Do etiologies of premature ovarian aging (POA) mimic those of premature ovarian failure (POF)?

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BACKGROUND: It is unknown whether etiologies differ between milder forms of premature ovarian senescence (the acronym given here ‘premature ovarian aging, POA’), and premature ovarian failure (POF).

METHODS: We assessed presumed pathophysiologies in 74 consecutive POA patients, diagnosed based on elevated age-specific baseline follicle stimulating hormone and/or abnormally low anti-Müllerian hormone levels (≤ 1.5 ng/ml). A genetic etiology was presumed with ≥ 34 triple CGG expansions on the FMR1 gene. An autoimmune etiology was assumed with at least one abnormality in a laboratory panel, involving antinuclear, antiphospholipid and thyroid antibodies, total immunoglobulin levels and anti-ovarian as well as anti-adrenal autoantibodies. A combined etiology was presumed with both autoimmune and genetic etiologies, and a patient was considered idiopathic when no abnormalities were found.

RESULTS: Twelve of 74 (16.2%) women demonstrated a genetic, 28 (37.8%) an autoimmune, 9 (12.2%) combined and 25 (33.8%) idiopathic etiologies.

CONCLUSIONS: Presumed underlying etiologies with POA follow a similar distribution pattern as reported for POF. POA and POF may, therefore, represent a continuum in phenotypical expression of different etiologies of premature ovarian senescence. Like POF, POA should be considered reason to investigate underlying etiologies.

Key words: diminished ovarian reserve / premature ovarian failure (POF) / premature ovarian aging (POA) / FMR1 gene / autoimmunity

Introduction

The terminology surrounding so-called premature ovarian failure (POF) has been somewhat confusing. POF is generally defined as occurrence of ovarian functional insufficiency before age 40. Criteria for ovarian functional insufficiency, in turn, are usually met when follicle stimulating hormone (FSH) levels exceed 40 mIU/ml and/or persistent amenorrhea is reached (Vegetti et al., 2000; Santoro, 2003). Some authorities recently have started utilizing the acronym ‘primary ovarian insufficiency (POI)’ for this condition, which represents the clinical end stage of ovarian function due to a large variety of processes with greatly varying etiologies (Welt, 2008).

Putting aside increasingly common iatrogenic forms of POF (Oktem and Oktay, 2007), etiologies of ‘spontaneously’ occurring POF can be classified into four large groupings: (i) idiopathic, (ii) genetic, (iii) autoimmune, and (iv) infectious (Table I).

The single most frequently reported etiology of POF is still ‘idiopathic’, suggesting that many causes of POF remain unknown. Among suspected etiologies, abnormal autoimmune function and genetic causes are uniformly reported as the most frequent (Table I). Genetic causes vary greatly but, as references in Table I, the most frequent one is represented by abnormally large triple CGG repeats on the fragile X (FMR1) gene.

Table I also reflects the close statistical association between POF and abnormal autoimmune function. What this association, however, in a physiological sense represents is not yet entirely clear: the risk of POF is most closely associated with autoimmune thyroid disease, but is, in general, associated with abnormal autoimmunity, and especially with autoimmunity to various glandular organs (Hoek et al., 1997;
The disruption of the pituitary-ovarian axis can cause POF (Altuntas et al., 2008). Other investigators have identified enolase within the ovary as a target antigen for autoimmune responses in POF (Sundblad et al., 2006), whereas commonalities of autoantigens in glandular organs have been considered possible causes for the observed frequency of abnormal autoimmune responses against multiple endocrine organs in association with POF (Hoek et al., 1997).

POF, therefore, quite obviously, represents the end stage of greatly varying ovarian pathophysiology, rather ‘arbitrarily’ defined by an upper age limit of 40 years and FSH levels of 40 mIU/ml. How ~1% of women (Vegetti et al., 2000; Santoro, 2003; Welt, 2008) reach this end stage of ovarian insufficiency, and what preliminary stages precede POF, is, however, largely unknown.

