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The FMR1 premutation and reproduction

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Objective: To update clinicians on the reproductive implications of premutations in *FMR1* (fragile X mental retardation 1). Fragile X syndrome, a cause of mental retardation and autism, is due to a full mutation (>200 CGG repeats). Initially, individuals who carried the premutation (defined as more than 55 but less than 200 CGG repeats) were not considered at risk for any clinical disorders. It is now recognized that this was incorrect, specifically with respect to female reproduction.

Design and Setting: Literature review and consensus building at two multidisciplinary scientific workshops.

Conclusion(s): Convincing evidence now relates the *FMR1* premutation to altered ovarian function and loss of fertility. An *FMR1* mRNA gain-of-function toxicity may underlie this altered ovarian function. There are major gaps in knowledge regarding the natural history of the altered ovarian function in women who carry the *FMR1* premutation, making counseling about reproductive plans a challenge. Women with premature ovarian failure are at increased risk of having an *FMR1* premutation and should be informed of the availability of fragile X testing. Specialists in reproductive medicine can provide a supportive environment in which to explain the implications of *FMR1* premutation testing, facilitate access to testing, and make appropriate referral to genetic counselors. (Fertil Steril® 2007;87:456–65. ©2007 by American Society for Reproductive Medicine.)

Key Words: Fragile X syndrome, FMR1, premutation, spontaneous premature ovarian failure, hypergonadotropic hypogonadism, primary hypogonadism, primary ovarian insufficiency, premature menopause, hypergonadotropic amenorrhea, low response to gonadotropin stimulation, diminished ovarian reserve, fragile X-associated tremor/ataxia syndrome, FXTAS, genetic counseling

Remarkable progress has been made to identify a constellation of clinically significant disorders associated with a dynamic triple repeat sequence mutation in the X-linked gene known as *FMR1* (fragile X mental retardation 1) (1–4). The fully expanded form of the mutation leads to fragile X syndrome, the most common cause of inherited mental re-

tardation as well as the most common known genetic cause of autism (5).

The form of the mutation that precedes the full mutation (i.e., the premutation) leads to two disorders that are distinct from fragile X syndrome. The first is an adult onset neurologic disorder now referred to as fragile X-associated tremor/ataxia syndrome, or FXTAS (6–17). FXTAS primarily affects males, consistent with an X-linked recessive disorder. The second disorder, the focus of this report, is premature ovarian failure, which affects approximately 15% of women who carry the premutation (18–21). In aggregate, these disorders have far-reaching adverse health implications for individuals and families identified through fragile X syndrome, premature ovarian failure, and/or the fragile X-associated tremor/ataxia syndrome.

Received July 5, 2006; revised and accepted August 4, 2006.

Supported by the American Society for Reproductive Medicine; the Intramural Research Program of the National Institute of Child Health and Human Development, Office of Rare Diseases, Office of Research on Women's Health, and the National Institute of Mental Health, National Institutes of Health; and by Centers for Disease Control and Prevention grant (U10/CCU 92513).

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These premutation-associated disorders have only recently been characterized and, thus, the overall expanded clinical phenotype of the *FMR1* mutations urgently requires focused research and the development of effective management strategies. With this in mind, on April 13, 2005, the National Institute of Child Health and Human Development (NICHD) convened a 3-day meeting entitled, “Workshop on Reproduction and the Fragile X Premutation.” The workshop was held at the William F. Bolger Center for Leadership and Development in Potomac, Maryland. The purpose was to [1] examine the basic science, clinical, and epidemiologic evidence regarding the fragile X premutation and its effects on reproduction, and [2] prepare recommendations outlining what might be done to move the related research agenda forward.

In addition, the Centers for Disease Control and Prevention (CDC) funded a focus group of experts regarding fragile X and reproductive endocrinology to meet in Atlanta, Georgia, on February 12 and 13, 2006. The purpose was to develop recommendations regarding [1] which patients in the practices of obstetricians, gynecologists, and reproductive endocrinologists should be screened for the *FMR1* premutation, and [2] what is the clinician’s role in genetic counseling and cascade testing of families in which the fragile X mutation is identified.

