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CASE REPORT

High performing male with fragile X syndrome with an unmethylated *FMR1* full mutation: The relevance of clinical and genetic correlations

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Key Clinical Message

A high performing male with an unmethylated full mutation in the fragile X messenger ribonucleoprotein 1 (*FMR1*) gene surpassed our expectations into young adulthood. Although initial genetic findings helped make a correct fragile X syndrome (FXS) determination, the report was insufficient. Ten years later, we repeated and conducted additional genetic and clinical studies to determine whether more information could assist with treatment and counseling. The genetic findings were very consistent with his high functioning and would have enabled us to be more confident about a good developmental outcome had they been available previously. As FXS enters the mainstream of well-understood genetic disorders and technological advancements improve genetic tests, it should be clearer to clinical providers what a full FXS assessment could include to provide high quality information for care. For individuals with FXS who are high functioning, their families and clinical professionals would benefit from knowing more genetic findings, including, most importantly, methylation status, but also the FMR1 protein (FMRP) level and mRNA level. While we now know that obtaining only the CGG repeat number is not always adequate to inform accurate clinical care, future studies are likely to show the benefit of studying other biomarkers, such as mRNA levels.

KEYWORDS

fragile X syndrome, unmethylated full mutation, high functioning, *FMR1*

1 | INTRODUCTION

Fragile X syndrome (FXS) results from the combination of a large CGG repeat trinucleotide expansion (>200 repeats) within the fragile X messenger ribonucleoprotein 1 (*FMR1*) gene and methylation of a CpG island proximal to the repeat that leads to silencing of the gene and loss or drastic reduction of the gene product, fragile X messenger ribonucleoprotein (FMRP).[1] Approximately one in 4000 males is thought to have FXS.[2] While the majority of males with FXS have a moderate to severe intellectual disability (ID), approximately 13% of males may be higher functioning, at least during the childhood years.[3] DNA studies on these high functioning males often show mosaicism with partial or complete failure of the expanded CGG repeat to methylate.[3,4] These males often produce enough FMRP to explain their lack of ID.[5] Here, we present a 22-year-old male, Patient A, who graduated from college and does not have ID or severe psychiatric problems despite having FXS. This patient demonstrates the need for increased availability of comprehensive genetic testing, including CGG repeat count, gene methylation status, FMRP level, and *FMR1* mRNA expression level to provide adequate understanding of the condition and for professionals to continue to learn more about the variability of FXS.

2 | METHODS

2.1 | Participant

Patient A was selected for this study because of a CGG repeat expansion in his *FMR1* gene and his high level of functioning. Neurodevelopmental, psychological, and molecular assessments had been performed throughout his childhood and adolescence.

2.2 | Genetic studies

2.2.1 | First study (2009)

A standard fragile X (FX) DNA study was ordered through the Duke Pediatric Genetics Clinic and the Duke Molecular Laboratory. As was the standard at the time, PCR and Southern blot were performed to determine the CGG repeat length in exon 1 of the *FMR1* gene and methylation status of the *FMR1* gene locus.

2.2.2 | Second study (2019)

FMR1 CGG sizing, methylation status, *FMR1* mRNA, and FMRP expression were performed at the New York State Institute for Basic Research in Developmental Disabilities. Genomic DNA was isolated from whole blood and used for *FMR1* repeat size analysis and Southern blot. CGG repeat primed PCR analysis (RP-PCR/CE)[6] and gene-specific PCR analysis (GS-PCR/CE) were performed to determine the FX CGG repeat size using AmplideX *FMR1* PCR/CE Kit (Asuragen, TX), followed by Southern blot analysis on EcoRI and EagI digested DNA probed with StB12.3.[7]

mRNA preparation followed the manufacturer protocol for PAXgene tube specimens, and *FMR1* mRNA was assessed using RT-PCR,[8] where *FMR1* mRNA levels were measured relative to b-glucuronidase (*GUS)* expression and normalized to the level of *FMR1* mRNA relative to *GUS* in a reference long-term lymphoblastoid (LTL) cell line. For quantitative FMRP (qFMRP) assessment, whole blood was spotted on dried blood spot (DBS) cards (GE Healthcare Life Sciences, Marlborough, MA). FMRP extraction from DBS followed previously published Luminex-based methods.[9] The FMRP was quantified and reported as the protein concentration in the extract (pM = pico mole/ L) and was further normalized to the number of white blood cells (WBCs) to give a more refined representation of the protein expression (reported as picogram FMRP detected per thousand WBCs).

