The Full (Mutation) Picture: One-Third of Patients with Fragile X Syndrome Present with Neurodevelopmental Disorders without Dysmorphism or Family History

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Background

Fragile X syndrome (FXS) has long been described as the most common single gene cause of intellectual disability (ID). In >99% of cases, the diagnosis is secondary to an *FMR1* full mutation (FM; >200 CGG repeats) resulting in aberrant *FMR1* promoter hypermethylation and loss of FMRP production.

Guidelines from the ACMG and AAP support *FMR1* analysis as a first-tier test for individuals with neurodevelopmental disorders (NDDs) including ID, developmental delay (DD), and autism spectrum disorder (ASD)[1,2]. Since the establishment of these guidelines in 2005 and 2011, respectively, several groups have called into question the utility of first-tier testing for FXS, citing a low diagnostic yield and frequent presence of suggestive clinical features (dysmorphism and/or family history) in affected individuals[3,4,5,6]. However, these studies are largely restricted to data from single institutions with low sample sizes.

In this report, we describe the presentation of FXS in a large NDD cohort from a pediatric-focused genetic testing laboratory.

Methods

Our study population comprised 135 individuals with an *FMR1* FM identified following 30,826 *FMR1* CGG repeat analyses performed between 2010-2021. The diagnostic yield and testing metrics (methylation analysis and presence of mosaicism) were assessed.

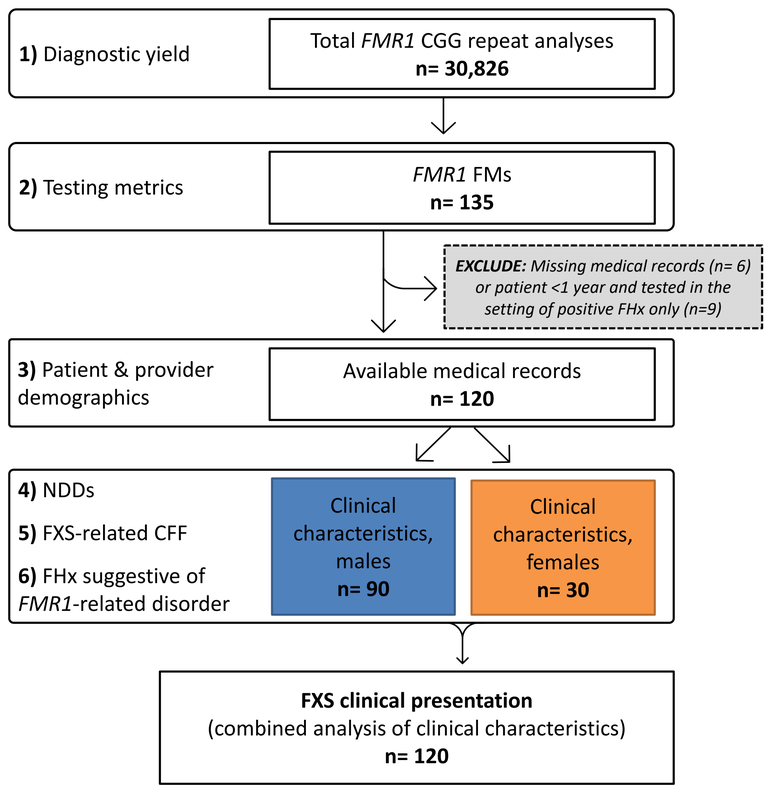
Retrospective chart review was then performed utilizing medical records and test requisition details provided at the time of initial test order. Pre-test genetic counseling session notes performed by the laboratory were used to corroborate provider details as available. Patients with missing medical records (n= 6) and those <1-year-old being tested in the setting of a positive family history of an *FMR1*-related disorder alone (n= 9) were excluded from analysis as available information was insufficient to draw meaningful conclusions.

