Case Report

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Identification of a male with fragile X syndrome through newborn screening

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Summary **A pilot newborn screening (NBS) study for fragile X syndrome was recently conducted at the University of California, Davis Medical Center. The screening study identified a case of a male with the full mutation completely methylated and no detectable expression of the fragile X mental retardation-1 (*FMR1*) gene. The patient was initially seen in clinic at the MIND Institute, for medical follow-up and a genetic counseling session at the chronological age of 3 months. Since then, he has been seen in clinic every six months for follow up, medical examination and developmental assessments. Longitudinally administered developmental testing of the infant has revealed persistent delays in development, consistent with fragile X syndrome. Cascade testing revealed that the patient's mother and two siblings also have the full mutation. The patient has been receiving speech and language therapy, combined with physical and occupational therapies on a weekly basis since the age of one year. He is currently being treated with 2.5 mg of sertraline, which has been demonstrated to be helpful for improving language in young children with the syndrome.**

Keywords: *FMR1* full mutation; trinucleotide repeat diseases; genetic counseling; cascade testing

1. Introduction

Fragile X syndrome (FXS) is the most common cause of inherited intellectual disabilities (ID), and is due to the expansion of a trinucleotide CGG repeat in the promoter region of the *FMR1* gene. Individuals with alleles harboring 200 or more CGG repeats are usually subject to hypermethylation of the *FMR1* gene, which impairs the production of *FMR1* protein (FMRP) and causes the abnormal neural development and subsequent intellectual disability (ID) typical of FXS. FXS affects as many as 1 in 5,000 males (*1*) and autism spectrum disorder (ASD) may be present in as many as 60% of these individuals (*2-4*). Although the parental first concern occurs usually at 12 months, the typical age at diagnosis is approximately 35-37 months (*5*) and in some countries can occur much later, particularly for females with FXS.

Newborn screening for *FMR1* mutation is not mandated in any state in the US, and, until recently, testing was costly and therefore not available for large population screening. In addition, until recently, the paucity of targeted treatments for fragile X-associated disorders and the lack of data on early intervention has diverted the attention and augmented the controversy. Population screening for fragile X mutations and, particularly newborn screening, has therefore been the object of ongoing controversy particularly regarding to the value of patients' discovery of their genetic status, at birth for the newborn and for other family members (*6,7*). There is also concern for identifying a carrier at birth since the premutation is associated with a neurodegenerative disorder, the fragile X-associated tremor ataxia syndrome (FXTAS) in aging for which currently, the diagnosis is not predictable. However, the premutation is also associated in some carriers with developmental problems that can benefit from early intervention (*8,9*).

In the past few years, significant advances in genomic testing, have improved the methods for

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detection of *FMR1* mutations, resulting in more accurate testing with low cost and timely return of test results and, have led to several large population screening studies (*10,11*). In addition, ongoing research, pursuing targeted treatment for fragile X-associated disorders, has revealed potential psychopharmacologic and educational interventions (*12-18*). Though screening and detection of *FMR1* mutations in the newborn period is not yet fully embraced (*19*), many parents took advantage of the opportunity to screen their newborn when made available as part of a voluntary screening during the state mandatory testing (*20*). Indeed two recent pilot newborn screening studies reported a high acceptance rate of > 70% (*11,21*) indicating that universal newborn screening for FXS may be more widely accepted and advisable than previously believed.

In this study, we report on the identification of a male with a *FMR1* full mutation through a pilot study of NBS conducted at the UC Davis Medical Investigation for Neurodevelopmental Disorders (MIND) Institute (*11,22*) and we provide a summary of the clinical involvement during the first 3 years of his life.

2. Newborn screening for FXS

Each year, roughly 1,700 babies are born in the Pediatric units at the University of California Davis Medical Center (UCDMC). A pilot NBS study for FXS was conducted using blood spots at the UCDMC, approved by the Institutional Review Board (IRB), as previously described (*11,23*). Parents of the newborns were approached by research assistants who explained the research and carried out the process of informed consent, during their stay on the Labor and Delivery units. According to the Institutional Review Board (IRB) approved protocol, a blood sample was obtained on individual filter paper (FTA or 903 blood spot cards, Whatman) and polymerase chain reaction (PCR) was performed to determine the CGG repeat size as previously described (*11*).