2.2.3 | Third study (2022)

Original archived DNA was retested in triplicate and reanalyzed using a new generation of assay employing CGG repeat primed PCR.[6] Methylation analysis was also performed using a PCR-based high resolution and sensitivity assay.[6] This assay estimates the percentage of methylation by comparing the methylation-sensitive restriction endonuclease digested PCR amplification products. The percentage methylation is determined by the ratio of methylated to total PCR product of each *FMR1* allele.

2.3 | Psychoeducational and behavioral evaluations

Records were obtained from the patient's schools and other medical centers. Two authors evaluated the patient using standardized measures.

3 | RESULTS

3.1 | Case report

Patient A is a Caucasian male who was initially evaluated at 9 years, 5 months. Chief concerns included inconsistent school performance, difficulty coordinating both sides of

his body, verbal apraxia, fine motor difficulties, anxious behaviors and possible attention-deficit/hyperactivity disorder (ADHD). He had an Individualized Education Plan (IEP) to address educational concerns. Physical examination was notable for enlarged ears, loose joints and flat feet. At a subsequent genetics evaluation, Patient A was found to have characteristics of Ehlers-Danlos syndrome,[10] and his FXS study showed an unmethylated expanded CGG repeat estimated to be in the range of 180–270 (Table 1).

3.2 | History

Patient A was born after a 39-week gestation weighing 9 lb and 5 oz. He had protruding ears without significant cartilage. He was poor at nursing and gagged at mealtimes until age six. He underwent otoplasties at age six.

Patient A began receiving speech-language therapy in preschool, and this continued through sixth grade when it was discontinued due to his age according to his mother. Speech was only 50% intelligible at kindergarten entry. People often asked him to speak more slowly and clearly, or to repeat himself. He qualified for occupational therapy (OT) services in kindergarten to address delays in handwriting and visual-motor skills. OT services continued until 10th grade. In first grade, he was diagnosed with a learning disability in reading and written language and began receiving special education services.

In fourth grade (age 9), he was diagnosed with ADHD, predominantly inattentive type, and anxiety and started on stimulant medication. He continued to receive special education services and classroom accommodations. A psychological evaluation showed discrepancies between his cognitive ability and academic achievement in reading comprehension, word attack skills, quantitative concepts, writing samples, and spelling (Table 2).

In middle school, Patient A participated in recreational soccer and horseback riding and played the saxophone. He received school-based physical therapy (PT) and made progress with coordination. He suffered from weekly headaches and increasing anxiety, particularly over bad weather conditions. He also had obsessive thoughts about food and television. Sertraline was started at age 12.

By high school, Patient A had excessive weight gain. He continued treatment for ADHD and anxiety and received monthly PT consultative services at school until mid-tenth grade when his IEP was eliminated. He experienced several episodes of dislocated joints involving an elbow and knee. He did not participate in athletics. He obtained his driver's license and drove to school daily. Patient A excelled in honors-level classes with testing accommodations and was inducted into the National Honor Society. He graduated 69th out of 300 students (GPA = 3.8), scored a 19 on the ACT and 980 on the Math and Verbal portions of the SAT (1000 is average).

Patient A graduated cum laude (GPA = 3.45) from college with a bachelor's degree in Food Services Management. He usually lived on campus and received over $30,000 annually in scholarships. Following his freshman and sophomore years, he secured an internship preparing food at the faculty club of a university. Initially, his parents provided transportation due to concerns about the long driving distance and his fatigue at the end of an 11-hour shift. The second summer, Patient A rented an apartment close to work and drove himself. Currently, Patient A works as a Food Service Supervisor at a local college and lives with

TABLE 1 Fragile X testing results and IQs.

