Academic Pathology: Volume 6
DOI: 10.1177/2374289519877550
journals.sagepub.com/home/apc

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Educational Case: Fragile X Syndrome with Size Mosaicism

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.

Keywords
pathology competencies, diagnostic medicine, genomics, chromosomal disorders, testing for genetic disorders, mutations, developmental abnormality, mosaicism, inherited diseases

Received April 19, 2019. Received revised August 18, 2019. Accepted for publication August 22, 2019.

Primary Objective

Objective GE2.1: Testing for Genetic Disorders: Describe the genetic and epigenetic causes, pathophysiology and clinical manifestations, and optimal laboratory tests used to diagnose the following specific genetic disorders: Mendelian, autosomal disorders (dominant and recessive), X-linked disorders, chromosomal disorders, and disorders of nonclassic inheritance.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic: Genomics (GE); Learning Goal 2: Chromosomal Disorders.

Secondary Objectives

Objective GM1.1: Types of Mutations: Describe different types of mutations that can occur in human disease and discuss how each of these can produce abnormalities in DNA transcription and/or alterations in the type or amount of protein produced.

Competency 1: Disease Mechanisms and Processes; Topic: Genetic Mechanisms (GM); Learning Goal 1: Genetic Mechanisms of Developmental and Functional Abnormalities.

Objective GE1.10: Mosaicism: Define mosaicism and explain how it affects the phenotype of a chromosomal disorder.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic: Genomics (GE); Learning Goal 1: Genes.

Objective GE4.1: Carrier Testing: Describe the role of preconception and prenatal carrier testing for genetic disorders depending upon family history and ethnic background.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic: Genomics (GE); Learning Goal 4: Reproductive Genetics.

Patient Presentation

A 2-year-old male with an unremarkable birth history is brought in by his parents with concerns for global developmental delays and behavioral issues. His mother first recognized signs of early developmental delays within the first year after birth. Rolling and crawling developmental milestones were delayed, but he started walking at the appropriate age. She feels

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he is still somewhat clumsy, but he is able to run and to climb stairs. The mother reported that he does not seem to have a desire to learn and is relatively nonverbal. Furthermore, the patient exhibits many oppositional behaviors. He is hyperactive, aggressive with peers, will bite his mother if he is excited or upset, and has frequent temper tantrums. He is an anxious child and will usually hide behind his mother in new situations. His mother describes that she is exhausted as he constantly demands her attention and involvement.

The patient started early intervention shortly after the onset of symptoms, but therapies were limited due to his oppositional behavior. A consulting developmental pediatrician did not find his symptoms consistent with an autism spectrum disorder. His physical examination was unremarkable and an audiology examination showed no evidence of hearing loss.

Diagnostic Findings

What Is your Differential Diagnosis Based on the Clinical History?

Intellectual disability (ID) is commonly encountered in the pediatric practice, and most children are identified initially because of delays in reaching multiple developmental milestones. The patient presentation showed motor, language, and social developmental delays as well as behavioral issues. Intellectual disability is a highly heterogenous medical condition, encompassing syndromic and nonsyndromic forms. Environmental etiologic factors can include problems during pregnancy, birth, or after birth. Examples include fetal drug or alcohol exposure, congenital infections, labor and delivery complications, childhood infections or injuries, malnutrition, or lead exposure. Genetic etiologic factors account for the majority of cases, however, and include Down syndrome and fragile X syndrome.

What Is the Difference Between Syndromic and Nonsyndromic Intellectual Disability?

Syndromic ID entails a set of clinical features and comorbidities that appear together with ID. Nonsyndromic ID is defined as the patient's ID being the sole clinical feature. Careful clinical assessment remains an important initial step in differentiating the 2 forms of ID, which can often be blurred due to subtle symptom manifestations. Details of pregnancy, birth, neonatal period, developmental history and progression, 3-generation pedigree, organ defects, and patterns of behavior can help in identifying the etiologic agent.

What Initial Testing Is Available for This Patient and Which Are Recommended?