Premature ovarian failure is a condition in which women develop loss of regular menstrual cycles, infertility, and ovarian hormone deficiency not normally observed until the age of menopause (22, 23). In approximately 90% of cases, no mechanism can be identified to explain the ovarian insufficiency. Through their presentations and discussion, the workshop participants provided compelling reasons to investigate the effects of the *FMR1* premutation on ovarian function. The effort should provide a portal to broader insights regarding ovarian function and pathophysiology. Furthermore, the convergence of disorders relating to a single underlying gene defect will provide synergy and serve as a paradigm for future investigations related to reproductive genetics.

FULL MUTATION AND FRAGILE X SYNDROME

The fragile X syndrome was shown to segregate in affected individuals who have a characteristic chromosomal fragility at Xq27.3, hence the name fragile X syndrome (24). Although inherited in an X-linked dominant pattern, the pattern differs from the usual properties of X-linked inheritance in some aspects. For example, a small proportion of males who were known to carry the mutation based on pedigree analysis had no cognitive disabilities. Also, the risk for fragile X-related mental retardation appeared to increase with each generation, a phenomenon known as anticipation.

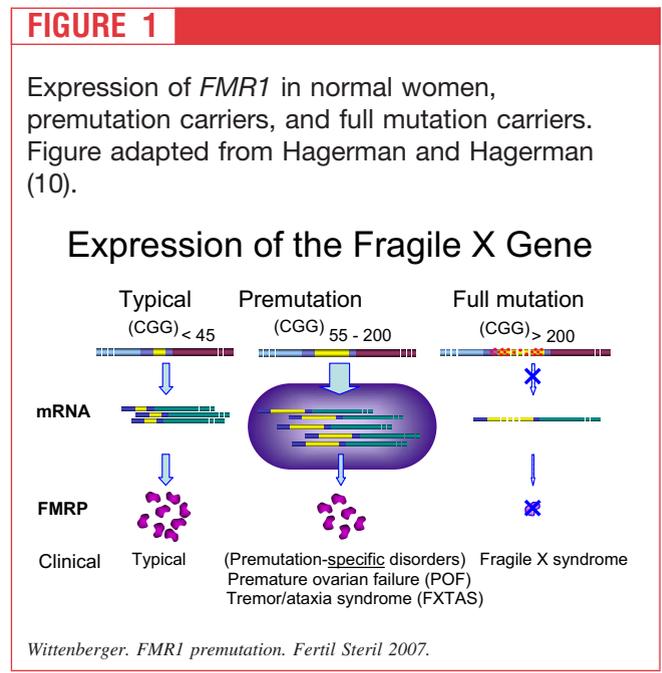
In 1991, the gene for fragile X syndrome was identified and the mutation was found to be due to an expanded sequence of CGGs, which was hypermethylated in affected

individuals (25, 26). The gene was named fragile X mental retardation 1 (*FMR1*) (26). Patients with mental retardation had more than 200 CGG repeats located in the 5’ untranslated region of the gene near the promoter region containing a CpG island. The hypermethylated expanded CGG repeat that extended to the promoter region was termed the full mutation.

The consequence of this hypermethylation associated with the full mutation is silencing of *FMR1*—little or no mRNA is produced and, hence, the corresponding gene product, the fragile X mental retardation protein (FMRP), is deficient or absent leading to the features of fragile X syndrome (Fig. 1) (27). Considerable research has determined that FMRP is an RNA binding protein that regulates translation of a unique subset of messages. The protein shuttles between the nuclear and cytoplasmic compartments and associates with translating polyribosomes (28–31).

Recent evidence suggests that one role of FMRP is to act as a translational suppressor. Consistent with a syndrome whose main feature is mental retardation, the *FMR1* product is highly expressed in the brain (26). FMRP binds with approximately 4% of mRNA in mammalian brains and appears to suppress translation of those messages, especially in dendrites (32). Therefore, lack of FMRP may result in over-expression of multiple mRNAs that in turn may lead to the characteristic fragile X syndrome phenotype.