Subject	*FMR1* PCR	Southern blot (kb)	Methylation status	*FMR1* mRNA	FMRP	IQ
Patient A (2009)	Allele expansion suspected due to broad expansion size range	3.31–3.58	Unmethylated	N/A	N/A	112 (RIAS)
Patient A (2019)	>200 CGG repeats	>3.4	Unmethylated	3.03	7.55 pM or 0.347 pg /10^3 WBC	91 (WAIS-IV)
Patient A (2022)	280–284	-	Unmethylated	–	–	91 (WAIS-IV)
Presumed normal male	10–44	2.8	Unmethylated	1.42 +/− 0.25	28 pM or 1.83 +/− 0.28 pg /10^3 WBC	85–115
Low premutation male	55- <100	2.9–3.4	Unmethylated	2.44 +/− 0.32	0.5 to 1	85–115
High premutation male	100–200	2.9–3.4	Unmethylated	7.21 +/− 0.63	0.5 to 1	85–115
Full mutation male	>200	5.7	Full Methylation	0	0	<70

TABLE 2 Summary of neurodevelopmental testing results.

Age at Evaluation	Test Administered	Age Standard Score
	Gross Motor/Visual-Motor Integration	
4 yrs, 10 mo	Peabody Developmental Motor Scales-Second Edition: Fine Motor Subtest[a]	97
5 yrs, 7 mo	Test of Visual Motor Skills-Revised	70
6 yrs, 5 mo	Beery-Buktenica-Developmental Test of Visual-Motor Integration-5: Beery Vmi	98
10 yrs, 5 mo	School physical therapist report[b]	
16 yrs, 9 mo	Beery-Buktenica Developmental Test of Visual-Motor Integration-6	
	Beery VMI	73
	Visual Perception	101
	Motor Coordination	90
	Language/Motor-Speech	
5 yrs, 1 mo	Preschool Language Scale-4[c]	
	Auditory Comprehension	109
	Expressive Communication	96
9 yrs, 1 mo	Clinical Evaluation of Language Fundamentals-4[d]	
	Receptive Language	93
	Expressive Language	83
	Core Language	87
11 yrs, 8 mo	Test of Language Development: Intermediate 4	
	Listening	89
	Organizing	92
	Speaking	86
	Grammar	72
	Semantics	104
	Spoken Language	86
18 yrs, 9 mo	Goldman-Fristoe Test of Articulation-3	
	Sounds-in-Words	56
	Sounds-in-Sentences	60
	Clinical Evaluation of Language Fundamentals-5: Pragmatics Profile SS[e]	6[e]
	Cognition	
6 yrs, 5 mo	Differential Ability Scales: School-Age Level	
	Verbal Reasoning	102
	Nonverbal Reasoning	110
	Spatial	95
	General Conceptual Ability	103
8 yrs, 9 mo	Reynolds Intellectual Assessment Scales	
	Verbal Intelligence Index	103
	Nonverbal Intelligence Index	122
	Composite Intelligence Index	112
	Composite Memory Index	102
16 yrs, 9 mo	Wechsler Adult Intelligence Scale-IV	
	Verbal Comprehension Index	102
	Perceptual Reasoning Index	98
	Working Memory Index	83
	Processing Speed Index	81
	Full Scale IQ	91

TABLE 2 (Continued)

Age at Evaluation	Test Administered	Age Standard Score
	Academic Skills	
6 yrs, 5 mo	Woodcock-Johnson-III, Tests of Achievement	
	Letter-Word Identification	116
	Passage Comprehension	108
	Writing Fluency	95
	Calculation	122
	Applied Problems	108
	Math Fluency	110
	Quantitative Concepts	110
	Writing Samples	117
8 yrs, 9 mo	Woodcock-Johnson-III, Tests of Achievement	
	Letter-Word Identification	99
	Passage Comprehension	92
	Calculation	99
	Applied Problems	111
	Broad Written Language	95
16 yrs, 9 mo	Woodcock-Johnson-IV, Tests of Achievement	
	Letter-Word Identification	90
	Passage Comprehension	94
	Calculation	104
	Applied Problems	108
	Broad Written Language	98
	Broad Oral Language	95

Note: For standard scores, mean = 100 and standard deviation =15. Scores between 85 and 115 are in the average range.

