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COMMITTEE OPINION

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Committee on Genetics

Carrier Screening for Fragile X Syndrome

ABSTRACT: Fragile X syndrome is the most common inherited form of mental retardation. The syndrome occurs in approximately 1 in 3,600 males and 1 in 4,000–6,000 females. Approximately 1 in 250 females carry the premutation. DNA-based molecular analysis is the preferred method of diagnosis for fragile X syndrome and its premutations. Prenatal testing for fragile X syndrome should be offered to known carriers of the fragile X premutation or full mutation. Women with a family history of fragile X-related disorders, unexplained mental retardation or developmental delay, autism, or premature ovarian insufficiency are candidates for genetic counseling and fragile X premutation carrier screening.

Fragile X syndrome is the most common inherited form of mental retardation. The syndrome occurs in approximately 1 in 3,600 males and 1 in 4,000–6,000 females from a variety of ethnic backgrounds. Mental retardation or impairment ranges from borderline, including learning disabilities, to severe, presenting with cognitive and behavioral disabilities, including autism. Most affected males have significant intellectual disability. Fragile X syndrome is the most common known cause of autism or "autisticlike" behaviors. Other associated phenotypic abnormalities include distinctive facial features in males (including long narrow face and prominent ears), enlarged testicles (macroorchidism), connective tissue problems, and speech and language problems. The abnormal facial features are subtle and become more noticeable with age, making phenotypic diagnosis difficult, especially in the newborn. Affected females may have a more subtle phenotype, and it is sometimes hard to establish the diagnosis based on clinical findings alone.

Fragile X syndrome is transmitted as an X-linked disorder. However, the molecular genetics of the syndrome are complex. The disorder is caused by expansion of a repeated trinucleotide segment of DNA, cytosine–guanine–guanine (CGG), that leads to altered transcription of the fragile X mental retardation 1 (*FMR1*) gene. The number of CGG repeats varies among individuals and has been classified into four groups depending on the repeat size: 1) unaffected, 2) intermediate, 3) premutation, and 4) full mutation (1, 2) (see Table 1). A person with 55–200 repeats usually is phenotypically normal and is said to have a premutation. When more than 200 repeats are present, an individual has a full mutation that results in the full expression of fragile X syndrome in males and variable expression in females secondary to X chromosome inactivation. The large number of repeats in a full mutation allele causes the *FMR1* gene to become methylated. Methylation "turns off" the regulatory region of a gene, thereby preventing DNA transcription and FMR1 protein production. The number of repeats and the status of gene methylation are determined by use of DNA-based molecular tests (eg, Southern blot analysis and polymerase chain reaction). In rare cases, the size of the triplet repeat and the methylation status do not correlate, making prediction of the clinical phenotype difficult. Fetal DNA analysis from amniocentesis or chorionic villus sampling (CVS) is reliable for determining the number of triplet repeats. However, in some cases, particularly in male fetuses, an analysis of *FMR1* gene

Table 1. Mutation in the Fragile X Mental Retardation 1 Gene

Status of Individual	Number of Triplet Repeats (Cytosine–Guanine–Guanine)
Unaffected	Less than 45
Intermediate (also called "grey zone")	45–54
Premutation	55–200
Full mutation	More than 200

methylation in full mutations from samples of chorionic villi may not be accurate. Gestational age differences in the methylation of specific genes in the chorionic villi may preclude determining the true methylation status of the *FMR1* gene (3). Therefore, findings of full *FMR1* mutations (greater than 200 repeats) on fetal DNA analysis from CVS may require a follow-up amniocentesis to accurately determine the methylation status of the gene.

Transmission of a disease-producing mutation to a fetus depends on the sex of the parent and the number of CGG repeats present in the parental gene. A woman who carries a premutation can transmit either her normal or premutation allele; the premutation allele may expand, resulting in the birth of an affected child. The larger the size of the premutation repeat, the more likely the expansion to a full mutation (Table 2). The smallest repeat size reported to expand to a full mutation in one generation is 59 repeats (4). Diagnosis of mutation size may vary by as many as 3 or 4 repeats. The frequency of premutation allele carriers (repeat size greater than 54) in the population has been reported to be as high as 1 in 157 in a large Israeli study of women (more than 36,000 individuals) without a family history of mental retardation or developmental abnormalities (5). The most recent prevalence data from the United States reported a carrier frequency of 1 in 86 for those with a family history of mental retardation and 1 in 257 for women with no known risk factors for fragile X syndrome (6).

