The surplus of males among patients with mental retardation was noted over a century ago, so it was not surprising when an X-linked gene for mental retardation was identified. However, research on this gene, FMR1 (fragile X mental retardation 1), has yielded several surprises since then. The first was a novel mutational mechanism associated with human disease: trinucleotide-repeat instability and expansion. In the case of FMR1, a CGG repeat tract exists in the 5′ untranslated region. In the general population, the number of repeats ranges from about 5 to 50 and does not change when transmitted through the generations. Repeat tracts with 50 to 200 repeats are unstable during transmission and are referred to as premutation alleles in reference to their general lack of associated phenotype and their propensity to expand to disease-causing lengths in offspring. Alleles with greater than 200 CGG repeats, full mutations, are associated with hypermethylation of the CGG tract and surrounding CpG island, resulting in transcriptional silencing; the consequent lack of the protein product, FMRP, causes mental retardation and macroorchidism, the hallmarks of fragile X syndrome.

The mental retardation associated with fragile X syndrome ranges from mild to severe. In addition to macroorchidism after puberty, patients present with subtle but characteristic somatic signs, such as prominent ears. Many patients display autistic-like features such as repetitive motor mannerisms, impaired verbal communication, and gaze aversion; however, they do not typically meet the full diagnostic criteria for autism. Social anxiety and shyness are also common.

Upon the cloning of the FMR1 gene, two key observations were made: that premutation alleles appeared to express the same amount of FMRP protein as did normal alleles and that premutation carriers were unaffected. However, a decade of data has indicated that premutation carriers have increased incidences of several fragile X–like phenotypes, including social anxiety, affective disorder, obsessive-compulsive disorder, and prominent ears. In
addition, premutation carrier females have an increased incidence of ovarian dysfunction. This feature is unique, however, as it is not apparent in females with full mutations. More recently, two molecular phenotypes of premutation cells have been identified, which may account for these phenotypes: increased \textit{FMR1} transcription and decreased FMRP protein levels.

**OVARIAN DYSFUNCTION**

The first observed sign of ovarian dysfunction related to fragile X syndrome appeared in Fryns’ characterization of obligate carrier females in Belgium in 1986. Of the 642 offspring of 134 carriers, there were 18 pairs of twins: 12 dizygotic and 6 of unknown zygosity. This twinning rate (1 in 35 births) is higher than the twinning rate reported for the general population (1 in 40 to 1 in 110 births). The following year, in a fragile X conference in Denver, it was anecdotally noted that several of the attending female carriers of fragile X mutations had experienced premature ovarian failure (POF). As a consequence of these two observations, and given \textit{FMR1}’s cytological location at Xq27.3, a region implicated in ovarian function, many researchers undertook to examine the possible relationship between fragile X mutations and ovarian dysfunction. The early studies did not distinguish between premutation and full mutation carriers, as our understanding of the molecular nature of the gene did not come until its cloning. These studies found that 13–28% of obligate fragile X carriers experienced POF, significantly higher than in the control group, with the entire distribution of age at menopause shifted toward a younger age in fragile X carrier women. Likewise, one study confirmed Fryns’ observation of increased twinning by reporting an increased rate of twin births from obligate fragile X carrier mothers (1 in 18 births). However, other studies were unable to detect a difference in twinning rate among women with fragile X mutations compared with controls.

Later studies, using molecular techniques to distinguish premutation carriers from full mutation carriers, indicated that the increased risks of POF and twinning are limited to premutation carriers, providing one possible explanation for differing results if the study groups varied in makeup of premutation and full mutation carriers. POF was experienced by 14 to 24% of premutation-allele carriers, whereas only 3 of 75 full mutation carriers experienced POF in the three studies combined. Likewise, the frequency of premutation carriers among women with idiopathic familial POF (6 to 13%) is higher than that for the general population (1 in 250). As expected, the two aspects of ovarian dysfunction (twinning and POF) are sometimes found in the same individual. Also, the premutation allele cosegregates with POF in families. Tables 1 and 2 summarize the reports of twin birth rates and POF prevalence, respectively, among fragile X carriers and controls. Table 3 summarizes reports of the frequency of premutation carriers among women with POF and controls.

Although these data provide convincing evidence of an association between ovarian dysfunction and premutation alleles, the association is far from complete. Many fragile X families do not have members with POF or twinning, and some population-based studies have failed to detect the association. Likewise, increased twinning was not observed in two studies of premutation carriers. Various mechanisms have been invoked to explain the discrepancy in results, including linkage disequilibrium, population differences, and imprinting mechanisms. Evidence has been presented that premutations inherited from paternal sources are associated with POF, whereas maternally inherited premutations are not. Although this study group was large, other groups failed to find similar phenomena, and the implied imprinting mechanism is at odds with many of the published pedigrees of fragile X families with POF. Again, population differences and ascertainment issues are likely to be key in resolving this issue.