In the remaining 120 patient cohort, patient/provider demographics were described, and clinical characteristics were analyzed including:

- **NDDs:** DD, ASD, ID
- **FXS-related craniofacial features (CFF):** Macrocephaly (>2 SD), long face, large/prominent ears, prognathism, high-arched palate, prominent forehead
- **Family history (FHx) suggestive of an *FMR1*-related disorder:** Maternal FHx of NDDs, known diagnosis of FXS, maternal carrier of *FMR1* premutation, FXPOI, FXTAS

Patient selection process, exclusions, and subanalyses associated with each data set are outlined in the accompanying flowchart.

SUBANALYSES:



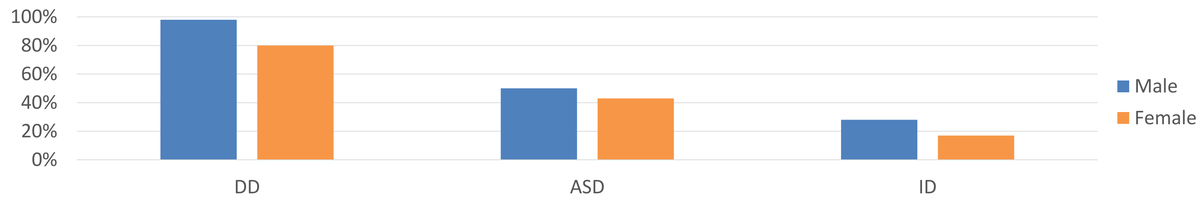
Results

SUBANALYSIS 1. Overall diagnostic yield of *FMR1* FMs: 0.44% (135/30,826)
SUBANALYSIS 2. Testing metrics: Methylation analysis results available in 93% of cases (repeat size mosaicism 14%, methylation mosaicism 6%, both size and methylation mosaicism 4%).
SUBANALYSIS 3. Demographics:

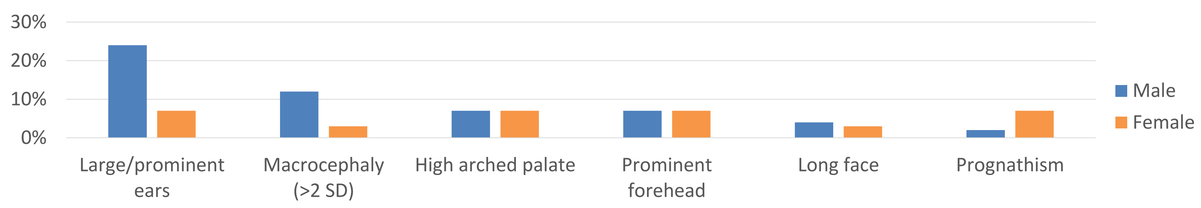
- Sex: 75% male, 25% female
- Median age at the time of test order: 5 years (range: 6 months-49 years)
- Ordering provider specialty: Developmental pediatrics (37%), neurology (33%), general pediatrics (22%), genetics (7%), psychiatry (1%)

Clinical Characteristics

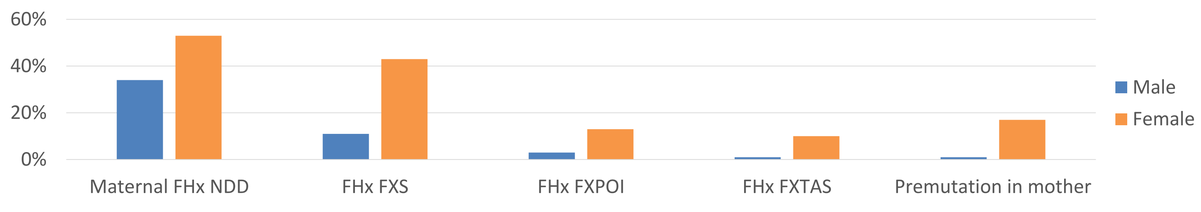
SUBANALYSIS 4. NDDs: One or more NDD in 100% of males and 93% of females.



