A pilot study of premature ovarian senescence: II. Different genotype and phenotype for genetic and autoimmune etiologies

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Objective: To assess whether abnormal autoimmune function and number of triple CGG repeats on the FMR1 (fragile X) gene, both historically associated with risk toward premature ovarian senescence, represent independent risk factors.

Design: Retrospective cohort study.

Setting: Academically affiliated, private fertility center.

Patient(s): Forty consecutive, new infertility patients, of which 11 presented with a primary diagnosis of repeat pregnancy loss, 23 with prematurely elevated, age-specific baseline follicle stimulating hormone (FSH) levels (i.e., premature ovarian aging) and 6 with premature ovarian failure.

Intervention(s): Determination of triple CGG repeats on both alleles of the FMR1 gene, assessment of ovarian reserve via FSH and anti-Müllerian hormone levels, and evaluation of autoimmune status by antiprophospholipid antibody panel, antinuclear antibody panel, total immunoglobulin levels (IgG, IgM, IgA), thyroid antibodies (antiaglobulin and antimicrosomal), antiovarian, and antiadrenal antibodies.

Result(s): Twenty-two of 40 patients (55%) demonstrated autoimmune abnormalities. Women with and without autoimmune abnormalities did not differ in age. Patients with autoimmune abnormalities, however, demonstrated significantly lower FSH levels and higher anti-Müllerian hormone levels. Although triple repeats on the lower count allele (allele-1) of the FMR1 gene did not differ statistically, autoimmune patients demonstrated in the higher count allele (allele-2) significantly fewer triple repeats, significantly fewer triple repeats \( \geq 30 \), and, in contrast to nonautoimmune patients, a normal mean level of triple repeats.

Conclusion(s): Abnormal autoimmune function and expansions in triple CGG repeats on the FMR1 gene represent distinctively different etiologies for premature ovarian senescence in infertile patients and may, indeed, constitute its two principal causes. (Fertil Steril® 2009;91:1707–11. ©2009 by American Society for Reproductive Medicine.)

Key Words: Premature ovarian failure (POF), premature ovarian aging (POA), follicle stimulating hormone (FSH), autoimmune, fragile X, FMR1 gene, fertility preservation

It has been suggested that the prevalence of premature ovarian aging (POA) in the general population is approximately 10% (1). We have demonstrated that in infertile women under treatment the risk for premature ovarian senescence is even significantly higher than that (2). Why such a large portion of the female population is affected by premature ovarian senescence is still largely unknown. Its most severe form, premature ovarian failure (POF), has a number of known causes. Among those are an autoimmune-induced form and POF associated with premutations and full mutations of the FMR1 (fragile X) gene (3, 4). Combined, these two etiologies may, indeed, account for a majority of POF cases (3–5).

Like premature ovarian senescence, the prevalence of autoimmune abnormalities in infertile women is surprisingly high (6, 7). Why so many infertile women are affected by abnormal autoimmune function is also still unknown, but may, at least in part, be a reflection of a higher prevalence of autoimmune diseases in women than men (8). Whether abnormal autoimmune function is causally related to female infertility has remained highly controversial (9, 10). Increasing evidence suggests, however, that many autoimmune diseases are characterized by decreased fecundity, at times even before their full clinical expression (6, 11–15).

The phenotypical expression of abnormal triple CGG expansions on the FMR1 gene includes premature elevations in basal follicle stimulating hormone (FSH) levels, early menopause, and POF (4). We recently extended this observation by demonstrating that, even in ranges starting at \( \geq 30 \)
repeats, generally still considered normal triple repeat numbers, a statistical correlation exists between triple repeat numbers and risk for premature decline in ovarian function. In its mildest form, patients may only demonstrate prematurely abnormal ovarian reserve tests, such as elevated FSH and abnormally low anti-Müllerian hormone (AMH) levels, or clinical evidence of abnormal ovarian function, such as resistance to ovarian stimulation with gonadotropins. In the most severe form, patients will express POF (16).

Considering the statistical correlation between number of CGG triple repeats on the FMR1 gene and risk for progressively serious forms of premature ovarian senescence (16), and the causal relationship between abnormal autoimmune function and premature ovarian senescence (3, 5), the question arises as to whether these two etiologies represent independent risks. We, therefore, investigated in an infertile female population the statistical relationship between triple repeat numbers on the FMR1 gene and abnormal autoimmune function.