<table>
<thead>
<tr>
<th>Population</th>
<th>Carriers</th>
<th>Premutations</th>
<th>Full Mutations</th>
<th>Noncarriers</th>
<th>Controls</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>17/735</td>
<td>1/34</td>
<td>1/139</td>
<td>1/140</td>
<td>8/589</td>
<td>45</td>
</tr>
<tr>
<td>Australia</td>
<td>1/34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>102</td>
</tr>
<tr>
<td>Belgium</td>
<td>19/624</td>
<td>0/40</td>
<td></td>
<td></td>
<td>1/176</td>
<td>103</td>
</tr>
<tr>
<td>Brazil</td>
<td>7/220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49</td>
</tr>
<tr>
<td>Italy</td>
<td>12/201</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Netherlands</td>
<td>3/116</td>
<td></td>
<td></td>
<td>14/205</td>
<td></td>
<td>46</td>
</tr>
<tr>
<td>United States</td>
<td>3/231</td>
<td></td>
<td></td>
<td>2/101</td>
<td></td>
<td>63</td>
</tr>
</tbody>
</table>

*Molecular status undetermined.*
Despite two case reports of precocious puberty in fragile X females,\textsuperscript{68,69} larger studies indicate that the age of menarche is normal in both premutation and full mutation carrier females.\textsuperscript{48,70}

**ENDOCRINOLOGY**

Data on endocrinology function in premutation carriers is limited. In vitro fertilization clinics report that premutation carrier clients have increased follicle-stimulating hormone (FSH) levels despite their young age and have a subnormal response to exogenous ovarian stimulation.\textsuperscript{71} Successful pregnancies have nonetheless been achieved, although donor eggs are sometimes required.\textsuperscript{72}

To date, only three other studies have examined the endocrinology of premutation carriers. In the first, endocrine serum concentrations were analyzed in nine randomly chosen premutation carriers from 31 to 40 years of age. Only one had normal menstrual cycles and a normal hormone profile. Two women had elevated FSH levels on day 1 of their cycle. Two had elevated progesterone levels at this time but had a surge of luteinizing hormone (LH) at day 14, consistent with a shortened cycle. Two other women also showed a shortened follicular phase of the cycle, as their hormone levels were postovulatory at day 14. And finally, one woman had no LH surge, suggesting that the cycle was anovulatory.\textsuperscript{73}

In the second endocrine study of fragile X carriers, FSH and estradiol serum levels were measured in 19 premutation carriers, 9 full mutation carriers, and 23 noncarriers. Participants ranged from age 16 to 53 years, although only three premutation carriers were under the age of 30. No differences were detected between full mutation carriers and noncarriers. However, FSH levels were elevated in premutation carriers compared with the levels in the other two groups.\textsuperscript{74} The FSH levels do not appear to be increased in premutation carriers younger than 30 years, although the small number of women in this group precludes any conclusions. Finally, levels of inhibin, an indicator of ovarian reserve, were not altered in premutation carriers, although the same subjects displayed earlier ages of menopause than did controls.\textsuperscript{46}

More endocrinology work has been reported on males with fragile X syndrome. Alterations in endocrine regulation associated with full mutations in males are widely reported but the results vary greatly with no consistent pattern of endocrine changes.\textsuperscript{75–85} Thus, it is not clear if the irregularities seen in premutation females are unique to premutations or are part of a spectrum of the endocrine problems seen in full mutation males.

**MODELS**

Several models have been proposed to explain the role of \textit{FMR1} in ovarian function. Fryns, in his initial 1986 report, speculated that fragile X carriers suffer from a breakdown in the regulation of the cortico-hypothalamic-pituitary axis. An alteration in the feedback mechanism between the ovaries and the brain could result in the higher FSH levels observed in fragile X carriers, thus resulting in increased twinning and faster depletion

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Prevalence of POF Among Fragile X Carrier and Noncarrier Females over the Age of 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Carriers</td>
</tr>
<tr>
<td>Italy</td>
<td>6/42</td>
</tr>
<tr>
<td>North America</td>
<td>10/47</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>30/106</td>
</tr>
<tr>
<td>United States</td>
<td>8/61</td>
</tr>
<tr>
<td>Worldwide</td>
<td>43/182</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Prevalence of Fragile X Premutation Carriers Among Females with POF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>POF</td>
</tr>
<tr>
<td>Australia</td>
<td>0/21*</td>
</tr>
<tr>
<td>Greece</td>
<td>4/33</td>
</tr>
<tr>
<td>Italy</td>
<td>7/108</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>4/50</td>
</tr>
<tr>
<td>United States</td>
<td>0/17</td>
</tr>
</tbody>
</table>

*POF cases who were also mothers of twins.
of the oocyte pool. In support of this model, FMRP is expressed in the cortex and the thalamus, both of which have significant neural inputs into the hypothalamus. More studies of fragile X carriers over a wider range of ages to determine when the altered FSH levels first appear may lend clues to whether the increased FSH levels are a cause or a result of early ovarian depletion. To date, only three premutation carriers under the age of 30 years have been examined, and although they did not have the increased FSH levels seen in older premutation carriers, this number is too small to draw any conclusions.

