Climbing the Branches of a Family Tree: Diagnosis of Fragile X Syndrome

Jeannie Visootsak, MD[1], Heather Hipp, MD[1], Heather Clark, MS[1], Elizabeth Berry-Kravis, MD, PhD[2], Tovi Anderson, PhD[1], and Dawn Laney, MS[1]

Objective To determine the average number of family members diagnosed with a *Fragile X Mental Retardation-1* (*FMR1*) mutation after a proband receives the initial diagnosis of fragile X syndrome (FXS).

Study design We reviewed pedigrees of families who had been evaluated at the Fragile X Syndrome Center at Emory University in Atlanta, Georgia. Through these pedigrees, we determined the number of additional family members diagnosed as *FMR1* premutation carriers or with full mutation FXS after the initial diagnosis in each proband.

Results The fragile X pedigree review identified 176 probands, including 108 males (61%) and 68 females (39%). A total of 785 family members were diagnosed with expanded fragile X alleles, including 278 males (35%) and 507 females (65%). These family members included 227 individuals with full mutation FXS (219 males and 8 females) and 558 premutation carriers (59 males and 499 females). After the initial diagnosis of a proband with FXS, on average at least 5 additional family members were diagnosed with an *FMR1* mutation.

Conclusion Our findings confirm that obtaining a detailed family history after diagnosis of a proband with FXS is likely to identify multiple family members with *FMR1* mutations. It is important that the pediatrician or other health care provider making a diagnosis of FXS recognize the value of a detailed family history for timely diagnosis and treatment of additional individuals who may be *FMR1* premutation carriers or have full mutation FXS. *(J Pediatr 2014;■:■-■).*

Fragile X syndrome (FXS; MIM 300624), the most common inherited form of intellectual disability, is caused by a CGG trinucleotide expansion in the promoter region of the *Fragile X Mental Retardation-1* (*FMR1*) gene. When this expansion contains more than 200 repeats (full mutation), it leads to decreased or absent levels of the fragile X mental retardation protein, resulting in FXS. Premutation carriers have unstable alleles with 55-200 repeats that can expand to the full mutation during transmission from mother to child.[1] FXS affects approximately 1 in 4000 males and 1 in 8000 females, and the prevalence of the premutation in the general population is 1 in 130-260 females and 1 in 250-810 males.[2]

The diagnosis of FXS is critically important not just to the affected child, but also to other siblings, parents, and immediate and extended family members in each generation. Fragile X–associated disorders are the clinical outcomes of the expansion mutation, which can result in 3 established medical conditions (FXS, fragile X–associated primary ovarian insufficiency [FXPOI], and fragile X–associated tremor ataxia syndrome [FXTAS]) that can occur across generations in a single family. Female premutation carriers are thought to be at risk for FXPOI, depression, anxiety, and such medical issues as hypothyroidism, fibromyalgia, and neuropathy.[3-6] Both males and females over age 50 who carry the premutation are at risk for FXTAS, a late-onset neurodegenerative disorder.[7,8]

Children with FXS typically present with developmental delay, autism, and/or behavioral problems (eg, hand flapping, decreased eye contact), and may be the first person in the family to be diagnosed with the condition.[1,9-11] The pediatrician has an opportunity to take a family history, inform the parents about the inheritance pattern of FXS, and encourage testing and evaluation for all at-risk members of the immediate and extended family. The present study was designed to determine the average number of family members diagnosed as premutation carriers or with full mutation FXS after the proband receives the initial diagnosis of FXS.

Methods

After receiving approval from Emory University's Institutional Review Board, 2 physicians and genetic counselors at the Fragile X Syndrome Center at Emory University in Atlanta, Georgia reviewed 176 previously completed pedigrees of families who had been evaluated at the center. Once the proband received the diagnosis of FXS, cascade testing was provided. Cascade testing identifies and

FMR1	*Fragile X Mental Retardation-1*
FXPOI	Fragile X–associated primary ovarian insufficiency
FXS	Fragile X syndrome
FXTAS	Fragile X–associated tremor ataxia syndrome

From the [1]Department of Human Genetics, Emory University, Atlanta, GA and [2]Departments of Pediatrics, Neurological Sciences, and Biochemistry, Rush University Medical Center, Chicago, IL

Supported by National Institutes of Health/*Eunice Kennedy Shriver* National Institute of Child Health and Human Development (1K23HD058043-01A1 to J.V.) and Fragile X Association of Georgia (to T.A.). J.V. and E.B-K. receive grant support from Novartis and Roche to conduct clinical trials and consult on use of mGluR5 blockers in individuals with FXS. E.B.-K. has received funding from Asuragen, Inc to develop testing standards for diagnosis of FXS. The other authors declare no conflicts of interest.