First-tier genetic investigations include chromosomal microarray (CMA) analysis, fragile X testing, urine metabolic screen for metabolic disorders, and brain imaging. Currently, the CMA analysis and fragile X testing is recommended as the first-line diagnostic test for individuals with ID. Chromosomal microarray is used to detect gains and losses of DNA across the whole genome, called copy number variants. The test does not have the ability to assess copy neutral mutations such as substitutions, translocations, and inversions. Chromosomal microarray analysis has a diagnostic yield of 15% in children with cognitive impairment, autism spectrum disorder, and multiple congenital abnormalities. The test methodology is based on using conventional DNA hybridization of differentially labeled patient and reference DNA. Chromosomal microarray analysis is able to detect autosomal aneuploidies, sex chromosome abnormalities, deletions, and duplications, and some that include single-nucleotide polymorphism genotyping can even detect consanguinity. Second-tier genetic tests involve "next-generation sequencing" (NGS) technology that allows for parallel sequencing of either the entire genome- or panel-specific genes. These tests include whole-genome sequencing, whole-exome sequencing, and disorder-specific gene panels based on initial clinical assessment. Although NGS is increasingly being used to discover the genetic etiology of ID in individuals, widespread adoption of NGS in the clinical setting is still slowed by the high degree of genetic heterogeneity causing ID, cost, turnaround time, and clinical interpretation.

The patient's urine metabolic screen and brain magnetic resonance imaging (MRI) were both normal. Given the patient's global developmental delays and behavioral issues, further genetic testing was undertaken. Chromosomal microarray analysis was negative for copy number alterations. Familial mental retardation 1 (*FMR1*) gene mutation analysis by polymerase chain reaction (PCR) for fragile X syndrome was performed next (Figure 1).

What Are the Abnormalities Present in Figure 1?

Test results show patient has fragile X syndrome and size mosaicism with a full mutation *FMR1* allele (>200 CGG repeats) and a premutation *FMR1* allele with 86 repeats (c.-129CGG[86]). Mosaicism refers to the presence of more than one genetically distinct cell population in a person. Size mosaicism, a form of mosaicism, has been described as a relatively common phenomenon in fragile X syndrome and is defined as the presence of both full and premutation repeat expansions within or across cell types.[1-5]

Questions/Discussion Points

What Are Some Classic Clinical Features of Fragile X Syndrome?

Fragile X mental retardation syndrome is the most common known form of inherited ID, with a frequency of 1 in 1550 males and 1 in 2500 females. Clinical features include delays in motor skills development, delays in language development, autism spectrum disorder, hyperactivity, anxiety, and moderate to severe intellectual disabilities. During and after puberty, affected individuals classically manifest a long and narrow