Reddy et al. (2008), in an elegant mouse study, recently demonstrated that premature ovarian aging can be induced by prematurely activating, and thus depleting, a primordial pool of oocytes in young mice. Building on the conceptual correlation between remaining primordial follicle pool size and ovarian reserve, first demonstrated by Faddy and associates (Faddy and Gosden, 1995, 1996; Faddy, 2000), others hypothesized about the existence of precursor stages to POF, simply representing parallel curves to normal ovarian aging, just moved toward younger ages (Nikolaou and Templeton, 2003; Gleicher, 2005).

Nikolaou and Templeton (2003), based on an epidemiologic study, which demonstrated an ~10% prevalence of premature menopause in the general female population, suggested that ~10% of all females should, therefore, also demonstrate precursor stages to POF. The diagnosis of POF, as noted earlier, is currently, however, only made up to age 40 years (Vegetti et al., 2000; Santoro, 2003). Considering that the average age of physiological menopause is 51 years (Santoro, 2003), it seems obvious that, in addition, there also must be a group of women, who between ages 41 and 51 years prematurely enter menopause. They, of course, also exhibit ‘early’ menopause, though currently may not qualify for a diagnosis of POF. Their precursor stages of declining ovarian reserve should, therefore, manifest themselves in an age range between that of women with a diagnosis of POF and those with normally timed menopause. Nikolaou and Templeton’s estimate of only a 10% prevalence of premature ovarian senescence in the general population, and of early observable precursor stages to menopause, would, therefore, seem conservatively low.

Only ~1% of women in the general population reach outright POF (Vegetti et al., 2000; Santoro, 2003; Welt, 2008). The remaining 9% (or more, based on above noted argument) will exhibit only milder forms of premature ovarian senescence. We coined for this group the acronym ‘premature ovarian aging (POA)’ and defined such patients by elevated age-specific baseline FSH levels (Barad et al., 2007). They are highly disproportionately concentrated among infertility patients (Barad et al., 2007), and other authorities have recently coined for these patients the acronym ‘occult POI’ (Streuli et al., 2008).

Though in the general population, thus, at least nine times (and among infertility patients a multiple of that) more prevalent than POF, nothing is known about the underlying etiologies of POA. Current understanding of premature ovarian senescence allows for two possible hypotheses how POF and POA interrelate: a first would suggest that among many possible causative processes for premature ovarian senescence, some favor POA, whereas others lead to POF. Under such a hypothesis, POA and POF should demonstrate greatly varying associated etiologies. A second concept would, however, suggest that POA and POF simply represent, within identical disease processes, varying phenotypical expressions on a continuum of
severity. This hypothesis, in turn, would mandate very similar underlying etiologies in women with POA and POF.

This study was, therefore, undertaken to clarify whether associated etiologies with POA and POF vary or follow a similar distribution pattern.

Materials and Methods

The study population involves 74 consecutive infertile women under age 38 years, who in our center’s electronic research database were identified to suffer from spontaneous POA. Age was restricted to below 38 years to exclude excessive effects from physiological ovarian senescence on outcome, reported to accelerate at 37 to 38 years (Faddy and Gosden, 1996). The study for the same reason also excluded women with iatrogentic POA (Oktem and Oktay, 2007).

A diagnosis of POA was defined by elevated age-specific baseline FSH levels, specific to our center’s patient population, as previously reported in detail (Barad et al., 2007), and/or anti-Müllerian hormone (AMH) levels < 1.5 ng/ml, previously reported as indicative of diminished ovarian reserve at all ages (Singer et al., 2008). Age-specific FSH cutoffs were < 7.0 mIU/ml under age 33 years, < 7.9 mIU/ml at ages 33–37 years, < 8.4 mIU/ml at ages 38–40 years and < 8.5 mIU/ml at, or above, age 41 years. These cutoffs have been demonstrated to discriminate between women who produce more or less oocytes under uniform ovarian stimulation protocols (Barad et al., 2007). Presumed associated POA etiologies in the study population were then recorded based on the review of medical records.