[a]OT noted poor quality of his fine motor skills.

[b]School physical therapist reported "very limited gross motor skills," mainly due to low muscle tone and poor left to right coordination.

[c]Speech intelligibility for words within normal limits. Less intelligibility during connected speech.

[d]"Has considerable difficulty with maintaining accurate productions in connected speech when he is not monitoring his rate."

[e]For scaled scores, mean = 10, standard deviation = 3.

his parents. Patient A aspires to have his own restaurant. He has several close friends, including some since childhood, but is not in a romantic relationship.

3.3 | Family history

Patient A is the younger of two children. His older brother does not have FXS or Ehlers-Danlos syndrome. The mother has the *FMR1* premutation (PM) allele with 79 CGG repeats. She worked as a special education teacher specializing in autism while completing a PhD in Curriculum and Instruction. Currently, she teaches in a demonstration school at a university.

Two sisters of Patient A's mother were diagnosed with bipolar disorder and subsequently found to be FX carriers. The maternal grandfather has an expanded *FMR1* allele with 77 CGG repeats. Several of his relatives were reported to have symptoms of Parkinson's or fragile X-associated tremor/ataxia syndrome (FXTAS). The maternal grandmother had a history of mood instability, and the maternal great grandmother was institutionalized once for depression.

Patient A's father's history includes childhood seizures, mitral valve prolapse (repaired) and flat feet. He is thought to have Ehlers-Danlos syndrome. He received speech therapy until second grade and has a sister with bipolar disorder. The father holds a PhD and is a college administrator.

3.4 | Physical examination

At age 20, Patient A's height was 181.8 cm (5′11 in, approximately 75th percentile). His weight was 120.7 kg (266 lbs, > 95th percentile). His BMI was 37.1 (obese);

head circumference was 58.9 cm (> 98th percentile). He had somewhat enlarged ears (7.0 cm in length, > 50th percentile), somewhat of a broad forehead, but not a long face. Patient A wore glasses for nearsightedness and had an intact palate. The heart and lung examinations were normal. He had soft skin and hyperextensible elbow and thumb joints. Patient A performed a self testicular examination using Prader beads and reported that his testicular volume was 20 mL (normal range). He refused a testicular examination in the clinic. The neurological examination was intact. He was well-related. The deep tendon reflexes of his upper extremities were symmetrical and of normal intensity. His knee and ankle reflexes were brisk and symmetrical. On an oral-motor assessment, he was able to move his tongue from side to side, touch his lips with his tongue, and pronounce words like "puh-tuh-kuh" and "linoleum." He was difficult to understand.

3.5 | Developmental and psychological evaluations

Patient A completed multiple evaluations (Table 2). Three composite IQs were in the average range, although his most recent Full Scale IQ (age 16 years, 9 months) was at the lower end of average.[11–13] His Processing Speed Index and Working Memory Index contributed to the lower IQ.

Patient A's academic skills, as measured by the Woodcock-Johnson Tests of Achievement,[14,15] have also remained in the average range. Visual-motor difficulties were noted and his visual-motor integration ability was significantly below average at age 16.[16–19] At age 10, he was noted to have limited gross motor skills due to low muscle tone and poor coordination.

Speech and language concerns involved his very poor intelligibility during connected speech. Apraxic-like characteristics were noted at age four. Patient A's latest speech and language assessment (age 18 years, 9 months) showed moderate dysarthria characterized by reduced articulatory precision, fast rate of speech, and abnormal resonance. Difficulties with articulation were apparent on the Goldman-Fristoe Test of Articulation-3 due to a phonological process known as gliding.[20] Patient A substituted w and r across all word positions and in pr and br clusters (e.g., saying "wabbit" for "rabbit"). His score on the Clinical Evaluation of Language Fundamentals-5 Pragmatics Profile parent report, a measure of verbal and nonverbal social communication skills,[21,22] was below average.

