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# When Whole Exome Sequencing Is Not Enough: Fragile X Premutation in a Child with ADHD and Speech Delay

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***Abstract:*** Background: Fragile X-associated disorders are among the most common inherited neurodevelopmental conditions encountered in pediatric practice. While whole exome sequencing (WES) has transformed the diagnostic evaluation of developmental delay and neurobehavioral disorders, repeat expansion disorders such as Fragile X syndrome remain undetectable by standard next-generation sequencing methodologies. This report describes a 7-year-old male child presenting with speech delay, attention deficit hyperactivity disorder (ADHD), behavioural concerns, and dysmorphic features including prominent ears, with a strong family history of developmental abnormalities. Initial WES identified variants of uncertain significance (VUS) in the CHD3 and DHX30 genes. Subsequent parental segregation studies demonstrated maternal homozygosity for the CHD3 variant and biparental heterozygosity for the DHX30 variant, reducing the likelihood of pathogenicity. Owing to persistent clinical suspicion, targeted FMR1 CGG repeat analysis was performed and revealed a premutation allele consisting of 173 CGG repeats. This case highlights the limitations of WES in detecting repeat expansion disorders and emphasizes the importance of phenotype-driven targeted molecular testing in children with neurodevelopmental disorders. The report further discusses the emerging neurobehavioral manifestations associated with Fragile X premutation in pediatric male patients and the diagnostic challenges posed by incidental VUS findings during genomic investigations.

***Keywords:*** Fragile X premutation, FMR1 gene, Whole exome sequencing, Developmental delay, Attention deficit hyperactivity disorder, Speech delay, Neurodevelopmental disorder, Variants of uncertain significance, CHD3, Diagnostic challenge

## INTRODUCTION

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism spectrum-related neurodevelopmental disorders. It results from CGG trinucleotide repeat expansion in the 5′ untranslated region of the Fragile X mental retardation 1 (FMR1) gene located on chromosome Xq27.3. Full mutation alleles (>200 CGG repeats) are associated with hypermethylation and transcriptional silencing of the FMR1 gene, leading to deficiency of fragile X mental retardation

protein (FMRP). In contrast, premutation alleles (55–200 repeats) were previously considered clinically silent but are now increasingly recognised to be associated with neuropsychiatric and neurodevelopmental manifestations, particularly in children and adults.

Advances in genomic medicine have substantially increased the utilization of whole exome sequencing in the evaluation of developmental delay, ADHD, autism spectrum disorders, and unexplained neurobehavioral conditions. However, conventional WES platforms are unable to reliably detect trinucleotide repeat expansion disorders such as Fragile X syndrome. Consequently, clinically relevant diagnoses may be overlooked if phenotype-guided targeted testing is not pursued.

This report presents a child with developmental delay and ADHD in whom WES demonstrated variants of uncertain significance in CHD3 and DHX30 genes, while subsequent targeted FMR1

testing identified a Fragile X premutation. The case underscores the importance of integrating clinical acumen with molecular diagnostics and highlights the interpretative complexities associated with incidental genomic findings.

## CASE PRESENTATION

A 7year old male child was evaluated for speech and language delay, behavioral difficulties, poor attention span, hyperactivity, and academic concerns. The child was the firstborn offspring of a non-consanguineous marriage. There was a significant family history suggestive of neurodevelopmental and behavioral abnormalities among extended relatives.

Developmentally, the child had delayed speech acquisition with persistent expressive language difficulties. Parents reported inattentiveness, impulsivity, hyperactivity, and behavioral dysregulation suggestive of ADHD. There was no significant motor delay. Social interaction was relatively preserved, although behavioral concerns had progressively increased with age.

On clinical examination, the child demonstrated dysmorphic facial features including relatively large and prominent ears. Neurodevelopmental evaluation revealed speech delay and behavioral abnormalities consistent with ADHD. There were no focal neurological deficits.

Given the developmental and behavioral phenotype along with dysmorphic features and family history, a genetic etiology was strongly suspected.

**Investigations**

Whole Exome Sequencing

Whole exome sequencing was performed using massively parallel sequencing technology. The clinical history provided to the laboratory included language and speech delay, ADHD, and neurodevelopmental concerns.

Phenotype-based analysis identified two heterozygous variants of uncertain significance:

1. CHD3 gene

- Variant: c.3160G>C (p.Ala1054Pro)
- Zygosity: Heterozygous
- Associated condition: Snijders Blok-Campeau syndrome

**DHX30 gene**

- Variant: c.208G>A (p.Ala70Thr)
- Zygosity: Heterozygous
- Associated condition: Neurodevelopmental disorder with variable motor and language impairment

The WES report explicitly stated that repeat disorders such as Fragile X syndrome are not amenable to detection by next-generation sequencing and therefore cannot be ruled out by the assay.

**Parental Segregation Analysis**

Sanger sequencing was subsequently performed in both parents for segregation analysis.

