



Clinical and Genetic Characteristics of Young Children with Fragile X Syndrome

So-Hee Lee, MD, Ji-Eun Jeong, MD, Yoon-Young Jang, MD, Jin-Kyung Kim, MD

Department of Pediatrics, Daegu Catholic University School of Medicine, Daegu, Korea

Received: March 24, 2021

Revised: May 23, 2021

Accepted: May 26, 2021

Corresponding author:

Jin-Kyung Kim, MD
Department of Pediatrics, Daegu
Catholic University School of
Medicine, 33 Duryugongwon-ro
17-gil, Nam-gu, Daegu 42472,
Korea
Tel: +82-53-650-4597
Fax: +82-53-650-4243
E-mail: kimjk@cu.ac.kr

Purpose: This study analyzed the clinical and genetic characteristics of young children with fragile X syndrome (FXS) and evaluated the significance of FXS genetic (FX) testing for children with global developmental delay (GDD).

Methods: FX testing was performed in 324 children aged <5 years and their family members between 2007 and 2020. Fourteen children (10 boys, four girls) with abnormal results were finally included in this report. We retrospectively reviewed their medical records and categorized them based on genetic test results. The results of an analysis of the expanded cytosine-guanine-guanine trinucleotide (CGG) alleles of fragile X mental retardation 1 (*FMR1*) were divided into four groups: normal, intermediate (IM), premutation (PM), and full mutation (FM).

Results: Twelve of the 14 children presented with FM (nine boys, three girls), and one each with PM and IM, respectively. Five of the children with FM and the one with PM belonged to two families. At the initial visit, the mean age of the nine boys with FM was 24.8±9.7 months. They presented with significant GDD and markedly delayed language development. Most of them had subtle physical features. Two girls with FM presented with less severe developmental delay than the boys with FM, and they were identified via sibling studies. However, one girl presented with FM resulting from maternal uniparental disomy and severe developmental delay.

Conclusion: Even in the absence of a family history, physicians should consider FX testing for children with unexplained GDD. If there is a family history of FXS, FX screening tests should be performed for all family members.

Keywords: Fragile X syndrome; Genetic testing; Intellectual disability

Introduction

Global developmental delay (GDD) is defined as a significant developmental delay in two or more of the following: gross or fine motor movement, speech/language, cognition, social/personal skills, and activities of daily living. The term GDD is reserved for children under the age of 5, and the term intellectual disability (ID) is applied to older children who can be evaluated using a relatively

reliable intelligence test. Thus, GDD is considered a predictor of the future diagnosis of ID [1].

Fragile X syndrome (FXS, OMIM*300624) is considered the most common inherited cause of ID, and is the most common monogenic cause of autism [2]. It is associated with attention deficit, hyperactivity, social deficit, autistic-like behavior, and psychiatric problems. Physical features include a long narrow face, prominent forehead, high arched palate, large ears, macrocephaly, promi-

ment jaw, flexible fingers, flat feet, and pubertal macroorchidism [3]. It can be difficult to detect, especially in young children, because the physical features of the disease are subtle and vague [2]. FXS results from the loss of function of the fragile X mental retardation 1 (*FMRI*) gene (OMIM*300624), caused by an expansion of cytosine-guanine-guanine trinucleotide (CGG) repeats in this gene. This is associated with hypermethylation of the *FMRI* promoter, leading to silencing of the *FMRI* gene and the consequent loss of its product, the fragile X mental retardation protein (FMRP). FMRP is a major regulator of the translation of many mRNAs involved in synaptic plasticity [3]. According to guidelines from the American College of Medical Genetics and Genomics, expanded CGG alleles can be divided into four groups: normal (6 to 44 CGG repeats), intermediate or gray zone (IM or GZ; 45 to 54 repeats), premutation (PM; 55 to 200 repeats), and full mutation (FM; > 200 repeats) [2]. Those with the PM or IM allele usually lack the typical clinical features of FXS. PM is meiotically unstable and therefore prone to expansion to FM when transmitted from the mother to the next generation. The risk of FM expansion thus increases with increasing numbers of CGG repeats. The presence of AGG interruptions in the CGG sequence influences *FMRI* stability, and the loss of these AGG interruptions appears to increase the instability of CGG repeats [4-6]. FXS tends to be diagnosed later because of the subtle clinical findings in young children with FXS. A late diagnosis can result in a lack of early intervention for rehabilitation and genetic counseling of family members [7].

In all children with unexplained GDD/ID, chromosomal microarray (CMA) and FXS genetic (FX) testing are recommended as first-tier diagnostic tests [1]. CMA provides a high diagnostic yield (15% to 20%) when used in the genetic testing of patients with unexplained GDD/ID or autistic spectrum disorder (ASD). In contrast, the FX test provides a low diagnostic yield (1% to 2%). Therefore, some reports have suggested that it may not be necessary to include FX testing in first-line diagnostic evaluations [8].

We retrospectively analyzed the medical records of children diagnosed with FXS between 2007 and 2020. This study aimed to analyze the clinical and genetic characteristics of young children with FXS, and to evaluate the significance of FX testing for children with unexplained GDD.

Materials and Methods

1. Subjects

FX testing was performed in 323 children (246 males and 77 females) with GDD under the age of 5 and their family members between 2007 and 2020. Of these 323, only 13 children (10 boys and

three girls) had abnormal results. One girl without GDD was tested because her sister was diagnosed with FXS. Thus, 14 children were included in the study.