In consideration of a patient population significantly affected by POA (Barad et al., 2007), and in view of recent authoritative recommendations to investigate women with evidence of POA for abnormalities in the fragile X (FMR1) gene (Wittenberger et al., 2007), our center has since January 2007 routinely investigated all newly presenting infertility patients for abnormalities on the FMR1 gene. Patients are, however, not routinely investigated for other potential genetic etiologies, representing risk towards premature ovarian senescence (Table I). A patient was, therefore, considered to have a ‘genetic’ etiology of POA only if the investigation of her fragile X (FMR1) gene revealed abnormally increased triple CGG repeats of ≥ 34 on at least one allele. This cutoff was chosen because we previously reported that triple repeat numbers beyond an upper cutoff of 32–34 denote risk for POA (Gleicher et al., 2008a, b). Streuli et al. (2008) more recently confirmed this observation at intermediate and premutation range CGG expansion ranges on the FMR1 gene. Since no other genetic tests were performed, what here is described as ‘genetic’ POA is, therefore, only reflective of FMR1 abnormalities. The overall percentage of genetically induced POA can, therefore, be expected to exceed the prevalence reported in this study.

An ‘autoimmune’ etiology was presumed if patients demonstrated at least one abnormality in an immunological panel, routinely drawn at our center on all new infertility patients. This is based on reports suggesting increased prevalence of abnormal autoimmune function among infertile women (Geva et al., 1997; Gleicher et al., 2007a, b), our centers reported high prevalence of abnormal autoimmune findings (Gleicher et al., 2008c) and the increased reported risk for miscarriages with subclinical levels of abnormal autoimmune function (Gleicher et al., 2007a, b). This investigation involves, as previously reported in detail (Gleicher et al., 2008a, b), an antinuclear antibody panel, a limited antiphospholipid antibody (APA) panel, thyroid antibodies, total immunoglobulin levels and anti-ovarian as well as anti-adrenal autoantibodies. Any woman with at least one abnormality in this panel was considered to potentially reflect an autoimmune etiology for POA.

Definition of subclinical abnormal autoimmune function is always difficult (Gleicher et al., 2007a, b). We, therefore, on purpose chose a, likely, too non-specific definition of abnormal autoimmune function, by classifying women as ‘autoimmune’ with only one abnormal laboratory finding. Such a definition, of course, will with considerable certainty contaminate this patient group of ‘autoimmune’ POA with potentially unaffected cases. It, however, as previously demonstrated, distinguishes clearly between ‘autoimmune’ and ‘genetic’ phenotypes of POA (Gleicher et al., 2008c). Although very likely too non-specific, this definition of subclinical autoimmunity however virtually guarantees that ‘genetic’ POA patients will not be contaminated by undiagnosed ‘autoimmune’ POA patients. Autoimmune data reported here may, therefore, be over-reported, whereas patients with a presumed genetic etiology, as previously noted, may be somewhat under-reported.

Patients were considered to be ‘genetic/autoimmune (combined)’ in their POA etiology if they demonstrated evidence for both etiologies, and ‘idiopathic’ if neither investigation revealed positive findings. Once the original study patients had been defined into these four groups, patient characteristics were compared, which included ages, FSH and AMH levels, peak estradiol levels during ovarian stimulation and oocyte yields.

Statistical analysis was performed with the SPSS 5.0 for Windows package (SPSS Inc., Chicago, IL, USA). Continuous data were presented as mean ± standard deviation. Categorical data were presented as counts (percentage). Differences between continuous variables with normal distribution were tested with analysis of variance. If distribution was not normal, the Kruskal–Wallis test was used.

All new patients at our center execute at initial visit an informed consent, which allows for the review of their medical records for study purposes. As long as the anonymity of patients and confidentiality of medical records are maintained, our center’s Institutional Review Board (IRB) policy, therefore, allows for expedited, automatic reviews and approvals of medical record studies. A confirmatory letter from the center’s IRB Chairman is available upon request.

