Fragile X Syndrome

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• No financial interests to disclose.
Objectives

• Epidemiology of intellectual disability
• Fragile X syndrome
  – Pathogenesis
  – Inheritance
• Related conditions
• Questions
Intellectual Disability

• Mental retardation
  – Static encephalopathy with serious deficits in the cognitive realm
Diagnostic Criteria for Mental Retardation

1. Significantly sub average intellectual functioning: an IQ of approximately 70 or below on an individually administered IQ test (for infants, a clinical judgment of significantly sub average intellectual functioning)

2. Concurrent deficits or impairments in present adaptive functioning (i.e. the person’s effectiveness in meeting the standards expected for his or her age by his or her cultural group) in at least two of the follow areas: communication, self-care, home living, social interpersonal skills, use of community resources, self-direction, functional academic skills, work, leisure, health, and safety.

3. The onset is before age 18 years.
   - Code based on degree of severity reflecting level of intellectual impairment:
     - Mild MR: IQ level 50-55 to 70
     - Moderate MR: IQ level 35-40 to 50-55
     - Severe MR: IQ level 20-25 to 35-40
     - Profound MRI: IQ level <20-25
     - MR, severity unspecified: when there is a strong presumption of MR but the person’s intelligence is untestable by standard tests

American Psychiatric Association, 1994
Genetics in OBGYN, Simpson and Elias
Epidemiology

• 3% of population has an IQ less than 2 SD below the mean
  – 80-90% of MR population have mild MR
  – 5% of MR population have severe to profound MR
• 30% more males have MR than females
• Genes on X chromosome
• MR more prevalent in children of lower SES and from disadvantaged minority groups
Etiology

- Genetic
  - Chromosomal – 40% of severe MR, 10-20% of mild MR
    - Down syndrome - most common genetic cause, 1 in 800 (general population)
  - Contiguous Gene Syndrome
  - Autosomal Dominant
  - Autosomal Recessive
  - Inborn errors of Metabolism
  - Nonmetabolic Syndromes of Unknown Cause
  - X-linked Conditions
    - Fragile X Syndrome

- Nongenetic –
  - Teratogenic
  - Ex - Maternal PKU (congenital, not genetic)

- Idiopathic – 40%
Case

- 34 yo woman – P0000
- Family history of MR and autism
- Premutation carrier (100, 96 CGG repeats), unaffected
- Counseling?
- Testing?
- Risk of affected male offspring?
- Risk of having female carrier offspring?
Fragile X Syndrome

- Most common cause of inherited MR
- Males – 1 in 2,500 -4,000
- Females – 1 in 6-8,000
- Accounts for 3-6% of MR among boys with a +FHX of MR and no birth defects

- Carrier frequency (premutation 61-200 repeats)
  - 1 in 350 females, 1 in 1000 males

ACMG 2005
Fragile X – major phenotypic features

- Age at onset – childhood
- Mental deficiency
- Dysmorphic facies
- Male postpubertal macroorchidism

- Fragile site at Xq27.3 – located in 5’ untranslated region of the first exon of a gene called FMR1 (fragile X mental retardation 1)
FIGURE 2-37  Location of genes on the X chromosome responsible for genetic diseases. (http://www.ncbi.nlm.nih.gov/diseases/)
Fragile X Syndrome

- FMR1 gene product is FMRP (expressed in many cell types – mostly in neurons); may chaperone a subclass of mRNAs from the nucleus to the translational machinery
- More than 99% of FMR1 mutations are expansions of a (CGG)n repeat sequence in the 5’ untranslated region of the gene; > 200 repeats results in hypermethylation of the CGG repeat sequence and the adjacent FMR1 promoter; this inactivates the FMR1 promoter, causing a loss of FMRP expression
• Expansion of repeated trinucleotide segment of DNA (cytosine–guanine–guanine, CGG) that leads to altered transcription of the fragile X mental retardation 1 (FMR1) gene.