Other models focus on the potential role of FMRP in the ovary itself. FMRP is expressed in germ cell development, in both primordial germ cells in the fetus, and in the granulosa cells of developing follicles in adults. Decreased expression of FMRP from premutation alleles during germ cell proliferation may adversely affect the number of oocytes produced, thus reducing the available pool in adults. Alternatively, the presence of the premutation tract itself may have some adverse effects on germ cells, perhaps destabilizing meiotic arrest or increasing the attrition rate, again causing a more rapid depletion of the germ cell pool. Finally, a model involving a selection against germ cells in which the premutation has expanded to full mutations, also reducing the germ cell pool, has been proposed.

A selection model is supported by an unusual inheritance pattern of fragile X syndrome. Affected patients always inherit the expanded repeat from their mothers and premutation-carrying males always pass on premutation alleles to their children. Furthermore, affected full mutation males produce sperm with only premutation alleles, and although full mutation germ cells are present in developing male fetuses, these are gradually replaced with premutation-bearing germ cells. This could be due to a proliferation advantage of FMRP-producing cells or to a selection against male germ cells with large trinucleotide repeat expansions. The latter model is supported by a similar phenomenon of maternal bias for large expansions in two other trinucleotide repeat disorders, myotonic dystrophy and Friedreich’s ataxia. Proliferation of germ cells with large repeat tracts may be influenced by the presence of an altered chromatin conformation associated with full mutation alleles. Myotonic dystrophy and Friedreich’s ataxia differ from fragile X syndrome in that larger paternal expansions (up to 2000 repeats) are observed in the two former cases, while the FMR1 repeat does not appear to be greater than around 120 repeats in sperm, which does not even approach the maximum premutation length, suggesting that a selection for FMRP-producing germ cells is an additional factor in fragile X patients.

The apparent lack of a similar phenomenon in females may be due to the X-linked nature of FMR1. In females, selection against germ cells with an expanded full mutation allele on the active X chromosome may reduce the germ cell pool, and expansion on the inactive X chromosome may allow passage of full mutation alleles to offspring. As the probability of expansion to full mutation range is directly correlated with the number of repeats, this model would predict a negative correlation between premutation repeat size and age at menopause. There is no evidence of such a correlation, but this relationship may be obscured by other genetic and environmental factors affecting age at menopause. Furthermore, the timing of expansion from premutation to full mutation range during oogenesis has not been elucidated; consequently, it is not possible to draw conclusions about the impact of premutation and full mutation alleles in germ cells.

More problematic is the apparent lack of association between full mutation alleles and ovarian dysfunction. One possible explanation is that X inactivation selects for inactivation of full mutation alleles, thereby silencing the effect of the lack of FMRP on germ cells that is evident in males with no such compensatory mechanism. Premutation-expressing oocytes, on the other hand, may have enough FMRP to escape this silencing but not enough for completely normal function at a later stage in development. Another mechanism is suggested by the observations that premutation cells produce more FMR1 messenger RNA (mRNA) than do normal cells (A. Kenneson et al, submitted). As a consequence, increased FMR1 mRNA is a molecular phenotype that distinguishes premutation alleles from both full mutations, which are not transcribed, and normal alleles. The enhanced transcription of FMR1, and consequent high level of FMR1 message, may act as a sink for transcription factors, translation factors, or CGG-binding proteins, with an ultimately detrimental effect on germ cells.

The cosegregation of POF and premutations in some families and the lack of POF in other fragile X families suggest that ovarian function is adversely affected by only a subset of premutation alleles. This model could partially account for the discrepant results of the various association studies between POF and fragile X mutations as the various study groups may differ in the proportion of alleles that are associated with POF. Differences between the POF and non-POF premutation alleles could be due to linkage disequilibrium with a nearby POF-causing mutation or to variations in the structure of the repeat itself, such as AGG interruption pattern or length of pure CGG tracts. Such changes may be responsible for subtle, but critical, changes in FMRP level. Other confounding factors could include modifying genes, perhaps also affecting FMRP levels, as well as the various genetic and environmental factors known to affect age at menopause. Whatever the mechanism of the association between
and ovarian function, the challenges raised by the differing results in various populations reflect the complexity at work in this particular genotype-phenotype relationship.

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