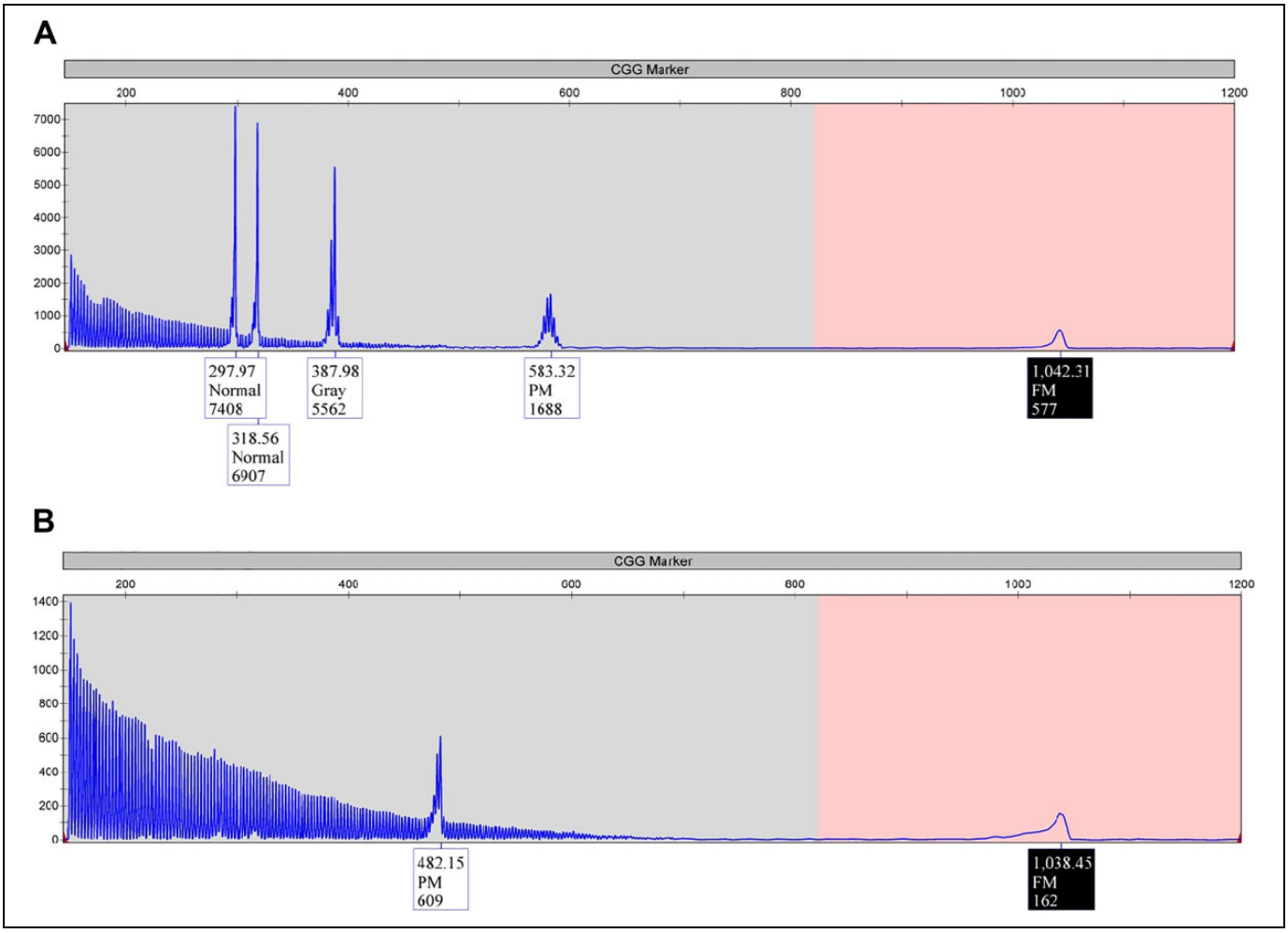

Figure 1. Triplet repeat PCR amplification. A, Positive control with 2 normal allele peaks (298 bp, 23 CGG repeats and 319 bp, 30 CGG repeats), one gray zone allele peak (388 bp, 55 CGG repeats), one premutation allele peak (583 bp, 120 CGG repeats), and one full mutation allele peak (1032 bp, >200 CGG repeats). B, Patient sample with one peak at 482 bp (86 repeats) and a second peak at 1038 bp (>200 CGG repeats). PCR indicates polymerase chain reaction.

face, large ears, hypermobile joints, and macro-orchism, especially in males. Girls with fragile X syndrome have similar mental and physical features to boys, although less severe and at lower rates. The significant phenotypic variation seen in one sex compared to the other is due to the *FMRI* gene location on the X chromosome. Males only have 1 X chromosome, whereas females have 2 X chromosomes. Therefore, all male X chromosomes would be potentially affected, while only 50% of female X chromosomes would be affected. The phenotypic variation between females is explained by random X-inactivation during embryonic development.[6-9]

Table 1. Mutant Allele Types Associated With Fragile X Syndrome.

Allele Type	Number of CGG Repeats	Methylation Status of *FMR1* Gene	Clinical Phenotype
Normal	<45	Unmethylated	None
Gray zone	45-55	Unmethylated	None
Premutation	56-200	Unmethylated	FXTAS, FXPOI
Full mutation	>200	Methylated	FTS

Abbreviations: FTS, fragile X syndrome; FXPOI, fragile X-associated primary ovarian insufficiency; FXTAS, fragile X-associated tremor/ataxia syndrome.

Outline the Etiology and Pathogenesis of Fragile X Syndrome

This X-linked disorder is associated with a disease-causing expansion of CGG trinucleotide units in the 5′ untranslated region of the *FMR1* gene with locus Xq27.3. The *FMR1* gene encodes an RNA binding protein involved in the regulation of neural messenger RNA stability and translation, which are important in brain development.

Four types of alleles are described, with different clinical manifestations depending on the number of CGG trinucleotide repeats (Table 1). Expansion of the number of CGG repeats to the full mutation range allele (>200 repeats) leads to hypermethylation of the *FMR1* gene promoter and ultimately transcriptional silencing. The premutation allele (56-200 CGG

repeats) is not typically associated with methylation of the *FMR* gene promoter. However, premutation alleles are unstable and can lead to trinucleotide expansion during maternal transmission. The allele can thus transition from a premutation to a full mutation allele, with odds depending on several factors including the range of repeats in the patient, number of AGG interruptions, and age of the mother. Clinical phenotypes are also specific to premutation carriers, including fragile X-associated tremor/ataxia syndrome or fragile X-associated primary ovarian insufficiency. Gray zone alleles (45-55) show mild instability and are precursors to premutation alleles, but they do not expand to a full mutation during oogenesis. Lastly, normal alleles contain up to 44 CGG repeats and are not associated with an increased risk of expansion in future generations.[10-15]

What Is the Etiology of Size Mosaicism in Fragile X Syndrome?