At age 16, standardized behavior checklists were completed by Patient A's mother. Concerns regarding anxiety, negative mood and peer relations were apparent (Table 3).[23–25] Patient A's T-scores on the self-report versions of these behavior checklists were all within the normal range.

TABLE 3 Elevated scales for patient A (age 16 years, 8 months) on three behavior checklists completed by his mother.

Child Behavior Checklist for Ages 6–18	T-score
Withdrawn/Depressed	78
Social Problems	66
Thought Problems	66
Conners 3-Parent	
Peer Relations	83
Multidimensional Anxiety Scale for Children-2, Parent	
Generalized Anxiety Disorder Index	69
Humiliation/Rejection	70

Note: Mean T-score = 50, standard deviation = 10. T-scores between 40 and 60 are in the average range. T-scores ≥70 are considered to be clinically significant.

3.6 | Genetics evaluation and molecular findings (Table 1)

In 2009, Patient A underwent a genetics evaluation. His hypermobility and positive paternal medical history were suggestive of Hypermobile Ehlers-Danlos syndrome. A FX DNA study, chromosome analysis, and a chromosomal microarray analysis were ordered because of his physical and cognitive characteristics. The karyotype (46, XY) and microarray analysis (arr snp/cn 1–22(1729384) x2, X (84594)x1,Y(8392)x1) were normal.

The Southern blot, using the EcoRI/EagI digest, revealed a broad band from roughly 3.31 Kb to 3.58 Kb in size, which was unmethylated. The expanded CGG repeat was estimated to range from 180 to 270. PCR was unable to size the repeats. Results were interpreted by the clinicians to represent a mosaic form of FX that was a PM and an unmethylated full mutation (FM) (Table 1).

The second FXS molecular, *FMR1* mRNA, and FMRP evaluation was done in 2019 due to Patient A's high level of functioning and our desire to seek more information. *FMR1* CGG repeat primed and gene-specific PCR analysis were performed to evaluate *FMR1* gene structure (AmplideX *FMR1* PCR/CE, Asuragen, TX), and showed a FM allele with >200 CGG repeats. The Southern blot showed a broad band above 3.4 kb indicating the unmethylated status of the allele (Figure 1).

The *FMR1* mRNA level was 3.03 relative to β-GUS when normalized to reference cell line's level of *FMR1*