0022-3476/$ - see front matter. Copyright © 2014 Mosby Inc. All rights reserved. http://dx.doi.org/10.1016/j.jpeds.2014.01.051

offers genetic testing to family members of the proband.[2,12] For example, the proband's siblings should be offered *FMR1* DNA testing, especially those with a history of intellectual disability, autism, and social/behavioral or learning disorders. Testing is also recommended for female relatives, who are at increased risk for infertility, premature menopause, and conceiving offspring affected with FXS.

Pedigree construction and analysis in the Fragile X Syndrome Center at Emory University was performed in a standard manner equivalent to the guidelines set by Finucane and coworkers[2,12] using a cascade method. Through these pedigrees, the number of additional family members diagnosed with premutation or full mutation FXS as confirmed by *FMR1* DNA testing was determined after the initial diagnosis in each proband. An anonymous summary sheet was developed to standardize the chart review procedures and to track the number of affected probands and family members. The probands and family members were categorized by sex as well.

Results

The fragile X pedigree review identified 176 probands, including 108 males (61%) and 68 females (39%). A total of 785 family members were diagnosed with expanded fragile X alleles, including 278 males (35%; 59 premutation and 219 FXS) and 507 females (65%; 499 premutation and 8 FXS). Overall, these family members included 227 individuals with full mutation FXS (29%) and 558 premutation carriers (71%). Although males were more commonly diagnosed as the initial proband, more females, particularly premutation carriers, were identified during subsequent analyses of family history and pedigree. After the initial diagnosis of a proband with FXS, an average of at least 5 additional family members were diagnosed with FXS or as a premutation carriers.

Discussion

Because FXS has no distinctive physical characteristics at birth to prompt diagnosis, it typically becomes evident only after the child exhibits developmental delay, behavioral problems, or learning issues. For these reasons, FXS is typically diagnosed at age 35-37 months in boys and 41 months in girls. On average, the diagnosis is made 18 months after the family first identifies concerns.[13,14] Late diagnosis delays families' understanding of their reproductive risk, which can be critical for family planning. In a national FXS survey study, 55% of parents already had another child before the first child was diagnosed with FXS.[14]

Our results indicate that on diagnosis of a proband with FXS, subsequent pedigree analysis will reveal additional individuals affected with full mutation FXS. This suggests that obtaining a detailed family history and recommending genetic testing for individuals within the family with a history of developmental delays, behavioral problems, and learning deficits will identify additional family members with FXS.[15] Earlier identification of FXS will allow the affected child to benefit optimally from early intervention and treatment for developmental and/or emotional disorders, provide access to current clinical trials targeting cognitive and behavioral deficits in FXS, and also alleviate potential parental stress associated with the diagnostic odyssey. Moreover, studies of the neurobiology and synaptic mechanisms in FXS have lead to the development of disease-specific pharmacologic treatments, with several drugs currently in Phase 2 and 3 clinical trials in children and adults with FXS, including metabotropic glutamate receptor 5 (mGluR5) blockers, gamma amino-butyric acid (GABA) agonists, and minocycline.[16-19] These various targeted treatments have shown promising preliminary results that may help with behavior, social functioning, and learning in individuals with FXS.

In the present study, identification of a proband with FXS and subsequent pedigree analysis led to diagnosis of >3-fold more premutation carriers, predominantly females. These individuals were previously not aware of their carrier status. By knowing their diagnosis, female carriers become aware not only of medical concerns related to the premutation, such as FXPOI and FXTAS,[6,7] but also of additional medical and psychiatric problems that can occur in some carriers, including neuropathy, migraines, sleep apnea, hypertension, hypothyroidism, fibromyalgia, anxiety, depression, and obsessive compulsive behavior.[3,5,20-22] Male carriers over age 50 are at increased risk for additional medical problems, including neuropathy, hypothyroidism, hypertension, arrhythmias, and sleep apnea, which may develop before the onset of FXTAS.[3,20,23] As such, identification of both male and female premutation carriers can lead to early diagnosis and treatment of these medical and psychiatric problems, potentially improving the individual's quality of life.