MATERIALS AND METHODS

The patient population of this study has previously been described (16). It involved 11 women with a history of repeated pregnancy loss, 23 with POA, defined by elevated age-specific baseline FSH levels, as previously reported in detail (2), and 6 classical POF patients. Their medical records were retroactively reviewed for patient characteristics, including age, FSH, and AMH levels, as well as evidence of abnormal autoimmune function.

In view of the reported high prevalence of abnormal autoimmune function in infertile women (6, 7), our center routinely evaluates newly presenting infertility patients for abnormal autoimmune function. Abnormal autoimmune function has been suggested to impede female fertility (9, 10) and to increase the risk of pregnancy loss (6, 17–20), a risk we strive to reduce via timely diagnosis and treatment (9). Women were, therefore, designated as autoimmune, or not, before data analysis for this study was initiated.

The patients autoimmune screen involves an investigation for autoimmune thyroid disease (21) (thyroid stimulating hormone, thyroid peroxidase, and thyroglobulin antibodies), an antinuclear antibody panel (18), a limited antiphospholipid antibody panel, consistent of antibodies to cardiolipin, phosphatidylserine and beta-2-glycoprotein in IgG, IgM, and IgA isotypes, as previously reported (22), and the investigation of nonspecific antiovian and antiadrenal autoantibodies, as previously described (23). All laboratory investigations were performed by commercial assays (Quest Diagnostics, Lyndhurst, NJ and LabCorp, Burlington, NC).

Detailed patient characteristics have previously been reported (16). A patient was considered to demonstrate evidence of abnormal autoimmune function if at least one immunologic laboratory test was reported as positive. Table 1 summarizes the laboratory findings in women characterized as autoimmune patients. Individual laboratory parameters were available for 30 to 39 patients.

CGG triple repeats also were recorded, as previously described (16). In brief, the allele with the lower triple repeat number was designated allele-1, and the one with the higher number as allele-2. We then analyzed mean triple repeat numbers for the higher count allele (allele-2) and the number of women in each group with ≥30 triple repeats on allele-2.

To avoid problems of abnormal distribution, rank values between individual patient groups were compared, using nonparametric testing (Mann-Whitney test). The prevalence of ≥30 repeats was compared with a two-sided chi-square test. Statistical analyses were performed, using SPSS for Windows, standard version 15.0. Continuous variables are presented as mean ± 1 SD.

All patients at our center sign at initial consultation an informed consent, allowing for the retroactive review of their medical records for clinical research purposes, as long as their anonymity is maintained. Such retroactive reviews of medical records, therefore, are exempt from review by the center’s institutional review board. A confirmatory letter from the Institutional Review Board chairman is available upon request.

RESULTS

Table 2 summarizes patient characteristics. Twenty-two of 40 patients (55%) demonstrated positive autoimmune findings and 18 (45%) did not. Women with autoimmune abnormalities did not differ in age (35.5 ± 4.0 years) from those without (mean 35.0 ± 6.4 years).

Women with autoimmune abnormalities, however, significantly differed in FSH and AMH levels, as well as triple repeat counts. Specifically, autoimmune patients demonstrated lower FSH levels (18.4 ± 36.1 vs. 33.4 ± 37.0 mIU/mL; \( P = .009 \)) and higher AMH levels (1.4 ± 0.8 vs. 1.0 ± 1.8 ng/mL; \( P = .023 \)). Although, as expected, allele-1 did

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**TABLE 1**

<table>
<thead>
<tr>
<th>Autoimmune findings</th>
<th>Number of positives(^a)</th>
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<tr>
<td>Antithyroid antibodies</td>
<td>6</td>
</tr>
<tr>
<td>Antiphospholipid antibodies</td>
<td>9</td>
</tr>
<tr>
<td>Antiovarian antibodies</td>
<td>4</td>
</tr>
<tr>
<td>Gammopathy</td>
<td>3</td>
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<tr>
<td>Autoimmune skin condition(^b)</td>
<td>1</td>
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\(^a\) One patient demonstrated more than one finding.

\(^b\) Psoriasis.

not differ between the two groups in number of triple repeats (25.6 ± 4.8 vs. 27.6 ± 4.2; P = .1), allele-2 showed significantly lower triple repeat numbers in autoimmune patients (29.1 ± 3.3 vs. 37.0 ± 15.0; P = .005). They also demonstrated significantly fewer triple repeats ≥ 30 (P = .017).