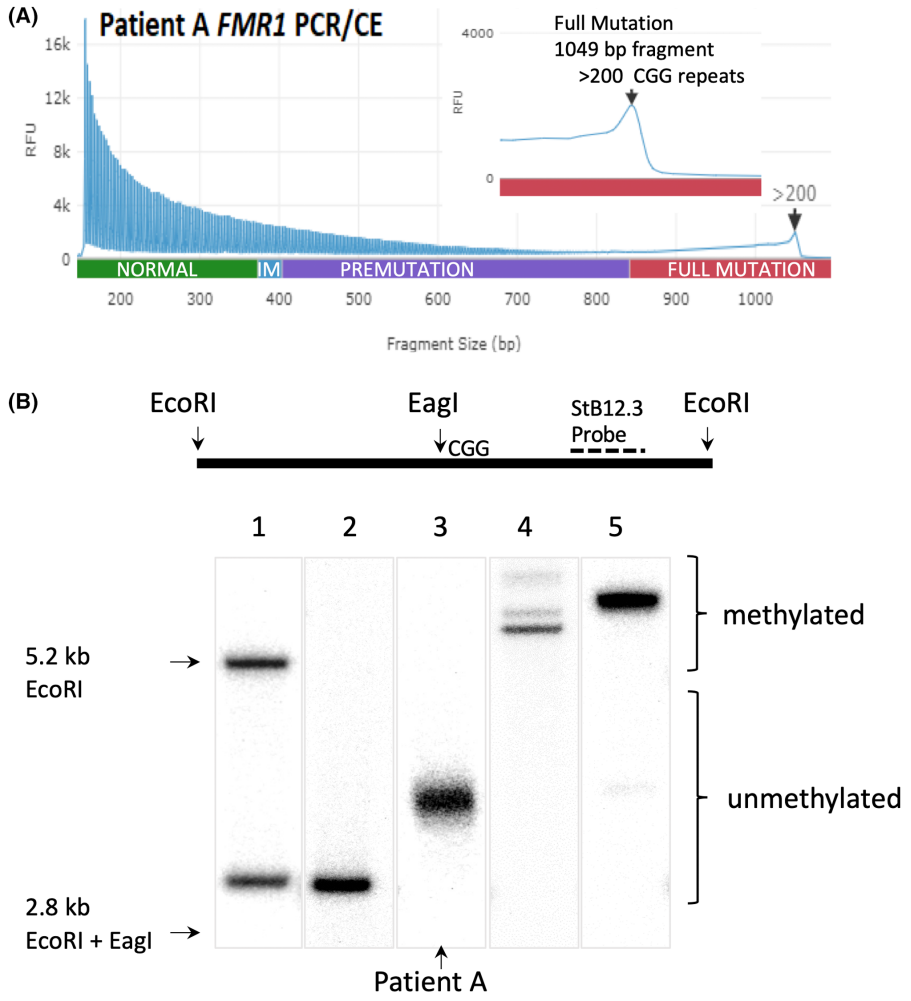

FIGURE 1 Molecular analysis of *FMR1* allele for Patient A. (A) AmplideX *FMR1* PCR/CE analysis of *FMR1* alleles with characteristic full mutation fragment of >200 CGGs. (B) Southern blot analysis of Patient A's DNA shows unmethylated full mutation allele. Lanes 1 and 2 show normal alleles in female and male (respectively) with characteristic unmethylated 2.8 kb EagI-EcoRI fragment detected by StB12.3 probe. Lane 1, 5.2 kb EcoRI digestion product of normal allele is a result of X-inactivation in a female. Lane 3 (Patient A), full mutation allele shows EagI-EcoRI fragment >3.4 kb indicating unmethylated status of the allele. Lane 4, full-mutation allele EcoRI fragments above 7 kb with >200 CGG are not cleaved by EagI due to methylation. Lane 5, full-mutation methylation mosaic allele EcoRI fragment at 9 kb is fully methylated; a faint EagI-EcoRI band at 3.7 kb contains >200 CGG and represents some unmethylated alleles in a male with methylation mosaicism.

mRNA (GUS), showing a 2.1 fold increase in *FMR1* mRNA level compared to males with normal CGG repeat numbers ($n < 55$ CGG).

The FMRP was detected at the level of 7.55 PM or 0.347 pg/10^3 WBC or about 19% of normal FMRP expression in males. The reference range for males with normal *FMR1* allele is 1.83+/−0.28 pg/10^3 WBC (Adayev, unpublished data) or 28 PM FMRP reported for males in a typically developing control group.[26]

In 2022, the original archived DNA was reanalyzed and showed 280–284 CGG repeats with no AGGs identified.[27] Methylation analysis using a PCR-based high resolution and sensitivity assay demonstrated 0% methylation.