• # of repeats varies – 4 groups - unaffected, intermediate, premutation, full mutation
  – 61–200 repeats - phenotypically normal, premutation
  – This condition occurs because the large number of repeats causes the FMR1 gene to become methylated and inactivated in these patients.
  – 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).
  – DNA methylation is a process that controls tissue specific gene expression. Methylation "turns off" the regulatory region of a gene, thereby preventing DNA transcription. Rarely, the size of the triplet repeat and the methylation status do not correlate, making prediction of the clinical phenotype difficult.
FIGURE 6-4 • Diagram of the FMR1 gene and the first exon in normal, premutation, and full mutation alleles. The oval immediately to the left of the start site of transcription represents the promoter region of the FMR1 gene. The open symbol represents active transcription, and the black symbol, silenced transcription. The vertical lines indicate CGG trinucleotides upstream of the methionine codon (AUG) at the translocational start site. (Reprinted with permission from Warren ST, Nelson DL: Advances in molecular analysis of fragile X syndrome. JAMA 271:536, 1994.)
# Fragile X syndrome

<table>
<thead>
<tr>
<th>Status of Individual</th>
<th>Number of Triplet Repeats (Cytosine–Guanine–Guanine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected</td>
<td>Less than 45</td>
</tr>
<tr>
<td>Intermediate</td>
<td>45-54</td>
</tr>
<tr>
<td>(also called &quot;grey zone&quot;)</td>
<td></td>
</tr>
<tr>
<td>Premutation</td>
<td>55–200</td>
</tr>
<tr>
<td>Full mutation</td>
<td>More than 200</td>
</tr>
</tbody>
</table>

ACOG committee opinion 2010
## Full Mutation Expansion from Maternal Premutation Allele

<table>
<thead>
<tr>
<th>Maternal Repeat Size</th>
<th>Full Mutation Expansion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-59</td>
<td>4</td>
</tr>
<tr>
<td>60-69</td>
<td>5</td>
</tr>
<tr>
<td>70-79</td>
<td>31</td>
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<tr>
<td>80-89</td>
<td>58</td>
</tr>
<tr>
<td>90-99</td>
<td>80</td>
</tr>
<tr>
<td>100-200</td>
<td>98</td>
</tr>
</tbody>
</table>

ACOG CO – 2010

ACOG committee opinion 2010
X-linked Inheritance

- ½ of offspring of carrier mothers will receive the mutation and all the daughters but none of the sons of carrier fathers receive the mutation.
- Risk of expansion of the CGG repeats in a premutation allele to a full mutation overlays the transmission pattern of FXS:
  - Expansion of the premutation to the full mutation during transmission through a carrier woman is positively correlated with size of the woman’s repeat.
  - Smallest repeat to expand to a full mutation in one generation is 59 repeats.
  - Risk of expansion to the full mutation from carrier men to their daughters is rare but has occurred.
  - Premutation males pass on the premutation to their daughters typically with only small expansions or contractions.

- Males affected
- Some carrier females mildly affected
- Affected males related through carrier females
- No male to male transmission
- 50% recurrence risk in males
X-linked Pedigree
Typical pedigree of fragile X syndrome. Note the presence of a transmitting male and anticipation with more affected individuals in later generations.

Ignatia B. Van den Veyver, MD Division of Maternal-Fetal Medicine and Reproductive Genetics, Department of Obstetrics and Gynecology and Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas (Vol, Chap 74)
ACMG/ACOG Recommendations
Fragile X Syndrome - ACOG

- Committee on Genetics recs re: testing for fragile X:
- DNA-based molecular test (Southern blot, PCR)
  - In rare cases where there is discordancy between the triplet repeat number and the methylation status, the patient should be referred to a genetic specialist.
- FHx, or hx of fragile X MR – genetic counseling, offered genetic testing to assess risk for having an affected child.
- Prenatal testing for fragile X syndrome by amniocentesis or CVS should be offered to known carriers of the fragile X premutation or mutation. Although it is reliable for determining the number of triplet repeats, CVS may not adequately determine the methylation status of the FMR1 gene.
- Testing for fragile X syndrome should be considered in any child with developmental delay of uncertain etiology, autism, or autistic like behavior or any individual with mental retardation of uncertain etiology.
- 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.
Fragile X syndrome: Diagnostic and carrier testing

Stephanie Sherman, PhD\textsuperscript{1,2}, Beth A. Pletcher, MD\textsuperscript{1,3}, and Deborah A. Driscoll, MD\textsuperscript{1,4}

Key Words: fragile X syndrome, genetic testing, FMR1, X-linked mental retardation
Individuals for Whom Testing Should Be Considered

Fragile X syndrome:

- Individuals of either sex with mental retardation, developmental delay, or autism, especially if they have (a) any physical or behavioral characteristics of fragile X syndrome, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.
- Individuals seeking reproductive counseling who have (a) a family history of fragile X syndrome or (b) a family history of undiagnosed mental retardation.
- Fetuses of known carrier mothers.
- Affected individuals or their relatives in the context of a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives. The cytogenetic test was used prior to the identification of the FMR1 gene and is significantly less accurate than the current DNA test. DNA testing on such individuals is warranted to accurately identify premutation carriers and to distinguish premutation from full mutation carrier women.
Ovarian dysfunction:

- Women who are experiencing reproductive or fertility problems associated with elevated follicle stimulating hormone (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.