Expanded CGG trinucleotide units in the 5′ untranslated region of the *FMR* allele can lead to instability and lead to size mosaicism. Mosaicism involves the presence of 2 or more populations of cells from a single zygote that differ in genetic constitution. Size mosaicism refers to an individual with a subpopulation of cells with a different CGG repeat allele expansion length, such that one population exists with a full mutation and another population with a premutation. This is a relatively common phenomenon with an estimated frequency between 10% and 40%. In addition to size mosaicism, variation in methylation status of full mutations can occur. Mosaicism has been shown to affect penetrance of the disorder and phenotype. Males with a higher percentage of cells carrying a methylated allele in individuals with size mosaicism had a lower IQ rating, suggesting that small increases in *FMR1* expression can have a significant impact on cognitive function.[4]

In our example (Figure 1), the patient shows size mosaicism with a full mutation *FMR1* allele (200 CGG repeats) and premutation *FMR1* allele (86 CGG repeats). This result is due to a deletion in the CGG region and flanking sequences of the *FMR1* allele in a subpopulation of cells with full mutation alleles that were expanded during meiosis. The mechanism leading to CGG instability and deletion is still not fully understood but is thought to involve DNA polymerase slippage and strand mispairing leading to CGG length reduction. Studies have also shown CpG methylation in the *FMR1* allele may be more susceptible to deletions.[1-5]

What Are the Limitations of Conventional Polymerase Chain Reaction Amplification Versus Triplet Repeat Primed Polymerase Chain Reaction Amplification?

After genomic DNA isolation from peripheral blood leukocytes and enzymatic digestion, the fragile X locus in this patient was sized by triplet repeat primed (TRP) PCR amplification followed by high-throughput capillary electrophoresis. In TRP PCR, the forward PCR primer is located upstream of the *FMR1* CGG region, while the fluorescently labeled reverse primers overlap the CGG repeats and adjacent nonrepeat sequence. Multiple PCR products are produced, creating a ladder of amplicons on electrophoresis. Conventional PCR amplification of CG-rich regions is difficult due to increased stability and formation of DNA secondary structures. This in turn impedes DNA melting and progress of the DNA polymerase, which becomes even more challenging with increasing numbers of CGG repeats. Triplet repeat primed PCR amplification increases the amount of full-length trinucleotide repeat products and circumvents preferential amplification of normal alleles in patients with multiple alleles, such as in females and mosaics.[12,15]

What Is the Role of Carrier Testing in the Mother?

Full mutation expansion from premutation alleles is only acquired via maternal meiosis, with an increased risk for full mutation expansion the larger the premutation sized allele. Paternal transmission of premutation alleles does not result in full mutation expansion, with rare exceptions. Therefore, this patient's mother is highly likely to be an obligate carrier of a premutation or full mutation allele. Fragile X carrier testing can provide risks of having a subsequent child with fragile X syndrome or risk of early menopause. Increasing trinucleotide repeat premutation length correlates with an increased risk of expansion into a full mutation in future generations. A repeat length of 50-59 is associated with 3.7% risk of expansion to a full mutation in offspring, while a repeat length of 90-99 is associated with 80.1% risk of expansion to a full mutation in offspring.[14,16]

Teaching Points

- Although ID is a highly heterogenous condition, genetic factors account for the majority of cases.
- Clinical assessment is critical in determining syndromic versus nonsyndromic ID.
- First-tier genetic testing includes CMA, fragile X testing, urine metabolic screen, and brain MRI.
- Fragile X syndrome is the most common known form of inherited ID and is an X-linked disorder caused by CGG trinucleotide repeat expansion in the *FMR1* gene.
- Gray zone alleles are 45-55 CGG repeats, premutation alleles are 56-200 CGG repeats, and full mutation alleles are >200 CGG repeats.
- Full mutation alleles cause hypermethylation of the *FMR1* gene promoter and results in transcriptional silencing.
- Expanded CGG trinucleotide units in the 5′ untranslated region of the *FMR* allele can lead to instability and size mosaicism.

- Mosaicism involves the presence of 2 or more populations of cells from a single zygote that differ in genetic constitution.
- Size mosaicism is a relatively common phenomenon in fragile X syndrome and is the presence of both full and premutation repeat expansions within or across cell types.
- Triplet repeat primed PCR amplification is used in fragile X testing and circumvents preferential amplification of normal alleles in patients with multiple alleles.
- Carrier testing is important in fragile X syndrome to estimate the risk of premutation expansion.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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