Results

The mean age of the study cohort was 33.1 ± 3.4 years (range 21–37). Forty-six (62.2%) of the cohort were Caucasian, 19 (25.6%) were Asian and 9 (12.2%) were African-American. Classifying their infertility diagnoses based on national reporting requirements to the Centers for Disease Control, 45 (60.8%) of the cohort members had as their ‘primary’ chart diagnosis so-called unexplained infertility, with FSH < 12 mIU/ml, normal hysterosalpingography and normal semen analyses. Of the remaining 29 patients, 26 (35.1%) had evidence of diminished ovarian reserve and 3 (4.1%) had abnormal pelvic factors.

Table II summarizes the distribution of immune tests leading to a diagnosis of immune factor. The most frequent immune abnormalities involved antiphospholipid antibodies (anticardiolipin and others combined), antithyroid autoimmunity and gammatopathies.

Table III summarizes patient characteristics in all four groups: among 74 women, 12 (16.2%) demonstrated a ‘genetic’, FMR1-related etiology, 28 (37.8%) an ‘immune’, 9 (12.2%) a ‘combined genetic/immune’ and 25 (33.8%) no demonstrable causes (i.e. ‘idiopathic’ etiology). Almost two-thirds of all patients (66.2%), thus, demonstrated either assumed genetic and/or immune causes for their POA.

The table also demonstrates that there were no significant differences between the four possible etiological backgrounds in age, FSH,
Discussion

With POA suggested to affect at least 9% of all women (nine times the prevalence of POF) and, depending on age, up to ~50% of infertile women under treatment (Barad et al., 2007), it is surprising that associated etiologies of POA so far have not been investigated. We very recently reported in a much smaller patient sample, a majority of POA cases associated with two independent etiologies—a genetic, linked to excessive triple repeats on the FMR1 gene, and abnormal autoimmune function (Gleicher et al., 2008a, c). Streuli et al. (2008), even more recently, confirmed the association between elevated triple CGG counts on the FMR1 gene and POA. The study reported here confirms this in a much larger patient population by demonstrating that approximately two-thirds of cases are associated with these two potential etiologies for POA.

In our preliminary pilot study, we were unable to reliably comment on the distribution of associated etiologies and could only conclude that women with associated genetic and autoimmune etiologies significantly varied in their respective clinical phenotypes (Gleicher et al., 2008c). The study reported here is of adequate sample size to further comment: by demonstrating that only approximately one-third of POA patients remain undefined in their etiology (i.e. ‘idiopathic’), the distribution of associated POA etiologies corresponds well to that reported for POF (Hoek et al., 1997; Vegetti et al., 2000; Santoro, 2003; Gleicher et al., 2007a, b; Welt, 2008). Like in POF, genetics and abnormal autoimmune function account for a majority of cases, though a considerable volume of cases still remains unaccounted for.

Table III deserves some additional attention: although individual parameters between the four investigated patient groupings did not differ statistically, this should not necessarily surprise since, as this study well demonstrates, patients may reflect more than one etiology leading to POA. For example, 9 of 74 (12.2%) of patients demonstrated genetic as well as autoimmune etiologies. We previously, furthermore, noted that genetic etiologies are likely underrepresented, whereas abnormal autoimmune function may be overrepresented in how patients were assigned to the four patient groups. These overlaps can be expected to blur distinct differences in patient characteristics, such as FSH and AMH.