Tremor/ataxia syndrome:

- Men and women who are experiencing late onset intention tremor and cerebellar ataxia of unknown origin, especially if they have (a) a family history of movement disorders, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.
APPROACHES TO TESTING

- DNA analysis is the method of choice if one is testing specifically for fragile X syndrome and associated trinucleotide repeat expansion in the FMR1 gene.
- For isolated cognitive impairment, DNA analysis for fragile X syndrome should be performed as part of a comprehensive genetic evaluation that includes routine cytogenetic analysis. Cytogenetic studies are critical, since constitutional chromosome abnormalities have been identified as frequently or more frequently than fragile X mutations in mentally retarded individuals referred for fragile X testing.
- For individuals who are at risk due to an established family history of fragile X syndrome, DNA testing alone is sufficient. If the diagnosis of the affected relative was based on previous cytogenetic testing for fragile X syndrome, at least one affected relative should have DNA testing.
American College of Medical Genetics

- Prenatal testing of a fetus should be offered when the mother is a known carrier to determine whether the fetus inherited the normal or mutant FMR1 gene. Ideally, DNA testing should be performed on cultured amniocytes obtained by amniocentesis after 15 weeks’ gestation. DNA testing can be performed on chorionic villi obtained by CVS at 10 to 12 weeks’ gestation, but the results must be interpreted with caution because the methylation status of the FMR1 gene is often not yet established in chorionic villi at the time of sampling. A follow-up amniocentesis may be necessary to resolve an ambiguous result.
- If a woman has ovarian failure before the age of 40, DNA testing for premutation size alleles should be considered as part of the infertility evaluation and prior to in vitro fertilization.
- If a patient has cerebellar ataxia and intentional tremor, DNA testing for premutation size alleles, especially among men, should be considered as part of the diagnostic evaluation.
CVS pitfall

- Chorionic villus sampling (CVS) - reliable for determining the number of triplet repeats, may not be reliable for diagnosis because of gestational age differences in ultimate methylation patterns in the trophoblast and may not adequately determine the methylation status of the FMR1 gene.
Limitations of Testing

• PCR – Accurate for # of repeats and detection of premutation carriers
  – Cannot assess methylation status

• Southern blot – Crude estimate of size of repeats, also accurate assessment of methylation status
FIGURE 6-5 • Southern blot analysis using EcoR1 and Eagl digestion, probed with StB12.3, using extended electrophoresis to illustrate several subtle specimen types. (1) Normal female. (2) Full mutation male. Note the combination of a predominant band with a diffuse smear. (3) Female with 28 and 52 repeats, with the smaller allele predominantly active. (4) Female with 26 and 52 repeats, with the larger allele predominantly active. (5) Female with 18 and ~80 repeats, with equal X-inactivation. (6) Normal male. (7) Normal male, underloaded and smiling due to DNA degradation. (The apparent line between lanes 6 and 7 is a photographic artifact.) (8) Normal female. (9) Normal male. (10) Normal male. (11) Affected male, underloaded and very diffuse. (12) Premutation male. (13) Female with 20 and 70 repeats, with the smaller allele virtually exclusively active. The only evidence of abnormality is the slow migration of the “5.2 kb” band. (14) Female with 27 and 42 repeats, with the larger allele somewhat more active. (15-17) Unremarkable normal females and males. Figure provided by Genetics and IVF Institute. (From Maddalena A, Richards CS, McGinniss MJ, et al.: Technical standards and guidelines for fragile X. Genet Med 3:200, 2001.)
Questions
Question #1

- A male that carries the FMR1 premutation will have daughters that are affected with Fragile X syndrome.
- A – True
- B – False
Question #1

• A male that carries the FMR1 premutation will have daughters that are affected with Fragile X syndrome.
• A – True
• **B – False
Question #2

- What is anticipation (from a genetic standpoint)?
- A – An individual that has a mixture of cells with repeat lengths ranging from premutation to full mutation
- B – An individual having a mixture of cells with and without methylation of the CGG repeat
- C – Increasing numbers of affected offspring are usually observed in later generations of an affected family
- D – An individual’s intense desire to hear Dr. Farley’s intriguing discussion about Fragile X syndrome
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Conclusions

• Indications for Fragile X testing
• Use ACMG, OMIM, GeneTest for review and options for prenatal diagnosis and counseling
Resources

- Genetics Home Reference
- OMIM (Johns Hopkins)
- American College of Medical Genetics
- ACOG CO -
Fragile X syndrome

What is fragile X syndrome?
Fragile X syndrome is a genetic condition that causes a range of developmental problems including learning disabilities and cognitive impairment. Usually, males are more severely affected by this disorder than females.