Women with an intermediate number of triplet repeats (45–54) do not transmit a full mutation to their sons and daughters, although there may be expansion to a premutation allele in their offspring. Genetic counseling for intermediate results may be useful. Males will transmit the unexpanded premutation gene to their daughters, but expansion to a full mutation is extremely rare. Empirically determined risks are available for the purposes of genetic counseling.

Fragile X-Associated Disorders

Carriers of premutation alleles do not display any of the classic phenotypic features associated with full mutation expansions. However, males and, to a lesser extent, females carrying a premutation are at an increased risk of a late-onset neurodegenerative disorder (with onset usually after age 50 years) characterized by progressive cerebellar ataxia and intention tremor, called *fragile X tremor ataxia syndrome* or *FXTAS*. The risk and the severity of the disorder appear to be related to the size of the premutation repeat, with the highest risk associated with larger repeats. The true incidence of this new neurologic syndrome among premutation carriers remains to be established and is an area of ongoing active research.

Table 2. Full Mutation Expansion from Maternal Premutation Allele

Maternal Repeat Size	Full Mutation Expansion (%)
55–59	4
60–69	5
70–79	31
80–89	58
90–99	80
100–200	98

Data from Nolin SL, Brown WT, Glicksman A, Houck GE Jr, Gargano AD, Sullivan A, et al. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. Am J Hum Genet 2003;72:454–64.

Women carrying a premutation are at an increased risk (20%) of premature ovarian failure or insufficiency (7). Fragile X-associated ovarian dysfunction is now commonly called *fragile X-associated primary ovarian insufficiency*. The carrier frequency of a fragile X premutation is approximately 3% for women with "sporadic" premature ovarian failure and 12% for women with a family history of premature ovarian failure (8). If a woman has a personal or family history of ovarian failure or an elevated follicle-stimulating hormone level before age 40 years without a known cause, fragile X premutation carrier testing should be offered (9, 10).

Preconception or Prenatal Carrier Screening

Current consensus guidelines from professional genetics organizations recommend carrier screening only for women with a family history of fragile X syndrome or undiagnosed mental retardation, developmental delay, or autism or for those with ovarian insufficiency (6). Although following these guidelines will not detect most premutation carriers in the population, it does target a higher prevalence group based on current data with regard to carrier frequency. However, patients without a family history may visit their obstetric providers informed about fragile X syndrome and ask to have screening to determine their carrier status. In addition, commercial laboratories have marketed the availability of *FMR1* mutation analysis directly to obstetricians. The current commercially available DNA assays for fragile X repeat expansion analysis are intended for diagnosis and not specifically for screening. Thus, they are costly, time consuming, and have limited utility for widespread population-based screening. Recent technical advances in high throughput DNA analysis offer the possibility of low-cost, screening-specific tests in the near future (11).

The American College of Obstetricians and Gynecologists' Committee on Genetics recommends testing for fragile X syndrome as follows:

- Women with a family history of fragile X-related disorders, unexplained mental retardation or developmental delay, autism, or premature ovarian insufficiency are candidates for genetic counseling and fragile X premutation carrier screening.

- If a woman has ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years without a known cause, fragile X carrier screening should be considered to determine whether she has an *FMR1* premutation.
- Women who request fragile X carrier screening, regardless of family history, should be offered *FMR1* DNA mutation analysis after genetic counseling about the risks, benefits, and limitations of screening.
- All identified carriers of a fragile X premutation (or full mutation) should be referred for follow-up genetic counseling to discuss the risk to their fetuses of inheriting an expanded full mutation fragile X allele and to discuss fragile X associated disorders (premature ovarian insufficiency and fragile X tremor ataxia syndrome). Prenatal and preimplantation diagnoses and donor eggs should be discussed.
- Prenatal testing for fragile X syndrome by amniocentesis or CVS should be offered to known carriers of the fragile X premutation or full mutation. Although amniocentesis and CVS are reliable for determining the number of triplet repeats, CVS may not adequately determine the methylation status of the *FMR1* gene.
- DNA-based molecular analysis (eg, Southern blot analysis and polymerase chain reaction) is the preferred method of diagnosis of fragile X syndrome and of determining *FMR1* triplet repeat number (eg, premutations). In rare cases where there is discordance between the triplet repeat number and the methylation status, the patient should be referred to a genetics professional.

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