With these important findings, direct DNA diagnosis became possible allowing accurate determination of repeat number and the methylation status of those repeats. The incidence of fragile X syndrome is approximately 1 in 4,000 males and 1 in 4,000 to 8,000 females (33–38). Female full mutation carriers are thought to be relatively protected from



the full effects of fragile X syndrome due to X-chromosome inactivation. However, approximately 70% of females with the full mutation have a borderline IQ or lower, and those with a normal IQ often have executive function deficits (39–41). Large population studies are needed to obtain more accurate estimates of the frequency of the carrier state and fragile X syndrome.

The American College of Medical Genetics (42) and the American College of Obstetricians and Gynecologists (38) recommend *FMR1* screening in the prenatal setting by amniocentesis or chorionic villus sampling only if specific family history indicators exist, such as fragile X syndrome or mental retardation of unknown cause, and they recommend testing the fetus of a mother known to be a carrier. The recommendations also urge consideration of *FMR1* testing in women with premature ovarian failure or elevated FSH levels before age 40.

PREMUTATION

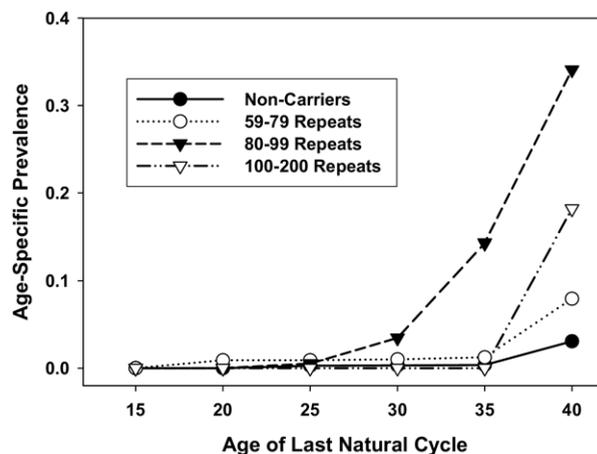
Once the full mutation was established as the molecular mechanism of fragile X syndrome, the alleles carried by unaffected obligate carriers as determined by pedigree analysis of families with fragile X syndrome were characterized and found to contain smaller expansions of approximately 55 to 200 CGG repeats. These smaller expansions, termed the premutation, were unmethylated. Moreover, *FMR1* functioned as evidenced by intact FMRP protein production. The term premutation was applied to the range of 55–200 repeats for two reasons: [1] carriers of premutations did not have fragile X-related mental retardation and [2] these alleles were at risk for expansion from this “pre” mutation form to the “full” mutation. Those who were noncarriers were found to carry even fewer repeats, the most common size being 29–30 repeats in the normal population (43).

PREMUTATION AND NEURODEGENERATION

It is now clear that distinct phenotypes can be associated with the *FMR1* premutation and that this situation should no longer be regarded as purely an unaffected carrier state. A progressive neurodegenerative disorder has been identified in male premutation carriers over the age of 50 (7). This disorder, termed the fragile-X associated tremor/ataxia syndrome (FXTAS), demonstrates both clinical and radiologic features. Patients may present with progressive intention tremor, ataxia resulting in frequent falls, autonomic dysfunction, parkinsonian features (masked facies, intermittent resting tremor, and increased tone or response to L-DOPA), cognitive deficits (memory problems and executive function deficits), psychological features (anxiety, mood lability, outburst or reclusive behavior), and peripheral neuropathy with decreased sensation in the lower extremities (11). Characteristic radiologic findings noted on magnetic resonance imaging (MRI) include global brain atrophy and deep cerebellar white matter hyperintensities—the middle cerebellar peduncle sign (8). Interestingly, on postmortem examination,

FIGURE 2

Age-specific prevalence of premature ovarian failure by CGG repeat length. Based on a data set of 429 *FMR1* premutation carriers and 517 non-carriers.



Wittenberger. *FMR1* premutation. *Fertil Steril* 2007.

pathognomonic eosinophilic inclusion bodies are present in cortical neurons and astrocytes (9, 17). In males with the premutation, the risk of developing FXTAS increases with age (13). Although FXTAS occurs in women with the premutation, it does so infrequently; women appear to be relatively spared from this disorder (13, 16). However, there is evidence that an unfavorable X-chromosome inactivation increases the risk of FXTAS in women (44, 45).