Affected individuals usually have delayed development of speech and language by age 2. Most males with fragile X syndrome have mild to moderate intellectual disability, while about one-third of affected females are intellectually disabled. Children with fragile X syndrome may also have anxiety and hyperactive behavior such as fidgeting or impulsive actions. They may have attention deficit disorder (ADD), which includes an impaired ability to maintain attention and difficulty focusing on specific tasks. About one-third of individuals with fragile X syndrome have features of autism spectrum disorders that affect communication and social interaction. Seizures occur in about 5 percent of males and about 5 percent of females with fragile X syndrome.

Most males and about half of females with fragile X syndrome have characteristic physical features that become more apparent with age. These features include a long and narrow face, large ears, a prominent jaw and forehead, unusually flexible fingers, flat feet, and in males, enlarged testicles (macroorchidism) after puberty.

How common is fragile X syndrome?
Fragile X syndrome occurs in approximately 1 in 4,000 males and 1 in 8,000 females.

What genes are related to fragile X syndrome?
Mutations in the FMR1 gene cause fragile X syndrome. The FMR1 gene provides instructions for making a protein called fragile X mental retardation 1 protein, or FMRP. This protein helps regulate the production of other proteins and plays a role in the development of synapses, which are specialized connections between nerve cells. Synapses are critical for relaying nerve impulses.

Nearly all cases of fragile X syndrome are caused by a mutation in which a DNA segment, known as the CGG triplet, is expanded within the FMR1 gene. Normally, this DNA segment is repeated from 5 to about 40 times. In people with fragile X syndrome, however, the CGG segment is repeated more than 200 times. The abnormally expanded CGG segment turns off (silences) the FMR1 gene, which prevents the gene from producing FMRP. Loss of a shortage (deficiency) of this protein disrupts nervous system functions and leads to the signs and symptoms of fragile X syndrome.

Males and females with 55 to 200 repeats of the CGG segment are said to have an FMR1 gene premutation. Most people with a premutation are intellectually normal. In some cases, however, individuals with a premutation have lower than normal amounts of FMRP. As a result, they may have mild versions of the physical features seen in fragile X syndrome (such as prominent ears)
GeneReviews® [Internet].

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FMR1-Related Disorders
Robert A Saul, MD, FACMG and Jack C Tarleton, PhD, FACMG.
Author Information

Summary

**Disease characteristics.** *FMR1*-related disorders include fragile X syndrome, fragile X-associated tremor/ataxia syndrome (FXTAS), and *FMR1*-related primary ovarian insufficiency (POI).

Fragile X syndrome occurs in individuals with an *FMR1* full mutation or other loss-of-function mutation and is nearly always characterized by moderate intellectual disability in affected males and mild intellectual disability in affected females. Because *FMR1* mutations are complex alterations involving non-classic gene-disrupting alterations (trinucleotide repeat expansion) and abnormal gene methylation, affected individuals occasionally have an atypical presentation with an IQ above 70, the traditional demarcation denoting intellectual disability (previously referred to as mental retardation). Males with an *FMR1* full mutation accompanied by aberrant methylation may have a characteristic appearance (large head, long face, prominent forehead and chin, protruding ears), connective tissue findings (joint laxity), and large testes after puberty. Behavioral abnormalities, sometimes including autism spectrum disorder, are common.

FXTAS occurs in males (and some females) who have an *FMR1* premutation and is characterized by late-onset, progressive cerebellar ataxia and intention tremor.

*FMR1*-related POI (age at cessation of menses <40 years) occurs in approximately 20% of females who have an *FMR1* premutation.

**Diagnosis/testing.** The diagnosis of *FMR1*-related disorders rests on the detection of an alteration in *FMR1*. More than 99% of individuals with fragile X syndrome have a loss-of-function mutation in *FMR1* caused by an increased number of CGG trinucleotide repeats (typically >200) accompanied by aberrant methylation of *FMR1*. Other mutations within *FMR1* that cause fragile X syndrome include deletions and point mutations. All individuals with FXTAS and *FMR1*-related POI have *FMR1* premutation trinucleotide repeats ranging from 55 to approximately 200. Both increased trinucleotide repeats and methylation changes in *FMR1* can be detected by molecular genetic testing.
FMR1 Gene

What Is a Gene?

