Fragile X syndrome

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What is fragile X syndrome?

Fragile X syndrome is one of the most common inherited forms of mental retardation. Both males and females can be affected, but females are usually less severely affected than males. Mental retardation ranges from mild to severe. Learning disabilities and behavioral problems are likely to be seen in this condition. Physically, individuals with fragile X may have a long, narrow face, large jaw with a prominent chin, large ears, and macroorchidism (large testes). The facial features become more apparent with age. Behavioral problems may include autism, shyness, social anxiety, hyperactivity, reduced attention span, and rapid, repetitive speech. Fragile X syndrome is the most common known genetic cause of autism. According to the National Fragile X Foundation, 2.5-6% of boys with autism have Fragile X syndrome. The incidence of fragile X syndrome is approximately 1 in 4,000 males and 1 in 4,000 to 8,000 females.

Genetics of Fragile X syndrome

Humans have 23 pairs of chromosomes, 22 "autosomes" and 1 pair of sex chromosomes. We get half of our chromosomes from our mothers and half from our fathers. Women have two X chromosomes, and men have an X chromosome and a Y chromosome.

The inheritance of fragile X syndrome is complex. It is an X-linked condition and generally follows and X-linked pattern of inheritance in which 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. The risk for mental retardation in families with the fragile X mutation appeared to increase with each generation, known as "anticipation".

In 1991, the gene for fragile X syndrome was identified and the mutation was found to be due to an expanded trinucleotide sequence in the DNA (CGG), which leads to altered transcription of the FMR1 (Fragile X mental retardation) gene. The protein produced by FMR1 is highly expressed in the brain.

The length of the CGG repeat on the FMR gene, located on the X chromosome, is what determines whether or not a person has symptoms of fragile X syndrome (table 1). In people who do not carry a change in the gene for fragile X, the number of CGG repeats usually measures between 6 and 50 on both chromosomes.

When there are 50-200 CGG repeats it is called a "premutation" and these individuals are premutation carriers. A premutation has different clinical significance than a full mutation. Women who carry a premutation are not expected to have mental retardation or significant health problems. In individuals who carry the premutation, the gene is still able to function, but the premutation makes the gene unstable and the number of repeats may increase in the next generation.

If the gene expands to more than 200 repeats, it is considered a "full mutation," and the gene is unable to work properly. Individuals with a full mutation have an inactive fragile X gene and are likely to exhibit features of fragile X syndrome. The large number of repeats causes the FMR1 gene to become methylated and inactivated in affected individuals.

Intermediate alleles (also know as "gray zone") is 45-54 repeats. This number varies slightly by laboratory. The smallest reported repeat length known to expand to a full mutation in one generation is 59 repeats. Consensus has not been reached for the intermediate zone end-points (i.e., 45–54 repeats or 40–54 repeats). The American College of Obstetricians and Gynecologists recently defined unaffected as 41 to 60, and premutation as 61 to 200 repeats.

Large changes in the repeat number usually only occur when the gene is passed from mother to child. A premutation will usually only change to a full mutation when passed from mother to child, although there are case reports of premutation males who have had daughters with full mutations (this is rare). If a woman who is a premutation carrier has children, there is a 50% chance with each pregnancy of passing on the X chromosome with the premutation and a 50% chance of passing on the X chromosome with the regular number of CGG repeats. If a fetus inherits the X chromosome with the fragile X gene, it may expand. The likelihood of expansion is related to the premutation size (table 2). A male child who inherits the full mutation would be expected to develop the fragile X syndrome. There are rare cases of males who are known to have the mutation based on pedigree analysis had no cognitive disabilities.

Males can also be carriers of fragile X syndrome, but in general the fragile X gene is not expected to expand into the affected range when passed on by the male. Men who are carriers of fragile X syndrome are called "transmitting males." Men pass the Y chromosome to their sons; therefore, no son will inherit the fragile X gene from his father. Men pass the X chromosome to their daughters. Consequently, typically all of the daughters of a male carrier of fragile X syndrome will also be carriers. These daughters will then be at increased risk to have affected children.

Females who possess the full mutation frequently are clinically affected, although often to a milder degree than males. The pattern of X inactivation (one X chromosome in each cell is "turned off") occurs randomly and influences the degree of severity of the syndrome in girls. However, approximately 70% of females with the full mutation have a borderline IQ or lower, and those with a normal IQ often have certain deficits.

Table 1—DNA Repeats as Indicators of Fragile X Disease Severity

Number of Repeats	Condition
5-44	Normal
45-54	Gray Zone, no measurable risk for affected children, but gene may be unstable
54-200	Premutation, carrier
200 and greater	Full mutation, affected

From Maddalena A, Richards CS, McGinnis MJ, et al. Technical standards and guidelines for fragile X: The first of a series of disease-specific supplements to the standards and guidelines for clinical genetics laboratories of the American College of Medical Genetics. Genet Med. 2001; 3(3):200-205.

Table 2—DNA Repeats Related to Disease Risk in Offspring

Number of Repeats	Risk of expansion to full mutation if passed to offspring by the mother
50 to 59	Negligible
60 to 69	17%
70 to 79	71%
80 to 89	82%
90 to 113	100%

From Fu Y-H, Kuhl DPA, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. Cell. 1991; 87:1047-1058.

Other conditions associated with FMR1 premutations

More recently, two disorders that are distinct from fragile X syndrome have been found to be associated with premutation status.