DISCUSSION

This study, once more, confirms the very high prevalence of autoimmune abnormalities in infertile women, who reach treatment. Although in the range of previously reported findings of up to 60% (6, 7), it most likely is a reflection of our center’s routine pursuit of autoimmune investigations, in attempts to prevent pregnancy loss after successful infertility treatments (10).

The most remarkable finding of this study, however, is the recognition that evidence of abnormal autoimmune function rather categorically separates infertile women with evidence of premature ovarian senescence into two distinct groups: women with autoimmune abnormalities demonstrate relative milder ovarian senescence than infertile women without autoimmune, characterized by lower (although still abnormally high) FSH levels and higher (although still abnormally low) AMH levels. Women with abnormal autoimmune function also demonstrate significantly fewer triple CGG repeats, and repeats ≥ 30, on their respective FMR1 genes and, indeed, demonstrate a mean range of triple repeats, previously identified as normal in regards to ovarian reserve (16).

These observations deserve further exploration: higher triple repeats have, of course, been associated with increased risk toward different degrees of premature ovarian senescence, including POA and POF (4, 15, 24, 25). The higher mean number, and more frequent repeats ≥ 30, in women without autoimmune abnormalities therefore suggest that, whatever risk toward premature ovarian senescence exists in these patients, it with considerable likelihood relates to the previously reported statistical association between increases in triple repeats (≥ 30) and premature ovarian senescence (16).

The opposite conclusion, however, has to be reached in women with abnormal autoimmune function (3, 5). Their statistically lower triple repeat numbers on the FMR1 gene suggest that their risk toward ovarian senescence is, most likely, independent of this genetic mutation and, therefore, not causally related to the FMR1 gene. By implication, this suggests that abnormal autoimmune function, per se, denotes risk toward premature ovarian senescence.

Abnormal autoimmune function and expansions ≥ 30 CGG triple repeats, thus, seem to reflect independent risks toward premature ovarian senescence. How the fact that both of these etiologies adversely affect ovarian function and, independently, lead toward POA remains to be determined. In association with POF, it has been suggested that a classical autoimmune reaction against ovarian antigens may be involved (3). It has also been suggested that crossreactive epitopes with other endocrine organs, the most frequently involved being thyroid, may be responsible for the abnormal immune response (3). Indeed, 10 patients demonstrated either antithyroid and/or antiovary antibodies (Table 1).

How FMR1 gene expansions may affect ovarian function is even more intriguing. Although the production of the gene’s product is interrupted in cases of full mutation (>200 triple repeats), milder expansions in intermediate and premutation ranges (45–200 triple repeats) are characterized by excessive transcription (i.e., elevated mRNA) of the gene product, the fragile X mental retardation protein (4). In premutations, such excessive transcription has been demonstrated to lead, primarily in males, to late age onset fragile X-associated tremor/ataxia syndrome (FXTAS). Women only rarely develop FXTAS, but experience a high prevalence of POF instead (4). As suggested by Sullivan et al. (26), premature

<table>
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<th>Table 2: Patient characteristics.</th>
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<tr>
<td><strong>Autoimmunity n = 22</strong></td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>FSH (mIU/mL)a</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
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<tr>
<td><strong>Triple repeats</strong>&lt;br&gt;Allele-1</td>
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<tr>
<td><strong>Allele-2b</strong></td>
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*Note:* NS, not significant.

a Large standard deviations are a consequence of very high b-FSH levels in POF patients.

b The mean range of triple CGG repeats in women with autoimmunity is in the 29–30 range, reported as normal for a large majority of patients, whereas the mean range in nonautoimmune patients is above the cut off of ≥ 30 triple CGG repeats, reported to denote risk for premature aging of the ovaries. For further detail, see text.

ovarian senescence, like FXTAS, may therefore, represent a late age onset condition, caused by excessive transcription of \( FMR1 \) gene product.