4 | DISCUSSION

This case study focuses on the remarkable functionality of Patient A. He presented with physical and developmental characteristics consistent with a mild FXS phenotype because his FXS gene is fully expanded but unmethylated.[3,4,28] Patient A typically would not have been evaluated for FXS because he did not have an ID, was not autistic, and did not have a known family history of FXS. It was the clinical decision of the pediatrician to refer him to genetics and of the geneticist to perform testing. His initial genetics report (2009) was consistent with PM/FM size mosaicism reported as a range without methylation of the gene. The inability to size the CGG repeat was the

limitation of the Southern blot at the time. However, as a Southern blot was performed, it was possible to identify that the larger CGG repeats were unmethylated. Although this genetics report was encouraging about his long term potential, some of us were hesitant to expect full functionality, since mosaic status can be associated with significant limitations and because he was clearly impacted by some characteristics of FXS.[4] Lack of methylation of the FM component of the gene should have encouraged all of us about his potential. Since Patient A's IQ was average, it seemed possible that most of his DNA would be in the PM range. This was not fully convincing because he had numerous characteristics consistent with the FM. His second DNA report was that of a FM male without methylation and was more consistent with limited reports of high functioning FXS males with greater than 200 CGG repeats but without methylation.[3,29–31]

Reports on high functioning males with FXS are few. Although we have followed at least 500 individuals with FXS and have encountered individuals with high functioning FXS,[8,32,33] Patient A is the highest functioning male that we treated. For over 10 years, he surpassed our expectations. He received high grades and was awarded scholarships. Despite having ADHD and anxiety, he functions well on standard medications. He is not as impaired by mental health concerns as some individuals described in the literature.[29,30] Articulation is a concern, but he can clarify his speech when concentrating. Past evaluations describing his speech disorder were inconsistent. As a four-year-old, his speech was described as apraxic-like and, as a teenager, dysarthric.

In a 1994 review by Hagerman et al. of 250 male patients with FXS, 29 (13.1%) of 221 who had psychological testing had IQs ≥70, the IQ cutoff for ID.[3] Seven had IQs greater than 85. None had an IQ above 100. Ten of these 29 males (out of 19 evaluated) had declining IQs below 70 on subsequent testing. Three of 22 high functioning males with both *FMR1* DNA testing and Southern blots (out of 17 subjects tested) had >200 CGG repeats that were unmethylated. All three had IQs above 70 (100, 94, 73). None of the individuals studied ($n = 36$) with an ID had this finding. Recently, Meng et al. reported on a cohort of males with FXS and methylation mosaicism, and they functioned higher than males with the FM or size mosaicism.[4] Patient A did have a drop in IQ, but he continued to test in the average range. His performance on the timed subtests may have contributed to the lower score at age 16.

High functioning males with FXS are not commonly seen. Hagerman et al.'s report detailed above and others suggest that as few as 2% of males with FXS may have this molecular finding.[3,28,31] On the other hand, these individuals may be under-identified since we would not evaluate cognitively intact males for FXS unless there is autism or a family history of FX.

We did not anticipate Patient A's current level of success so, to better understand his high functionality, we performed additional studies.[34,35] The second set of studies clarified Patient A's FX status. A fully expanded unmethylated FM was more consistent with his clinical phenotype.[3] The third study demonstrated that testing methods have improved over time to make more accurate results possible.

Patient A was reported to have about 20% of normal FMR1 protein production. Greater than 35% of FMRP production has been associated with a mean IQ of 85 among males with FXS.[5,36–38] This information suggests that modestly increased FMRP may improve cognitive functioning for some individuals.[5]

Patient A's mRNA result was approximately double the normal level. This was lower than anticipated. Both males with high PMs and unmethylated FMs have high mRNA levels.[8,30,38,39] Up to a six fold increase in the amount of mRNA produced in response to a low protein level might have been expected.[8,40–42]

As Patient A has an elevated mRNA level, he could be at risk for developing FXTAS. This late onset disorder is characterized by problems with movement and cognition and is generally associated with the *FMR1* PM[43]. We are aware of three case studies of men with FXTAS who presented with the fully unmethylated FM allele.[30,39,44]

5 | CONCLUSIONS

About 13% of males with the *FMR1* FM present without an ID.[3] One reliable subgroup of high functioning males will have the *FMR1* FM without methylation.[3,4] These cases are most likely to be detected when relatives with FXS are diagnosed.[3,31,45] Treating Patient A for over 10 years has been of utmost importance and has raised our standard for what we thought was possible for patients with FXS. This patient's case history raised numerous clinical and genetic counseling concerns, including the difficulty of obtaining comprehensive genetic and psychological evaluations. Over the span of many years, Patient A received these services.