PREMUTATION AND PREMATURE OVARIAN FAILURE

While women with the premutation have a low risk of developing FXTAS, premature ovarian failure is a relatively common finding. The prevalence of premature ovarian failure in women who carry the *FMR1* premutation is estimated to be between 13% and 26% (18, 21, 46–48). Premutation carriers have been identified in 0.8% to 7.5% of women with sporadic premature ovarian failure and in up to 13% of women with familial premature ovarian failure (49–52).

Some of the variation in the estimates of penetrance is probably due, in part, to the increasing probability of premature ovarian failure with increasing number of CGG repeats. Surprisingly, however, this relationship is nonlinear. Indeed, the risk appears to increase with increasing premutation repeat size between 59 and 99, thereafter the risk of premature ovarian failure plateaus or even decreases for women with repeat sizes over 100 (Fig. 2) (21, 53).

Preliminary evidence suggests that there may also be an increased risk of premature ovarian failure among women who carry intermediate-size alleles, those between approximately 41 and 58 repeats (54, 55). However, more data are

needed to confirm these findings. There appears to be no *FMR1* premutation parent of origin effect on the relative risk of premature ovarian failure (21, 56, 57). In contrast with fragile X syndrome and FXTAS, unfavorable X-inactivation does not appear to increase the risk for premature ovarian failure (20, 21).

PREMUTATION AND ALTERED OVARIAN FUNCTION

Premature ovarian failure may be preceded by months to years of altered reproductive function. Consistent with the hypothesis that there is a continuum of impaired ovarian function in women with the *FMR1* premutation, premutation carriers have significantly elevated FSH levels compared with controls, particularly during their thirties (21, 58–60). Welt et al. reported that women carrying the *FMR1* premutation had significantly elevated serum FSH levels across the early, mid, and late follicular phase of the menstrual cycle (21.9 ± 3.5 vs. 11.2 ± 0.5 IU/L, $P < .001$) (60). Furthermore, the group found other serum markers evidencing impaired ovarian function in these women (inhibin B, inhibin A, progesterone), consistent with both impaired follicular and luteal function (60). Additionally, in two separate studies using survival analysis, premutation carriers entered menopause approximately 5 years earlier than non-carrier women (20, 21).

Currently, the mechanism of the impaired ovarian function related to the *FMR1* premutation is unclear. Attractive possibilities include decreased number of ovarian follicles in the initial pool, an accelerated rate of atresia of follicles, or some other mechanism impairing follicle function. All these mechanisms are consistent with the hypothesis that a continuum of impaired ovarian function exists in women with the *FMR1* premutation.

INHERITANCE OF THE FRAGILE X MUTATION

The fragile X mutation is located on the X chromosome and therefore follows the basic pattern of X-linked inheritance. That is, women who carry the mutation transmit it to 50% of their offspring. Men who carry the mutation transmit it to all of their daughters and to none of their sons. Layered onto this pattern is the complication of the meiotic instability of the repeat sequence. As the mutation is passed from mother to offspring, it has the tendency to expand in size. The risk to expand from the premutation to the full mutation is dependent on the size of the repeat that is carried by the mother (61, 62). For example, a repeat size of 59–79 expands to the full mutation less than 50% of the time, whereas a repeat size of >90 expands to the full mutation more than 90% of the time (62). This dependency of expansion on parental repeat size explains the noted “anticipation” pattern with respect to the risk of fragile X syndrome.

The smallest repeat length known to expand to a full mutation in one generation is 59 repeats (62). To date, most of the data on premutation expansion rates have been based

on families with fragile X syndrome, that is, premutations known to expand to the full mutation. It is unknown if the risk of expansion varies based on ascertainment.

In contrast, the repeat sequence is transmitted from fathers to daughters in a relatively stable manner. It usually expands by relatively fewer repeats compared with female transmissions and can also contract (63). Intriguingly, the premutation transmitted by fathers only rarely expands to the full mutation. Evidence suggests that large repeat sequences are highly unstable in developing sperm. This explains the observation that only premutation-size alleles are found in sperm of pre- and full mutation males (64).