If a repeat size in either the premutation range (between 55 and 200 CGG repeats) or full mutation range (> 200 CGG repeats) was discovered, the family was contacted by the genetics counselor at the MIND Institute after the infant reached 2 months of age. The genetic counselor also invited the family of the newborn to the MIND Institute for an appointment to provide confirmatory genetic testing, genetic counseling for the family, a medical examination, and developmental assessments for the infant. Family members who wished to participate in further research, tracking the development of children with fragile X gene mutations were scheduled to visit at six-month intervals for medical exams and developmental assessments at no cost (*11,23*).

PCR on blood spot was performed as previously described (*11*). Genomic deoxyribonucleic acid (DNA) was isolated from 3 ml of peripheral blood lymphocytes using standard methodology (Qiagen, Valencia, CA). Repeat size and methylation status were determined using PCR and Southern blot analyses using the *FMR1*-specific probe StB12.3. as described in previous studies (*24,25*). *FMR1* mRNA expression levels were measured by quantitative reverse transcription-PCR (qRT-PCR) as described (*26*).

3. Case report

3.1. *Clinical history*

The identified newborn was born vaginally at 38 weeks 6 days gestation and his birth weight was 7lb 8oz, his Apgars were 7 and 9. Meconium was present at birth. His mother had gestational diabetes mellitus and received insulin during the last four months of pregnancy.

The patient demonstrated restless and fitful sleep as an infant, in addition to significant irritability and tantrums. Throughout development he has been very interested in social interaction. He can be hyperactive and also perseverative in his behavior and language. His developmental milestones included sitting at ten months, crawling by 11 months, and cruising at one year, but he was not walking independently until 18 months. He began using single words at two years of age. He was referred for early intervention in the first year of life and he received speech and language therapy and physical therapy (PT) and occupational therapy (OT), which included sensory integration, on a weekly basis.

He demonstrates poor eye contact; also must lean his head back in order to see what is in front of him due to his congenital bilateral ptosis. A developmental pediatrician recommended surgical correction at a later date.

The patient has been seen in clinic at the MIND Institute, for medical examination and developmental assessments, beginning at 3 months of ages. On his first examination (at 3 months) the patient presented with a hydrocele on the right testicle, which resolved spontaneously. At 6 months his head circumference was 42.5 cm, at 12 months it was 45 cm (97th percentile) and at 36 months it was 48 cm (50th percentile).

Developmental assessments show global delays in various domains of ability. The Mullen Scales of Early Learning (MSEL) were administered at 6, 12, 24, and 30 months of age, as were the Vineland Adaptive Behavior Scales (Table 1 and Table 2). At 27 months he was evaluated for autism spectrum disorder (ASD) using the Autism Diagnostic Observation Schedule (Module I, some words). His Social Affect and Restricted and Repetitive Behavior Total was 11 (cut off for autism is 12, for autism spectrum, 8). His intervention intensified with Applied Behavioral Analysis (ABA) therapy, administered at preschool, because of his ASD diagnosis.

The Preschool Language Scales evaluation placed

Table 1. Vineland Adaptive Behavior Scales (VABS)

Age (months)	Communication	Daily Living Skills	Socialization	Motor Skills	ABC
6	74	67	66	61	64
12	84	88	73	65	74
24	90	96	92	90	90
30	84	82	91	90	84

Table 2. Mullen Scales of Early Learning (MSEL)

Age (months)	Gross Motor Skills	Visual Reception	Fine Motor Skills	Receptive Language	Expressive Language	ELC
6	3	4	2	4	3	63
12	10	8	6	9	6	60
24	16	16	18	16	14	63
30	16	19	21	24	20	60

Table 3. Molecular and clinical measures

Case	Age (years)	Gender	Category	FSIQ	IQ test	CGG size	AR	*FMR1* mRNA (Std. Err)
Proband	3	Male	FM	62	Binet	390, 460, 780, 1130	N/A	0.01 (0.05)
Mother	36	Female	FM	103	WASI	28, 270, 400, 570, 650	0.88	1.4 (0.02)
Brother	4	Male	FM	49	Mullen	223, 389, 532, 1000	N/A	0
Sister	14	Female	FM	65	Binet	33, 431, 561, 1093	0.77	1.46 (0.15)

FM= full mutation; AR= Activation Ratio (percent of cells carrying the normal allele on the active X chromosome).

him at a 21 months developmental level although he was 32 months at time of testing. He was entered into a controlled trial of sertraline for young children with FXS at 2 years of age but he was later found to have randomized to placebo so he was subsequently treated with 2.5 mg of sertraline since this has been shown to be helpful in young children with FXS (*27*). The patient is also currently taking a multivitamin and vitamin D.