Although most children with the premutation do not exhibit neurodevelopmental deficits, recent studies have suggested that as many as 10%-20% experience attention deficit hyperactivity disorder, learning problems, shyness, anxiety, or autism.[24-26] Thus, awareness of the premutation status in immediate and extended family members will help identify related medical, psychosocial, and/or neurodevelopmental problems that can be addressed with appropriate treatments.

The multigenerational mutation process and variable phenotype associated with the *FMR1* mutation have significant implications beyond the immediate concerns of the proband, who is typically a child with FXS. For these reasons, pediatricians should be familiar with the variable clinical manifestations of fragile X–associated disorders, including FXS, FXPOI, and FXTAS. Cascade testing is recommended once a child receives the diagnosis of FXS.[2,12] For instance, if a male child had FXS, then testing for his mother would be recommended to determine whether she is a premutation carrier or possibly has full mutation FXS herself. If a premutation carrier, she is at risk for developing FXPOI and other medical issues, such as hypothyroidism and autoimmune disorders. Importantly, knowing her carrier status will allow her to make informed decisions regarding family planning. In addition, the cascade testing would indicate subsequent

genetic testing for the proband's grandparents, who could be at risk for medical problems associated with the premutation, including FXTAS. The pedigree analysis would document the presentation of any clinical symptoms in the maternal grandmother or maternal grandfather, which may indicate which person should be tested first. Cascade testing would identify any other family members who also may be affected with premutation or full mutation FXS, allowing them to seek early and appropriate treatment associated with their fragile X mutation.

The pedigree review from the Fragile X Syndrome Center at Emory University revealed that on average, at least 5 family members are subsequently diagnosed with FXS or identified as an *FMR1* premutation carrier after diagnosis of each proband. Because FXS is typically diagnosed by pediatricians in children presenting with developmental delay and/or behavioral problems, our findings emphasize the importance of pediatricians obtaining a detailed family history for each patient affected by FXS and encouraging testing and evaluation of all at-risk family members. Alternatively, pediatricians may choose to refer families for genetic counseling to facilitate cascade testing. Using the family history to identify at-risk family members is an effective and efficient way to facilitate the diagnosis of individuals who may have full mutation FXS or be premutation carriers. This approach also reduces the diagnostic odyssey of symptomatic relatives (eg, FXTAS, FXPOI) who are being evaluated, often without attention to the family history.

A limitation of this study is the possible refusal of affected family members to undergo evaluation. Thus, our finding of 5 affected relatives per family represents a minimum number of individuals expected to be identified through a pedigree analysis. All of our participants were clinic and/or research patients at a single FXS center (Emory University). It is possible that their experiences differ from those of FXS families at other clinics and families who do not participate in research. Our sample may be biased in that it represents families who are active in the FXS community and proactive in seeking specialty clinic care for their child. We did not collect data on parental socioeconomic status, which might contribute to factors involved in communicating the diagnosis and its inheritance pattern to other family members. Participants who were identified as premutation carriers through cascade testing underwent *FMR1* testing through our research study; thus, we do not have their clinical information related to symptoms associated with FXPOI or FXTAS. Our results indicate high number of affected family members in a typical FXS family, emphasizing the importance of a detailed family history in FXS and similar inherited conditions, and of subsequent testing of at-risk family members. ■

We would like to thank the Fragile X Association of Georgia for their support of individuals with Fragile X syndrome and their families.

Submitted for publication Oct 9, 2013; last revision received Dec 30, 2013; accepted Jan 24, 2014.

Reprint requests: Jeannie Visootsak, MD, 2165 N Decatur Rd, Decatur, GA 30033. E-mail: Jvisoot@emory.edu