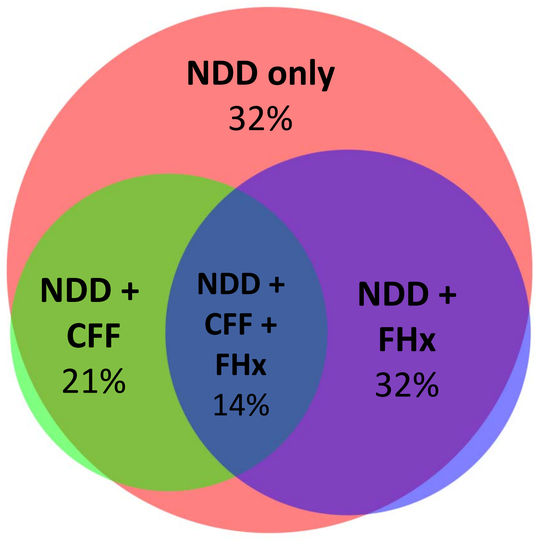
SUBANALYSIS 5. FXS-related CFF: One or more CFF in 40% of males and 23% of females.



SUBANALYSIS 6. FHx suggestive of *FMR1*-related disorder: One or more suggestive FHx element in 49% of males and 73% of females.



COMBINED ANALYSIS. FXS clinical presentation: Representation[7] of overlapping clinical characteristics (the presence of at least one NDD, CFF, and/or FHx feature) in males and females demonstrating approximately one-third (32%) of individuals with *FMR1* FMs present with NDDs only at the time of initial test ordering.



Limitations

Despite limitations inherent to retrospective study designs, such that data may be limited or missing, we believe that our results provide a summary of the actual ordering practices and clinical recognition of FXS-related features from a broad range of specialists and institutions at the time of initial test order.

Importantly, it is not possible to clarify for each included case what features were not reported as abnormal due to the presence of normal findings, versus omission of abnormal features that were not assessed or not yet present at the time that patients were initially evaluated.

Conclusions

Several groups have recently suggested that individuals with FXS typically present with clinical features sufficient to suspect the diagnosis on evaluation and family history alone. They have proposed that, in the absence of a suggestive presentation, *FMR1* analysis should be transitioned from first-tier to second-tier testing. The authors indicate that the low diagnostic yield compared to genome-wide analyses (CMA, exome sequencing) further supports a transition in guideline recommendations. However, our study indicates that approximately one-third of individuals with an *FMR1* FM have only NDDs, lacking other recognized FXS-suggestive features. Therefore, although encompassing a small percentage of the NDD population at large, a considerable number of individuals with FXS would receive a delayed diagnosis if testing was ordered as second-tier. In this setting, we must consider the value of an earlier diagnosis, including genetic counseling for recurrence risk, medical implications for other family members, and potential promise of drug treatment trials. Furthermore, we recognize the long wait times for a clinical evaluation and possibility of losing a patient to follow-up after initial testing is non-diagnostic. As such, the relatively low cost of *FMR1* analysis may outweigh the high cost of a delayed or missed diagnosis, justifying the current first-tier status of this testing.

With phenotypes such as NDDs that are characterized by high genetic heterogeneity, we face a greater need for the adoption of genome-wide technologies with the ability to detect repeat expansions as well as other diagnostic genomic variants. One technology with this capability is optical genome mapping (OGM), which can simultaneously detect structural and copy number variants, absence of heterozygosity, triploidy, and *FMR1* FMs[8,9]. Additionally, OGM provides an estimated CGG repeat number for non-FM alleles, as well as the upper and lower limits of plausible repeats with 99% probability. Therefore, OGM can prioritize borderline alleles (such as large premutation alleles) for further confirmatory testing. As comprehensive genome-wide technologies are adopted, this data suggests that *FMR1* CGG repeat analysis continue to be offered as a first-tier test in individuals with NDDs.

References

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