As mentioned above, the smallest repeat to expand to the full mutation in one generation is approximately 59 repeats. The initial mutation leading to instability is usually not observed in a family with fragile X syndrome as the mutational process occurs over multiple generations. Thus, it has been difficult to determine the lowest size repeat that can be unstable. Other factors in addition to repeat size also play a role in stability, most importantly interruption of the CGG repeat with interspersed AGG sequences (65). Although beyond the scope of this review, such factors make it difficult to define the lower boundary of the premutation, which is most commonly defined as 55 repeats (42).

Alleles from 45 to 54 repeats, comprising the so-called gray zone or intermediate range, may or may not be unstable and may have the chance of expanding to the premutation in several generations (62, 66).

GENETIC COUNSELING FOR *FMR1*

The National Society of Genetic Counselors recently updated their recommendations for health care professionals who provide genetic counseling and risk assessment regarding *FMR1* and fragile X syndrome (63). This is a challenging area to explain to patients and their families because of the complex inheritance involving repeat expansion and the different phenotypes that can be expressed related to the gene. Furthermore, genetic counseling needs may vary depending on how the change in *FMR1* was discovered in the family and the size of the CGG repeat.

These data carry significant reproductive ramifications for women carrying the *FMR1* premutation allele. In addition to concerns female premutation carriers have regarding the risk of having an affected child, they may be unable to conceive. Women with an *FMR1* premutation may feel pressured to have children earlier to minimize this risk. Identification of fragile X mutation carrier status allows women to make informed reproductive decisions. Genetic counseling further provides an opportunity to discuss the diagnosis in terms of risks to other family members. Clearly, learning of carrier status is difficult for a woman and may raise feelings of anxiety, guilt, or altered feelings of self-worth (67–69).

Considerable research has been done to understand the impact of *FMR1* carrier status information on self-concept

(67, 68), coping (69), and attitudes about testing and informing at-risk family members (70, 71). Genetic counseling should thus include assessment of coping behaviors, suggestions for adaptive coping, and resources to deal with the feeling of being “at risk” and then processing the test results when they become available (63). Adaptive coping behaviors have been identified that come into play in response to genetic testing (69, 72–74).

Part of the genetic counseling process includes developing strategies for informing other family members (63). When key family members refuse or are unable to relay information to at-risk relatives, this raises difficult ethical issues. Using a family network approach permits relatives to be informed initially by a family member, whom they know, with follow-up by a genetic counselor (75).

At the appropriate time in the counseling process, reproductive options need to be addressed, including child-free living, adoption, foster care, egg donation, embryo adoption, parenting a child with fragile X syndrome, and prenatal testing. A summary letter by a genetic counselor including contact information can be provided at the time of the disclosure (63). In addition, informational resources for patients and professionals about *FMRI* and fragile X syndrome are available in printed form and online at www.fragileX.org (63).

Most experience in genetic counseling regarding *FMRI* has been derived from families who are seeking a diagnosis for a relative with fragile X syndrome. Few data are available concerning genetic counseling of individuals identified through population screening or because of infertility or neurologic symptoms. These individuals may have little or no prior knowledge of fragile X syndrome. Therefore, women with infertility are in a situation that differs in important respects from women who learn of their carrier status as a result of a family member being identified as having fragile X syndrome. There is a need for prospective study of preconception *FMRI* screening in women seeking evaluation and treatment of infertility.

An important issue in counseling women about *FMRI* testing is how much information should be given to women who are at low risk of a positive result prior to testing, for example, having idiopathic premature ovarian failure and no family history of premature ovarian failure or fragile X syndrome. Should full genetic counseling of *FMRI* be provided in order to prepare the woman for a positive finding? or, is it acceptable to give minimal information about *FMRI* and the implications of the test and defer full genetic counseling until the test is positive? Currently, there are no available data to address this issue.

CLINICAL MANAGEMENT OF THE *FMR1* PREMUTATION

Women with premature ovarian failure who are premutation carriers need to understand that approximately 5%–10% will conceive subsequent to the diagnosis without medical inter-

vention (76). As has been demonstrated in a recent case report, women who have premature ovarian failure related to an *FMRI* premutation are at risk of having a child with fragile X syndrome should they conceive (77). Thus, a diagnosis of premature ovarian failure cannot be considered an absolute barrier to conception.

