is highly probable that mutations of such genes exist in women and the search for them will depend on the ovarian phenotype observed. However, we cannot exclude that a heterogeneous syndrome such as POF might be secondary to multiple gene abnormalities.

Therefore, in the future, the identification of the ovarian transcriptome in those patients compared with normal young adults also appears necessary to better understand the role of different genes in follicular and ovarian development. This strategy using DNA chips would allow for the qualitative and quantitative analysis of gene expression and the understanding of gene function and detection of gene alterations (Ben-Shlomo et al., 2002), but would be based on the initial extraction of the ovarian mRNA, therefore justifying the ovarian biopsy. Finally, our knowledge of POF syndrome...
should be improved by the study of familial cases enabling a linkage analysis of such cases, with the determination of the gene defects associated with the syndrome.

In conclusion, the clinical presentation associated with the hormonal investigations, the ultrasonographic description and the histological ovarian phenotype constitute the global phenotyping of our POF patients and are mandatory in the identification of such new genetic mutations.

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References