**Results demonstrated:**

**CHD3 variant**

- Mother: Homozygous for c.3160G>C (p.Ala1054Pro)
- Father: Variant absent

**DHX30 variant**

- Mother: Heterozygous
- Father: Heterozygous

The maternal homozygosity for the CHD3 variant in the absence of significant maternal neurodevelopmental manifestations reduced the likelihood of pathogenicity. Similarly, the presence of the DHX30 variant in both clinically unaffected parents suggested limited clinical significance.

Fragile X Molecular Analysis

Because of persistent clinical suspicion based on the child's phenotype, targeted FMR1 CGG repeat analysis was undertaken using triplet repeat primed PCR and fragment analysis.

**The study revealed:**

- 173 CGG repeats in the FMR1 gene
- Classification: Premutation range

The report concluded that the child carried an FMR1 premutation allele associated with Fragile X-associated disorders.

**Differential Diagnosis**

The differential diagnoses considered included:

- Fragile X-associated neurodevelopmental disorder
- Snijders Blok-Campeau syndrome
- DHX30-associated neurodevelopmental disorder
- Syndromic ADHD with developmental delay
- Autism spectrum disorder-associated genetic syndromes

Although WES initially suggested possible CHD3 and DHX30-related conditions, segregation analysis and

clinical correlation favored Fragile X premutation-associated neurodevelopmental manifestations as the principal diagnosis.

**Outcome and Follow-Up**

The child continued to receive multidisciplinary developmental intervention, including speech therapy, behavioral therapy, educational support, and ADHD management. The family received detailed genetic counseling regarding the implications of Fragile X premutation status, inheritance patterns, future reproductive risk, and the possibility of premutation expansion in subsequent generations.

Prenatal genetic evaluation was later performed during a subsequent pregnancy and did not reveal a pathogenic expanded FMR1 allele in the fetus.

## DISCUSSION

Fragile X syndrome remains one of the most important inherited neurodevelopmental disorders encountered in pediatric neurology and developmental pediatrics. While full mutation alleles are classically associated with intellectual disability, autism spectrum disorder, macroorchidism, and dysmorphic features, increasing evidence suggests that premutation carriers may also exhibit clinically significant neurobehavioral manifestations.

Historically, FMR1 premutation alleles were considered largely asymptomatic. However, emerging literature has demonstrated associations between premutation status and ADHD, anxiety disorders, executive dysfunction, autism spectrum traits, speech delay, and learning difficulties in pediatric populations. Male premutation carriers may display variable neurodevelopmental phenotypes even in the absence of full mutation expansion.

The present case is particularly instructive because initial WES demonstrated variants of uncertain significance in CHD3 and DHX30 genes, potentially leading clinicians toward alternative diagnostic pathways. CHD3-related Snijders Blok-Campeau syndrome is characterized by developmental delay, speech impairment, macrocephaly, hypotonia, and dysmorphic facial features. Similarly, DHX30-associated disorders involve language impairment and neurodevelopmental abnormalities. Nevertheless, segregation analysis demonstrated maternal homozygosity for the CHD3 variant and biparental heterozygosity for the DHX30 variant, thereby reducing the likelihood that these variants were causative.

This case therefore illustrates an increasingly common challenge in genomic medicine: the identification of incidental or uncertain variants during exome sequencing that may not fully explain the clinical phenotype. Over interpretation of VUS findings can potentially delay accurate diagnosis if careful phenotype-driven evaluation is not maintained.

A particularly important teaching point in this case relates to the limitations of WES. The sequencing report itself clearly acknowledged that repeat expansion disorders such as Fragile X syndrome cannot be reliably detected by conventional next-generation sequencing methodologies. Despite this known limitation, reliance solely on exome sequencing without targeted FMR1 analysis could have resulted in missed diagnosis.

The case emphasizes the continued importance of meticulous clinical examination and phenotype-guided targeted molecular testing, even in the era of advanced genomic technologies. Children presenting with ADHD, developmental delay, speech impairment, dysmorphic facial features, and positive family history should continue to undergo Fragile X testing regardless of WES findings.

Additionally, this report contributes to the growing body of evidence suggesting that Fragile X premutation status may not always represent a benign carrier state in pediatric males. Recognition of these subtler neurodevelopmental phenotypes has important implications for early intervention, family counseling, and reproductive planning.

**Learning Points**

- Whole exome sequencing cannot reliably detect trinucleotide repeat expansion disorders such as Fragile X syndrome.
- Fragile X premutation alleles may be associated with ADHD, speech delay, and neurodevelopmental abnormalities in pediatric males.
- Variants of uncertain significance identified on exome sequencing require cautious interpretation and clinical correlation.
- Segregation analysis can help clarify the pathogenic significance of genomic variants.
- Phenotype-driven targeted molecular testing remains essential in children with developmental and behavioral disorders.

Patient Perspective

The family expressed relief after obtaining a clearer explanation for the child's developmental and behavioral concerns. They appreciated the importance of targeted genetic testing and genetic counseling in understanding recurrence risk and future family planning.

**Ethics Statements**

Patient Consent for Publication

Written informed consent for publication of clinical details and genetic findings was obtained from the patient's parents/guardians

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