The fragile X mental retardation protein has so far not been well investigated in its effect on ovarian function. Our previously reported observation that the risk of premature ovarian senescence increases with increasing \( CGG \) expansion numbers (i.e., potentially with increasing transcription of the gene product), suggests that Sullivan’s suggestion may be correct, and that this protein may play an important role in premature ovarian senescence. Indeed, one could further speculate that it also may play a role in the still poorly understood normal, physiologic ovarian aging process (16). The protein has been better investigated in the mature testis and is highly expressed in spermatogonia, although not in Sertoli cells. It also appears to support germ cell proliferation in the testis (27).

A potential weakness of this study lies in the currently still rather diffuse definition of what constitutes abnormal autoimmune function, especially at subclinical levels (10). This weakness is, however, practically universal to the definition of all subclinical or preclinical autoimmune conditions. For example, up to 40% of first-degree relatives of patients with autoimmune diseases express subclinical autoimmune abnormalities, and will in a large majority never express clinical disease (28). Women demonstrate significantly more subclinical (and clinical) autoimmune than males, and the reasons for this difference have remained controversial (8). We already noted the reported high prevalence of such abnormalities in infertile females (6, 7).

Considering the lack of specific markers for “abnormal autoimmune function,” and the relatively small number of investigated patients, it is rather remarkable how statistically obvious the designation of patients as autoimmune, or not, separated out two distinct patient populations with entirely different genotype (number of triple repeats on the \( FMR1 \) gene) and phenotype (FSH and AMH levels). In view of the small study size, the data reported here should, however, nevertheless still be considered as preliminary, and await further confirmation.

If confirmed, these findings would further validate autoimmune abnormalities as a potential independent cause for female reproductive failure, including infertility, because premature ovarian senescence is, of course, even at relatively mild levels, a potential cause of female infertility. We have previously pointed out that abnormal autoimmune function is frequently overlooked, and definitely underappreciated as a cause of female infertility (9, 10). At the same time, these observations, however, also validate the predictive value of triple \( CGG \) repeats on the \( FMR1 \) gene in forecasting ovarian function (16). If the number of triple repeats can statistically so well discriminate between women affected and not affected by autoimmunity, its specificity as a predictor of female reproductive potential appears promising.

In combination, therefore, one can speculate that abnormal autoimmune function, and excessive \( CGG \) triple repeats on the \( FMR1 \) gene, independently, can lead to premature ovarian senescence. Considering the reported approximately 23% prevalence of excessive triple repeats (\( \geq 30 \)) in an infertile population with evidence of premature ovarian aging (16), and the 55% prevalence of abnormal autoimmune observed here, corresponding to reports in the literature (6, 7), our preliminary data would suggest that, in combination, autoimmune abnormalities and number of triple repeats on the \( FMR1 \) gene should be responsible for a majority of cases of premature ovarian senescence. Clinically this means that, if confirmed, women with evidence of abnormal autoimmune function, like women with \( \geq 30 \) \( CGG \) expansions on the \( FMR1 \) gene, are at risk for premature ovarian senescence and, therefore, should be prospectively followed for early clinical evidence in confirmation of such a diagnosis.

In contrast to widely expressed sentiment, and even authoritative policy statements (29), the investigation of subclinical autoimmune abnormalities in young women, and especially in women with established infertility, therefore, would appear indicated after all. Any improvement in early diagnosis of insipient premature ovarian senescence will also decrease the diagnosis of so-called unexplained infertility (30) and accelerate appropriate clinical interventions. Further studies to establish predictive values of autoimmune testing, and assessment of triple repeats on the \( FMR1 \) gene, therefore, appear urgently indicated.

If confirmed by further studies, other and wider clinical applications can also be foreseen: one, for example, can expect differences in clinical responses to treatments, leading to the introduction of phenotype- and genotypes-specific fertility therapies of premature ovarian senescence. Based on the very obvious differences in FSH and AMH levels between the two groups of patients described here, it would not be surprising to see differences in response to ovarian stimulation and/or different stimulation protocols.

Fertility profiling, based on triple repeat numbers and autoimmune abnormalities, however, may not only benefit women with already established infertility. The ability to potentially predict premature decreases in ovarian function (and therefore female infertility) may facilitate early diagnosis of subfertility, which in turn, would allow women to adjust reproductive planning and/or to take fertility preserving steps, like oocyte, ovarian, or embryo cryopreservation (4). A fertility profile, consistent of autoimmune testing and assessment of triple \( CGG \) repeats on the \( FMR1 \) gene, could thus become a universal screening test for the fertility potential of young women.

REFERENCES