The discovery of the *FMRI* premutation presents clinicians with many challenges. The risk of premature ovarian failure is increased, but not absolute. Many women who have their family building plans ahead of them may be distressed at the prospect of impaired fertility and will want more information concerning their ovarian function. Currently, it is unclear what, if any, evaluation of ovarian function should be undertaken in an asymptomatic woman who is discovered through screening or cascade testing to carry the premutation. Such women will have to weigh decisions about the timing of their family building. They will need to consider avoiding risk factors that are known to decrease the age at menopause, such as smoking. It should also be recognized that use of hormonal contraception may mask the development of premature ovarian failure.

Research is needed to determine the best course of action to evaluate the risk of altered ovarian function among asymptomatic premutation carriers. No validated tests are of proven predictive value with regard to infertility or premature ovarian failure in the population of women who are asymptomatic. No doubt even after appropriate counseling, many women will want to proceed with some form of evaluation of ovarian function. If so, tests that might plausibly provide an indication of ovarian function could be considered (e.g., serum FSH level, or ovarian follicle count by transvaginal ultrasound). Counseling must be given that the predictive value of such tests is based on general experience in all women, not specifically those with the premutation.

METHODS TO DETECT THE *FMR1* PREMUTATION

Evaluation of carrier status of the fragile X mutation seeks to determine the number of CGG repeats and, if in the full mutation range, to assess the methylation status. Most laboratories use both polymerase chain reaction (PCR) analysis and Southern blot analysis to detect repeat size and to identify possible deletions of *FMRI* (63). To identify *FMRI* allele size(s) and methylation status, two restriction enzymes are used, one of which one does not cut methylated DNA (78). PCR has the advantage of lower cost and can give an accurate repeat size in the normal, intermediate, and premutation ranges. PCR has the disadvantage of not being able to detect longer repeat sizes (42, 63, 79, 80).

When screening specifically for a premutation in *FMRI*, the cost can be significantly reduced by first screening only with the PCR-based test. It has been estimated that approximately 80% of women would be successfully screened with this test alone, whereas 20% would require addition of

Southern blot analysis (80, 81). However, carriers with a mosaic pattern of premutation and full mutation are difficult to identify using just one DNA method (42). PCR testing could identify the premutation but not the full mutation and lead to inappropriate risk assessment. Thus, it has been recommended that in a clinical setting, both PCR and Southern blot analysis should be performed (42).

Four allelic forms of *FMRI* with respect to CGG repeat size have been described. They are referred to as [1] normal or common, [2] “gray zone” or intermediate, [3] premutation, and [4] full mutation (42). Consensus has been reached regarding the premutation size of 55–200 repeats and the full mutation at >200 repeats both in the literature (15, 82) and at the workshop and focus group meetings that form the basis of this report. However, consensus has not been reached for the gray or intermediate zone end-points (i.e., 45–54 repeats or 40–54 repeats). As stated previously, the issue with respect to the intermediate alleles is the inability to assess transmission stability of such alleles in this range (42). These approaches are generally applicable in traditional prenatal genetic diagnosis (amniotic fluid, chorionic villi), although the American College of Obstetricians and Gynecologists recently defined unaffected as <40, intermediate as 41 to 60, and premutation as 61 to 200 repeats (38).

FMRI PREIMPLANTATION GENETIC DIAGNOSIS

Preimplantation genetic diagnosis (PGD) permits the selection of embryos free of the full mutation or premutation. However, there are significant challenges to employing this technique to detect the fragile X mutation. First, family studies must be informative. Ideally, the number of CGG repeats on the normal *FMRI* allele of the mother and father should differ. In the approximately 40% of cases in which the genetic situation is not directly informative, polymorphic DNA markers linked to *FMRI* must be employed (83, 84).

Another challenge to preimplantation diagnosis in fragile X syndrome is that women who carry the *FMRI* premutation tend to have elevated baseline FSH levels (58–60). Consequently, women with the premutation typically do not respond well to exogenous gonadotropin stimulation, and this limits the number of embryos available for PGD selection (84).