Genetic counseling and cascade testing were carried out for the family members. The mother and the additional 2 siblings were found to also have the full mutation. The sister was enrolled into a study of an metabotropic glutamate receptor 5 (mGluR 5) antagonist and the brother was enrolled in a clinical trial using ganaxolone (*16*).

3.2. *Molecular measures*

DNA molecular testing results on the newborn and his immediate family members, including the mother and two siblings (one male and one female). DNA was isolated from 3ml of blood using standard protocol (Qiagen, Valencia, CA). Using Southern Blot and PCR analysis (*24,25*) the presence of a full mutation was detected in all of them as depicted in Figure 1. CGG repeat number, Activation Ratio, methylation and *FMR1* mRNA levels are reported in Table 3.

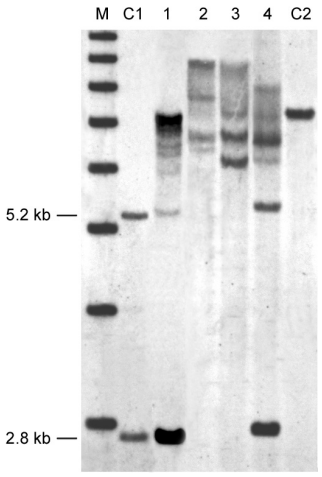

Figure 1. Southern blot analysis of genomic DNA isolated from a normal control female (C1), probands' mother (Lane 1), male proband (Lane 2), proband's brother (Lane 3), proband's sister (Lane 4) and from a full mutation control (C2). M= DNA marker, 1 kb ladder. Normal unmethylated band (2.8 kb) and normal methylated band (5.2 kb) shown on the left. (C1) and a full mutation control sample is shown on the right (C2). Southern blot analysis was carried out on 7-10ug of with Eco RI and Nru I restriction enzymes. Fragments were separated on an agarose gel, transferred on a nylon the membranes, which were hybridized with a FMR1-specific genomic probe, StB12.3. Additional details of the method are as described in (*25*).

4. Discussion

The identification of a child with FXS at the time of birth can lead to earlier intervention, which can be beneficial for the development of the child (*27,28*). This case began intervention before the end of the first year and the mother benefitted from cascade testing, demonstrating how testing can be beneficial not only for the proband, but for the family as well. The mother is a single parent and she is raising her 3 children

without much family support. Thus, the relationship that she established with the MIND Institute has been helpful and supportive to her and her family.

This child has done well with interventions. The cause of his congenital ptosis may be related to his FXS, although he has a more severe ptosis than what has been previously reported (*29*). Although his visuo-spacial development would have likely improved with earlier surgery, it was thought to be of high risk. Children under 5 years old with ASD have been documented to have low serotonin levels in the frontal regions of the brain by positron emission tomography (PET) imaging (*30,31*). The newborn has had the advantage of an early intervention treatment trial of sertraline which enhances serotonin and stimulates neurogenesis and Brain Derived Neurotropic Factor (BDNF) in the CNS (*32*). In a previous retrospective study of sertraline in young children with FXS those who received treatment with sertraline had higher receptive and expressive language than those who did not receive sertraline (*27*). Additional treatments are available to the newborn and his family. Because early treatment with minocycline, which lower the elevated matrix metallopeptidase 9 (MMP-9), levels observed FXS and has shown efficacy in children with FXS (*15*), he will also undergo a trial of minocycline in the near future in addition to sertraline. Mother has also treated him with antioxidants and infant massage therapy.

Newborn screening can lead to cascade testing and the identification of other family members with an *FMR1* mutation can be beneficial information to other family members (*23*). Although young premutation carriers can be identified who may develop medical problems such as FXTAS or fragile X-associated primary ovarian insufficiency (FXPOI) with age, they can be followed closely for developmental problems that they might demonstrate and subsequently benefit from treatment. In addition, there are a number of interventions that may help to avoid the aging problems of some carriers and such interventions have been reviewed recently (*33,34*).

In conclusion, we have identified a newborn with FXS through newborn screening. To our knowledge this is the first case of a full mutation with FXS identified through newborn screening reported in the literature.

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