Assuming that POA and POF, indeed, share etiologies, our limited ability to search for all underlying etiologies is, of course, a reason for

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**Table II** Distribution of immune findings

<table>
<thead>
<tr>
<th>Laboratory finding</th>
<th>Number</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Gammopathy</td>
<td>15</td>
<td>20.3</td>
</tr>
<tr>
<td>ACA</td>
<td>12</td>
<td>16.2</td>
</tr>
<tr>
<td>Other APAs</td>
<td>9</td>
<td>12.2</td>
</tr>
<tr>
<td>Antithyroglobulin antibody</td>
<td>10</td>
<td>13.5</td>
</tr>
<tr>
<td>Antiperoxidase antibody</td>
<td>9</td>
<td>12.2</td>
</tr>
<tr>
<td>Anti-ovarian antibody</td>
<td>4</td>
<td>5.4</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>Anti-adrenal antibody</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Total with immune abnormalities</td>
<td>37</td>
<td>50.0</td>
</tr>
<tr>
<td>Without immune abnormalities</td>
<td>37</td>
<td>50.0</td>
</tr>
</tbody>
</table>

The total percentage exceeds 100% because some patients demonstrated more than one immune abnormality.

1Defined as either abnormally high or low IgG, IgM or IgA.

2ACA, anticardiolipin antibody in either IgG, IgM or IgA.

3IgG, IgM or IgA isotypes of antiphosphatidylserine and/or β-2 glycoprotein. Among those, 28 (38%) showed only immune abnormal antibodies (APA) and 9 (12%) also demonstrated elevated CGG repeat numbers. ACAs and APAs, in combination, represent 21 patients (28.4%) and, therefore, the single most frequently detected autoimmune finding.

4Includes women with only autoimmune (n = 28) and autoimmunity as well as a genetic POA (n = 9).

5Includes women with genetic (n = 12) and idiopathic (n = 25) POA.

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**Table III** Patient characteristics of individual groups

<table>
<thead>
<tr>
<th></th>
<th>Genetic n = 12</th>
<th>Immune n = 28</th>
<th>Genetic/immune n = 9</th>
<th>Idiopathic n = 25</th>
<th>Total n = 74</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.8 ± 2.6</td>
<td>33.4 ± 2.9</td>
<td>32.7 ± 3.4</td>
<td>32.6 ± 4.3</td>
<td>33.1 ± 3.4</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>13.6 ± 6.4</td>
<td>17.1 ± 27.3</td>
<td>27.4 ± 42.4</td>
<td>14.9 ± 14.2</td>
<td>17.0 ± 23.7</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>41.8 ± 13.6</td>
<td>56.8 ± 25.7</td>
<td>50.1 ± 24.4</td>
<td>50.1 ± 20.6</td>
<td>51.2 ± 22.4</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>2.6 ± 6.8</td>
<td>1.3 ± 1.3</td>
<td>1.1 ± 0.8</td>
<td>1.2 ± 1.0</td>
<td>1.4 ± 2.9</td>
</tr>
<tr>
<td>CGG repeats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele-1</td>
<td>28.6 ± 4.3</td>
<td>26.7 ± 4.4</td>
<td>29.0 ± 4.4</td>
<td>28.8 ± 2.9</td>
<td>28.0 ± 4.0</td>
</tr>
<tr>
<td>Allele-2</td>
<td>41.7 ± 6.1</td>
<td>30.0 ± 2.2</td>
<td>44.8 ± 15.1</td>
<td>30.4 ± 1.8</td>
<td>34.1 ± 8.5</td>
</tr>
</tbody>
</table>

All values are presented ± 1 SD. The four groups did not vary statistically in any of the listed parameters (P > 0.05 for all comparisons).
caution (Table I). Another reason is the previously already addressed difficulty in defining abnormal autoimmune function, especially when clinically not overt, as is the case in most POA patients (Gleicher et al., 2007a, b). Limited autoimmune investigations, therefore, may fail to identify women with abnormal autoimmunity, and a low diagnostic threshold, as chosen for this study, may have the opposite effects and create false-positive identifications.