Lack of methylation of the fully expanded *FMR1* mutation is the main genetic finding that predicts a good outcome. Often in our clinic, a FM status is available (>200 repeats) but without a description of the methylation status. Without knowing this, it is difficult to know which individuals, especially children, may be high functioning. This diminishes our capacity to provide adequate developmental care. At our institution, a second study may be sent to a reference laboratory for

methylation status, when the patient is high functioning. Genetics laboratories should encourage obtaining methylation status when high functioning males are seen. In Patient A's case, it was initially mistakenly thought that the patient was a true PM/FM mosaic. Even though we knew that the FM was not methylated, we thought that his high functioning was due to his PM status and his clinical findings were due to his FM status. There was some concern that there could have been a small amount of methylation of the larger repeat DNA in other tissues or that methylation status was not reported as it was below the level of detection. A decade later, with improved and more sensitive testing, CGG repeats were reported to be completely in the range of the FM and no methylation was detected. This second report was consistent with his high functionality, his clinical findings, and literature findings.[3]

Greater knowledge about the FMRP level would have also been useful, although we may have thought that such low FMR1 protein production would have been consistent with ID.[5,36] This patient appears to be an outlier compared to most reported cases.[5] There is one published report of a patient with low protein production (25% in peripheral blood cells and 50% in fibroblast cells) and IQ scores of 90 and 84. He had numerous medical and psychiatric concerns as an older man.[44] Another report described a male who had a FM and methylation mosaicism with unmethylated alleles in both the PM and FM range. He produced 20% of normal FMRP and had a Full Scale IQ of 107, placing him in the average range.[46]

We may eventually understand more about how *FMR1* mRNA levels correlate with overall functioning and adult onset FXTAS. *FMR1* mRNA levels appear to vary greatly in patients with unmethylated FMs.[8] Additionally, information on mRNA levels seems to be relatively limited and understanding variations in these levels may be important.[41]

In our experience, FXS testing varies across laboratories and some only provide information on whether CGG repeat size is greater than 200. This is a disservice for high functioning individuals with FXS. They should have a thorough genetics assessment including methylation status.[34] In addition, several laboratories were contacted before we found one that would test the patient's protein and mRNA level using a blood sample. In the area of clinical trials, it may eventually become necessary to stratify patients based on the severity of their condition, and this clinical research would also call for more detailed laboratory studies.[47,48]

We anticipate that Patient A will remain fully independent but numerous issues regarding genetic counseling for FXS, mental health and overall wellness still need to be addressed. His daughters would carry the gene for this condition at least in the PM state.[33,39] He will continue to need follow-up care for anxiety and ADHD as well as health problems such as obesity. He may be at risk for FXTAS and would do well to avoid toxins such as alcohol.

AUTHOR CONTRIBUTIONS

Meg Shieh: Conceptualization; formal analysis; investigation; writing – original draft; writing – review and editing. **Keren Amkraut:** Writing – original draft. **Gail A. Spiridigliozzi:** Data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Tatyana Adayev:** Data curation; formal analysis; investigation; methodology; visualization; writing – review and editing. **Kaylea Nicholson:** Data curation; formal analysis; investigation; writing – original draft. **Allyn McConkie-Rosell:** Data curation; formal analysis; investigation; writing – review and editing. **Marie T McDonald:** Formal analysis; investigation; writing – review and editing. **Malinda Pennington:** Validation; writing – original draft; writing – review and editing. **Siby Sebastian:** Formal analysis; investigation; validation; writing – review and editing. **Ave M. Lachiewicz:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Nothing to declare.

DATA AVAILABILITY STATEMENT

De-identified data will be made available upon request.

ETHICS STATEMENT

This project was approved by the Duke IRB. The subject signed informed consent. Protocol ID: Pro00094584. Date of issue: 8/13/2018. The data reviewed in preparation of the manuscript were de-identified when possible.

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