RECOMMENDATIONS FOR FMRI TESTING

A strong case can be made for offering screening for the *FMRI* premutation to all women with premature ovarian failure (35, 77). Current American College of Medical Genetics (ACMG) practice guidelines recommend testing the repeat region of *FMRI* in women who are experiencing reproductive problems associated with elevated FSH levels, especially if they have [a] a family history of premature ovarian failure, [b] a family history of fragile X syndrome, or [c] male or female relatives with undiagnosed mental retardation (42). Family history of tremor/ataxia syndrome due to

the *FMRI* premutation or undiagnosed movement disorders such as tremor and cerebellar ataxia would also raise suspicion that ovarian failure or ovarian insufficiency may be related to an *FMRI* premutation.

The American College of Obstetricians and Gynecologists (ACOG) in their most recent committee opinion on the subject stated, “If a woman has ovarian failure or an elevated follicle-stimulating hormone level before the age 40 years without a known cause, fragile X carrier screening should be considered to determine whether she has a premutation” (38). Guidelines published in 2006 by the European Society for Human Genetics and the European Society of Human Reproduction and Embryology suggest that testing *FMRI* as part of the diagnostic workup of female infertility may be relevant, but specific recommendations were not provided (85).

Increasingly, clinicians are responsible for engaging patients in discussions regarding available genetic tests based on their personal history, family history, or their ethnic group. For a limited but growing number of diseases, clinicians are responsible for discussing available genetic tests to every couple planning to become pregnant (86). Nearly universal screening for cystic fibrosis emerged in the wake of a 1997 NIH Consensus Development Conference concluding that this should be offered to couples currently planning a pregnancy (87). By 2001, the ACOG and the ACMG recommended a panel of mutations to be tested and developed model resources for disseminating information to patients and providers (88). Carrier screening and fetal diagnostic testing affords the family an opportunity to make informed decisions regarding their health care. In this context, primary providers of reproductive health care need to be able to engage patients in discussions regarding available genetic tests (86).

The prevalence of *FMRI* premutations in the general population is approximately 1 in 300 (81, 89), although a recent meta-analysis suggests the premutation prevalence in women may be as high as 1 in 129 (90). Based on opinions developed in 2005, the ACMG does not recommend widespread prenatal or preconception screening for *FMRI* premutations except as part of a well-defined clinical research protocol (42). However, participants in this workshop and focus group recommended screening focused on higher risk individuals. This includes women with premature ovarian failure, family history of mental retardation or autism, or features of fragile X syndrome. Specialists, namely those in reproductive medicine, could provide a supportive environment to explain the meaning and implications of test results and facilitate access to testing and referral to genetic counselors.

Recommendation against widespread population screening at present centers on concerns regarding the limited resources available to perform effective patient teaching and counseling associated with this complex disorder. Concerns have also been raised with respect to the lack of knowledge

about the stability of increased CGG repeat alleles identified in the general population, particularly those in the intermediate range (35, 91). This is problematic as the frequency of intermediate alleles is high in the general population (e.g., in one report it was 1 in 52 using a definition of intermediate repeat size of 41–60) (92).

Several groups have addressed screening for carrier status at the population level (35, 81, 93–98). Two studies from the United States have examined the question regarding population-based screening for premutations in *FMRI*. One concluded that this is both clinically desirable and cost-effective (81). An earlier study concluded that the cost of testing needed to be reduced in order to make *FMRI* population screening cost-effective (95). A study from England and Wales concluded that preconception and prenatal screening for *FMRI* abnormalities are feasible and acceptable by affected families and by the general population (99). The reports suggest the need for prospective clinical studies.

A study in Israel offered preconception or prenatal testing because of the high prevalence of premutation and full mutation alleles in the general population. They concluded that such screening was cost-effective and should be carried out on a wide scale (98). More studies need to be conducted to estimate the prevalence of premutations among different racial and ethnic groups, but the incidence of 1 in 100 in the Israeli population made for a favorable cost-benefit comparison.

Given the relatively high prevalence of premutation alleles in the general population, consideration might be given to screening potential gamete donors (63). Theoretically, men carrying an *FMRI* premutation could avoid passing this on to their daughters through the use of sperm-sorting techniques to select only Y-bearing spermatozoa (63). However, the procedure is controversial, and clinical experience with this technique is still limited (100, 101).