A gene is a unit of heredity that is passed down from parent to child. Genes are located on chromosomes that are in all of our cells, including the sperm and egg that make a baby.

What Is a Gene Made Of?

Genes are made of molecules or chemicals called DNA. The pattern of DNA will determine if the gene is working properly. The DNA has to be in a certain pattern or order, like the numbers in a phone number.

How Does a Gene Work?

A gene has different parts that work together like a factory or machine. It has a "promoter" that turns the gene on, like a light switch. It has sections that are just "filler" and act as place holders, called "introns." The sections that are used to make a protein or do a job are called "exons."

What Do Genes Do?

The job of a gene is to either make a protein, the building blocks of all the structures in the body, or to regulate other proteins in the body.

How Does a Gene Make Proteins?
• QUESTIONS ??
References

- Genetics Home Reference
- ACOG
- ACMG
- OMIM
- Genetics in OBGYN - Elias
Extra slides – Do not print beyond this point

• Notes are following
Fragile X syndrome

- CVS pitfall
- Fragile X syndrome - X-linked recessive
- Expansion of repeated trinucleotide segment of DNA (cytosine–guanine–guanine, CGG) that leads to altered transcription of the fragile X mental retardation 1 (FMR1) gene.
- # of repeats varies – 4 groups - unaffected, intermediate, premutation, full mutation
  - 61–200 repeats - phenotypically normal, premutation
  - This condition occurs because the large number of repeats causes the FMR1 gene to become methylated and inactivated in these patients.
  - 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).
  - Chorionic villus sampling (CVS) - reliable for determining the number of triplet repeats, may not be reliable for diagnosis because of gestational age differences in ultimate methylation patterns in the trophoblast and may not adequately determine the methylation status of the FMR1 gene.
  - DNA methylation is a process that controls tissue specific gene expression. Methylation "turns off" the regulatory region of a gene, thereby preventing DNA transcription. Rarely, the size of the triplet repeat and the methylation status do not correlate, making prediction of the clinical phenotype difficult.
Fragile X syndrome

- Triplet repeat expansion – massive expansion nearly always occurs during female gametogenesis; Normal number of repeats up to 60; several thousand occur in fragile X; > 200 copies of the repeat lead to excessive methylation of cytosines in the promoter of FMR1; this interferes with replication or chromatin condensation or both, producing the characteristic chromosomal fragile site, a form of DNA modification that prevents normal promoter function or blocks translation

- Somatic mosaicism- a mutation affecting morphogenesis and occurring during embryonic development might be manifested as a segmental or patchy abnormality, depending on the stage at which the mutation occurred and the lineage of the somatic cell in which it originated – ex: NF1 is sometimes segmental, affecting only one part of the body. Segmental NF1 is caused by mosaicism for a mutation that occurred after conception. In such cases the patient has normal parents, but if he or she has an affected child, the child’s phenotype is typical for NF1, that is, not segmental. In such cases, the mutation has to be in the patient’s gametes and therefore must have occurred before separation of germline cells from the somatic cell line that carries the mutation.
Fragile X

- Sex-specific anticipation – Every child of an affected mother has a more severe form than the mother did, may only have a mild expression of the disease and may not know she is affected.
- DNA methylation – the major form of DNA modification in the human genome involves methylation of cytosine residues (to form 5-methylcytosine), specifically when they are located immediately 5’ to a guanine (i.e. as the dinucleotide 5’-CG-3’). Hotspot for mutation in the human genome as it upstream from coding exons.
- Haplotype effect – a given set of alleles at a locus or cluster of loci on a chromosome is referred to as a haplotype; the set of HLA alleles at the different class I and class loci on a given chromosome together from a haplotype; risk of premutation expansion to a full mutation increases as the repeat length of the premutation increases. Not all premutations, however, are equally predisposed to expand. Although premutations are relatively common, progression to a full mutation has been observed only on a limited number of haplotypes; that is, there is a haplotype predisposition to expansion. This haplotype predisposition may relate partly to the presence of a few AGG triplets embedded within the string of CGG repeats; these AGG triplets appear to inhibit expansion of the string of CGG repeats, and their absence in some haplotypes, therefore, may predispose to expansion.