References

1. Chonchaiya W, Schneider A, Hagerman RJ. Fragile X: a family of disorders. Adv Pediatr 2009;56:165-86.
2. Finucane B, Abrams L, Cronister A, Archibald AD, Bennett RL, McConkie-Rosell A. Genetic counseling and testing for *FMR1* gene mutations: practice guidelines of the national society of genetic counselors. J Genet Couns 2012;21:752-60.
3. Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, et al. Expanded clinical phenotype of women with the *FMR1* premutation. Am J Med Genet A 2008;146A:1009-16.
4. Bailey DB Jr, Raspa M, Olmsted M, Holiday DB. Co-occurring conditions associated with *FMR1* gene variations: findings from a national parent survey. Am J Med Genet A 2008;146A:2060-9.
5. Hunter JE, Rohr JK, Sherman SL. Co-occurring diagnoses among *FMR1* premutation allele carriers. Clin Genet 2010;77:374-81.
6. Sullivan SD, Welt C, Sherman S. *FMR1* and the continuum of primary ovarian insufficiency. Semin Reprod Med 2011;29:299-307.
7. Hagerman P. Fragile X–associated tremor/ataxia syndrome (FXTAS): pathology and mechanisms. Acta Neuropathol 2013;126:1-19.
8. Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, et al. Fragile X–associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. Am J Hum Genet 2004;74:1051-6.
9. Bagni C, Tassone F, Neri G, Hagerman R. Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. J Clin Invest 2012;122:4314-22.
10. Angkustsiri K, Wirojanan J, Deprey LJ, Gane LW, Hagerman RJ. Fragile X syndrome with anxiety disorder and exceptional verbal intelligence. Am J Med Genet A 2008;146A:376-9.
11. Harris SW, Hessl D, Goodlin-Jones B, Ferranti J, Bacalman S, Barbato I, et al. Autism profiles of males with fragile X syndrome. Am J Ment Retard 2008;113:427-38.
12. McConkie-Rosell A, Abrams L, Finucane B, Cronister A, Gane LW, Coffey SM, et al. Recommendations from multidisciplinary focus groups on cascade testing and genetic counseling for fragile X–associated disorders. J Genet Couns 2007;16:593-606.
13. Bailey DB Jr, Raspa M, Bishop E, Holiday D. No change in the age of diagnosis for fragile X syndrome: findings from a national parent survey. Pediatrics 2009;124:527-33.
14. Bailey DB Jr, Skinner D, Sparkman KL. Discovering fragile X syndrome: family experiences and perceptions. Pediatrics 2003;111:407-16.
15. Sorensen PL, Gane LW, Yarborough M, Hagerman RJ, Tassone F. Newborn screening and cascade testing for *FMR1* mutations. Am J Med Genet A 2013;161A:59-69.
16. Berry-Kravis E, Knox A, Hervey C. Targeted treatments for fragile X syndrome. J Neurodev Disord 2011;3:193-210.
17. Hagerman R, Lauterborn J, Au J, Berry-Kravis E. Fragile X syndrome and targeted treatment trials. Results Probl Cell Differ 2012;54:297-335.
18. Paribello C, Tao L, Folino A, Berry-Kravis E, Tranfaglia M, Ethell IM, et al. Open-label add-on treatment trial of minocycline in fragile X syndrome. BMC Neurol 2010;10:91.
19. Utari A, Chonchaiya W, Rivera SM, Schneider A, Hagerman RJ, Faradz SM, et al. Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. Am J Intellect Dev Disabil 2010;115:433-43.
20. Hamlin AA, Sukharev D, Campos L, Mu Y, Tassone F, Hessl D, et al. Hypertension in *FMR1* premutation males with and without fragile X–associated tremor/ataxia syndrome (FXTAS). Am J Med Genet A 2012;158A:1304-9.
21. Bourgeois JA, Seritan AL, Casillas EM, Hessl D, Schneider A, Yang Y, et al. Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. J Clin Psychiatry 2011;72:175-82.

22. Roberts JE, Bailey DB Jr, Mankowski J, Ford A, Sideris J, Weisenfeld LA, et al. Mood and anxiety disorders in females with the *FMR1* premutation. Am J Med Genet B Neuropsychiatr Genet 2009;150B:130-9.
23. Goodrich-Hunsaker NJ, Wong LM, McLennan Y, Tassone F, Harvey D, Rivera SM, et al. Adult female fragile X premutation carriers exhibit age- and CGG repeat length-related impairments on an attentionally based enumeration task. Front Hum Neurosci 2011;5:63.
24. Hessl D, Wang JM, Schneider A, Koldewyn K, Le L, Iwashi C, et al. Decreased fragile X mental retardation protein expression underlies amygdala dysfunction in carriers of the fragile X premutation. Biol Psychiatry 2011;70:859-65.
25. Renda MM, Voigt RG, Babovic-Vuksanovic D, Highsmith WE, Vinson SS, Sadowski CM, et al. Neurodevelopmental disabilities in children with intermediate and premutation range fragile X cytosine-guanine-guanine expansions. J Child Neurol 2012;29:326-30.
26. Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, et al. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr 2006;27:S137-44.