THE *FMRI* PREMUTATION AND REPRODUCTION: FUTURE DIRECTIONS

A need exists for the relevant professional societies and patient advocacy groups to establish standardized clinical definitions, terminology, and testing recommendations that will facilitate patient care and research. Specific goals with respect to applying the current knowledge to the clinical arena would be to [1] make recommendations regarding the indications for *FMRI* premutation testing in various populations; [2] develop standardized terminology and define the continuum of altered ovarian function that can be experienced by women who carry an *FMRI* premutation; and [3] make recommendations regarding the clinical evaluation of ovarian function in women found to be carriers.

Areas of needed clinical research can be stratified into two general perspectives: [1] management of asymptomatic women known to carry an *FMRI* premutation, and [2] man-

agement of women with infertility and altered ovarian function who have unknown *FMRI* carrier status.

Asymptomatic Women Who Carry an *FMRI* Premutation

For asymptomatic women who carry an *FMRI* premutation, research is needed that would achieve the following:

1. Determine the natural history.
 - a. Determine the nature of the onset of altered ovarian function. How often does this occur among young girls? What percentage experience primary amenorrhea or fail to ever establish regular menses during puberty?
 - b. Determine the prevalence of infertility. If regular ovulatory menstrual cycles are present, do premutation carriers experience an increased rate of infertility?
 - c. Determine the specific clinical phenotype of premature ovarian failure in premutation carriers as compared with other mechanisms. What are the long-term health consequences of premature ovarian failure in premutation carriers?
 - d. Determine the factors, both genetic and environmental, that increase the risk of altered ovarian function among premutation carriers. How much of this risk is explained by CGG repeat length?
2. Delineate the clinical evaluation of the reproductive axis that should be offered to an asymptomatic premutation carrier.
3. Evaluate the behavioral and psychological impact that the knowledge of increased risk of premature ovarian failure has on young women who carry the premutation.

Symptomatic Women with Unknown *FMRI* Premutation Status

For women who present with infertility or altered ovarian function and who may or may not be an *FMRI* carrier, research is needed that would achieve the following:

1. Determine the prevalence of the premutation among subsets of women with specific reproductive disorders:
 - a. Women with premature ovarian failure in various ethnic groups. To date, most of the information has come from White and Northern European or Ashkenazi Jew extractions.
 - b. Women with oligomenorrhea or polymenorrhea.
 - c. Women with infertility and regular menstrual cycles who have increased serum FSH or other measures indicative of altered ovarian function.
 - d. Women who respond poorly to gonadotropin stimulation.
2. Evaluate the behavioral and psychological impact of carrying the *FMRI* premutation. That is, how does a woman not only understand the cause of her reproductive disorder but also react to knowledge of an increased risk of mental retardation among offspring.

3. Define the genetic counseling and information needs of a woman with reproductive disorders related to premutations in *FMR1*, both pretest and post-test.
4. Determine if the expansion rates to the full mutation differ between alleles ascertained through the premutation state and those ascertained via a case of fragile X syndrome.
5. Through research determine the mechanism of the altered ovarian function among premutation carriers. Is the impaired ovarian function associated with the *FMR1* premutation caused by an inadequate endowment of follicles at birth, an accelerated loss of follicles, or some other mechanism? Is it feasible to parallel the clinical studies outlined above with studies in animal model systems in order to identify the pathophysiologic mechanisms by which the *FMR1* premutation impairs ovarian function? Can animal models identify the cell types and stages of development affected? Can they determine the mechanism of toxicity and the contribution of genetic and environmental factors to premutation-related altered ovarian function?

CONCLUSIONS

Convincing evidence relates the *FMR1* premutation to altered ovarian function and loss of fertility. An *FMR1* mRNA gain-of-function toxicity seems to underlie this altered ovarian function. Major gaps in knowledge regarding the natural history of the altered ovarian function in women who carry the *FMR1* premutation make counseling about reproductive plans a challenge.

The panels conclude that women with premature ovarian failure are at increased risk of having an *FMR1* premutation and should be informed of the availability of fragile X testing. Specialists in reproductive medicine can provide a supportive environment in which to explain the implications of *FMR1* premutation testing, facilitate access to testing, and make appropriate referral to genetic counselors.

Acknowledgment: One of the authors (L.M.N.) is a commissioned officer in the United States Public Health Service.

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