Fragile X–associated tremor/ataxia syndrome, or FXTAS, is a progressive neurodegenerative disorder has been identified in male premutation carriers over the age of 50. Patients may present with progressive intention tremor, ataxia, autonomic dysfunction, parkinsonian features, cognitive deficits, psychological features (anxiety, mood lability, outburst or reclusive behavior), and peripheral neuropathy with decreased sensation in the lower extremities. Characteristic radiologic findings noted on MRI include global brain atrophy and deep cerebellar white matter hyperintensities. Postmortem examination reveals pathognomonic eosinophilic inclusion bodies in cortical neurons and astrocytes. In males with the premutation, the risk of developing FXTAS increases with age. Although FXTAS can occur in women with the premutation, it is not common. There is evidence that an unfavorable X-chromosome inactivation increases the risk of FXTAS in women.

Premature ovarian failure (POF) affects approximately 15% of women who carry the premutation. 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. In approximately 90% of cases, no mechanism can be identified to explain the ovarian insufficiency.

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. The risk seems to increase with increasing premutation size between 59 – 99; There is preliminary evidence that suggests a possible increased risk of premature ovarian failure among women who carry intermediate-size alleles, those between approximately 41 and 58 repeats. Unfavorable X-inactivation does not appear to increase the risk for premature ovarian failure.

About 5-10% of women with premature ovarian failure who are premutation carriers will conceive subsequent to the diagnosis without medical intervention. Women who have premature ovarian failure related to an FMR1 premutation are at risk of having a child with fragile X syndrome should they conceive. Therefore, it is important to offer appropriate testing and genetic counseling to these women.

Genetic Counseling

The genetics of Fragile X syndrome is challenging to explain to patients and their families because of the complex inheritance involving repeat expansion and the different phenotypes that can be expressed. Genetic counseling needs may vary depending on how the mutation was discovered in the family and also on the size of the CGG repeat.

Fragile X test results carry significant reproductive ramifications for women carrying the FMR1 premutation allele. In addition to concerns that female premutation carriers have regarding the risk of having an affected child, they may be unable to conceive using their own eggs. These women 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.

Learning of carrier status is difficult and may raise feelings of anxiety, guilt, or altered feelings of self-worth. Genetic counseling includes an assessment of coping behaviors, suggestions for adaptive coping, providing resources to deal with the feeling of being "at risk", and processing the test results when they become available. Reproductive options are 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 is usually provided after the genetic counseling session. In addition, other written materials, online resources and support group information can be provided as needed.

Genetic testing for Fragile X
Testing for the fragile X mutation is done by determining the number of CGG repeats and, if in the full mutation range, assessing 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 FMR1.

PGD for Fragile X
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" meaning that ideally, the number of CGG repeats on the normal FMR1 allele of the mother and father should differ. In the approximately 40% of cases in which the family is uninformative, polymorphic DNA markers linked to FMR1 are used to differentiate the chromosomes. Another challenge is that women with a premutation tend to be poor responders, limiting the number of embryos that are usually available for PGD.

Who should be tested?
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.

NSGC position statement on genetic screening:

The NSGC recognizes the increasing availability of laboratory screening tests which identify individuals who are carriers of gene mutations potentially resulting in genetic disorders in their offspring. The NSGC therefore supports the following recommendations:

1. Individuals seeking genetic counseling should be offered genetic screening tests after clinical research trials have been satisfactorily completed and the individuals:
    - have a family history of a specific genetic condition for which testing is available, or
    - have reason to suspect a family history of a genetic condition for which testing is available, or
    - are members of a high risk subpopulation;
2. Pilot studies to explore the scientific, educational, counseling, social and ethical aspects of screening should be completed prior instituting large scale screening programs;
3. Clinicians should evaluate test accuracy, informativeness, specificity, sensitivity, and laboratory proficiency prior to sending specimens;
4. Genetic counseling services by a Board Certified/Board Eligible genetic counseling professional should be an integral component of any genetic screening program. (Adopted October 1994).

The American College of Medical Genetics and the American College of Obstetricians and Gynecologists 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.

American College of Medical Genetics (ACMG) practice guidelines recommend offering Fragile X testing to women with elevated FSH levels, especially if they have a family history of POF or fragile X syndrome, or relatives with mental retardation of unknown etiology. Family history of tremor/ataxia syndrome or movement disorders of unknown etiology also raise suspicion of fragile X.

The American College of Obstetricians and Gynecologists (ACOG) in their most recent committee opinion on fragile X testing 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".

ASRM guidelines for screening gamete donors in 2006 states "oocyte donors may be tested for fragile X at the discretion of the program." There is no mention of formal genetic counseling. If ovum donor programs plan to screen their clients for Fragile X syndrome, they must be prepared with the appropriate resources for follow-up.

The prevalence of FMR1 premutations in the general population is approximately 1 in 300, although a recent meta-analysis suggests the premutation prevalence in women may be as high as 1 in 129. Recommendation against widespread population screening at present is mostly due to limited genetic counseling resources. 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. 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).

Increasingly, clinicians are discussing genetic tests with patients based on their personal history, family history, or their ethnic group. Carrier screening prior to pregnancy allows the family an opportunity to make informed decisions regarding their health care. Because of its unique nature (complexity, affect on family as well as individual, potential for psychological sequelae, potential for genetic discrimination) genetic testing should be reviewed *prior* to offering the test to discuss risks, benefits, and possible outcomes with anyone undergoing testing. Any positive genetic test should be followed up with formal genetic counseling provided by a board certified/board eligible genetic counseling professional. This is especially true for complicated conditions such as fragile X syndrome. Genetic counselors can be located at www.nsgc.org. Genetic counselors affiliated with the GC SIG of ASRM can be located at www.asrm.org.

Healthcare providers in reproductive medicine can provide a supportive environment in which to explain the indications, risks, and benefits for fragile X testing to their patients, facilitate access to testing, and make appropriate referral to genetic counselors when indicated. A partnership with a genetic counselor can enhance your practice.

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