It should, however, be noted that our definition of abnormal autoimmunity does not significantly differ from historical definitions in investigations of POF (Hoek et al., 1997). This study, therefore, defines abnormal autoimmune function just as well (or as poorly) as past studies of POF. The study design utilized here would, therefore, appear entirely appropriate for a comparison of possible associated underlying etiologies. Having made this statement, it is also important to note that association does not necessarily imply causation. In defining etiologies, we, therefore throughout this manuscript, have utilized careful terminology by discussing associated and presumed etiologies of both POA and POF. Although the data reported here, indeed, suggest that POA and POF demonstrate similar underlying pathophysiology, nothing in the study reported here proves that any of these presumptive etiologies are really causally associated with either POA or POF.

It is, nevertheless, impressive how similar the distribution of potential etiological associations is between POF and POA: except that ‘genetic’ and ‘immune’ etiologies, combined, reflect similar percentages, it seems quite obvious that abnormalities in triple CGG repeats on the FMR1 gene represent the dominant genetic defect in both conditions. Such excessive expansion sizes have for long been considered the most frequent genetic cause of POF (Wittenberger et al., 2007), and we recently reported a similar association with milder forms of ovarian senescence; i.e. POA (Gleicher et al., 2008a, b), recently also confirmed by Streuli et al. (2008).

On the autoimmune side, autoimmune thyroid disease has been reported as the single most frequently associated autoimmune abnormality in POF (Hoek et al., 1997; Goswami et al., 2006; Sundblad et al., 2006). Not surprisingly, autoimmune thyroid laboratory abnormalities are also among the most frequent autoimmune findings in this study of POA patients (Table II).

Although POA, within a continuum of premature ovarian senescence, as a milder phenotypical stage of POF makes hypothetical sense, the occurrence of POF is generally perceived as a quick and unpredictable process, with no known universal precursor stages (Hoek et al., 1997). By definition, POA is, however, a rather slowly progressing process (Nikolaou and Templet, 2003; Gleicher, 2005). This then should point toward an assumption of different etiologies and pathophysiology for POA and POF, respectively.

Some of the arising confusion may, however, be explainable: since the currently utilized definition of POF allows for a diagnosis only till age 40 (Hoek et al., 1997; Veggetti et al., 2000; Santoro 2003; Welt, 2008), only more severe cases of POA will ever have the opportunity of a POF diagnosis because only more severely affected ovaries will reach complete exhaustion by age 40 years. The large majority of milder POA patients will pass age 40 with diminished ovarian function [and clinical evidence of premature ovarian senescence, if properly investigated (Barad et al., 2007)], but will not meet minimum criteria for a diagnosis of POF by age 40. Considering that the time period between accelerated decline in ovarian function at ~25 000 remaining follicles (normally at age ~37.5 years) and menopause (~1000 remaining follicles) is believed fixed (Faddy and Gosden, 1995, 1996; Faddy, 2000), time of menopause becomes predictable (Faddy and Gosden, 1996) and may in many cases occur before age 51, the average age of menopause in the general population (Santoro, 2003). Under current clinically prevalent terminology, such patients experience so-called ‘early menopause’, but will not be diagnosed with POF, even though they, too, of course, reach the same end-point of total ovarian insufficiency (Welt, 2008) and do so before age 51 years.

This arbitrary definition of POF distorts the study of premature ovarian senescence and demonstrates well the dangers of arbitrarily selected cutoffs in defining diseases. It also suggests why the current definition of POF may benefit from revisions, which would allow for a diagnosis of POF above age 40.

The current belief that POF occurs mostly suddenly, and lacks precursor stages (Hoek et al., 1997), may, therefore, be simply based on observational biases. Assuming a diagnosis of POF in women with normally aging ovaries up to age ~51 years, POA, based on the data of this study, may represent the natural precursor stage for POF; if different etiologies were responsible for POA and POF, the two patient populations should demonstrate distinctly different associated etiologies. The similarity in observed distribution of associated etiologies between POA and POF, however, supports the contention that POA may represent a milder precursor stage to POF within a continuum of prematurely diminishing ovarian reserve. This study, therefore, supports the hypothesis that POA defines a frequent precursor stage to POF.

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