2. Methods

We retrospectively reviewed the medical records of the 14 children with abnormal *FMRI* CGG test results. Medical and physical examinations were performed, and the language and cognitive abilities of the children were examined. The Bayley Scales of Infant Development (BSID-II) were used to assess cognitive function, which provided the mental development index (MDI) and the psychomotor development index (PDI). A significant delay was defined as a development index (DI) < 70, and a mild delay was defined as $70 \leq DI < 85$. A $DI \geq 85$ was considered normal [9]. The Korean Social Maturity Scale, a measure of personal and social skills needed in everyday life, was used to obtain the social maturity quotient (SQ), upon which basis social maturity was classified as normal ($SQ \geq 85$), mild deficit ($70 \leq SQ < 85$), or significant deficit ($SQ < 70$) [10]. Language abilities were assessed using the Sequenced Language Scale for Infants. A significant speech development delay was defined as a developmental score two standard deviations (SDs) away from the mean score, and a mild speech developmental delay was defined as a developmental score between 1 SD and 2 SDs from the mean [11]. The Korean version of the Childhood Autism Rating Scale (CARS) was used to assess ASD. A score of 30 or above on CARS was considered clinically significant [12]. The (CGG)_n repeats of the *FMRI* gene were analyzed by polymerase chain reaction (PCR) using AmpliEx PCR CE/*FMRI* (Asuragen, Austin, TX, USA) and the products were separated by capillary electrophoresis with ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were analyzed using the GeneMapper version 4.0 software (Applied Biosystems).

3. Ethical statement

The study was approved by the Institutional Review Board of Daegu Catholic University Medical Center, Daegu, Korea (IRB No. CR-20-076). The requirement for informed consent was waived because of the retrospective nature of the study.

Results

1. Study subjects

In total, nine boys and three girls tested positive for the FM allele. Two of the three girls (patients 10 and 12) had an FM allele and a normal allele, but one girl (patient 11) presented with only FM alleles, resulting from maternal uniparental disomy (UPD) of the en-

tire X chromosome. The mother of the girl with only FM alleles had one normal allele (30 CGG repeats) and one PM allele (65 CGG repeats). Her father had a normal 32-CGG-repeats allele. CMA analysis and short tandem repeat analysis confirmed the maternal inheritance of the duplicated X chromosome, consistent with maternal UPD. In addition, one girl (patient 13) presented with the PM allele, and one boy (patient 14) with the IM allele. FX testing was also performed on six mothers of the children with the FM allele. All of them had a PM allele.

This study focused on 12 children with FM (Table 1). They were full-term with a mean birth weight of 3.6 ± 0.44 kg. There were no specific perinatal problems in any of the children. The mean age at the first visit of the nine boys was 24.8 ± 9.7 months (range, 11 to 45), and the mean age of the three girls was 34.3 ± 8.3 months (range, 25 to 41). The initial presentations included gross motor developmental delay in two children and language developmental delay in the other 10 children. The nine boys with FM began walking independently after 12 months (mean age, 16.9 ± 3.0 ; range, 15 to 24). A girl with FM (patient 10) presented with delayed language development and none of the typical clinical features of FXS. She was evaluated using the FX test because her younger brother (patient 6) had been diagnosed with FXS at our clinic; she was found to be heterozygous for FM at the *FMRI* locus. Later, her male cousin (patient 7) visited our clinic for delayed development and was also diagnosed with FXS. The remaining three girls (patients 11, 12, and 13) belonged to one family. The first sister (patient 11) had only FM alleles (288 and 288 repeats), the second (patient 12) was heterozygous for the FM allele (30

and 282 repeats), and the third (patient 13) presented with one PM and one normal allele (31 and 99 repeats) (Table 2).

2. Analysis of clinical and genetic characteristics of 14 children

Medical records revealed that all nine boys presented with both large ears and a prominent forehead, consistent with the physical features of children with FXS. However, we could not detect these

Table 1. Demographic data of the 12 study participants

Characteristic	Value
Sex (male/female)	9/3
Age at first visit (mo)	27.1 ± 9.9 (11–45)
Male	24.8 ± 9.7 (11–45)
Female	34.3 ± 8.3 (25–41)
Initial presentation	
Delayed motor development (n)	2
Delayed language development (n)	10
Birth history	
Gestational age (wk)	39.5 ± 1.2
Birth weight (kg)	3.6 ± 0.44
Developmental history	
Age of independent walking (mo)	
Male	16.9 ± 3.0 (15–24)
Female	14.0 ± 3.6 (11–18)
Maternal FX test	6
Family history of FXS	6 (2 family)

Values are presented as mean \pm standard deviation (range).
FX, fragile X; FXS, fragile X syndrome.

Table 2. Molecular diagnostic findings and results of psychological testing for all 14 patients evaluated in this study

No.	Age (yr) ^a	Sex	(CGG) _n repeat	Maternal (CGG) _n repeats	Walking alone ^b	Age (yr) ^c	MDI	PDI	SQ	L-R ^d	L-E ^d	CARS
1	11	M	342	24/98	24	22	< 50	< 50	49.5	9	8	NA
2	17	M	> 450	NA	18	40	< 50	< 50	55	13	13	NA
3	19	M	209	30/84	15	20	50	60	65	10	10	NA
4	24	M	353	31/86	15	25	< 50	65	64	9	8	32.5
5	25	M	274	NA	15	27	< 50	< 50	65.1	14	10	NA
6	25	M	275	30/88	15	27	< 50	< 50	54.1	13	10	NA
7	25	M	278	NA	17	26	< 50	< 50	NA	10	7	NA
8	32	M	294	29/92	18	33	< 50	< 50	61.16	10	9	25
9	45	M	284	NA	15	48	< 50	< 50	65.7	18	17	25
10	41	F	26/271	30/88	13	43	< 50	< 50	69.4	24	25	NA
11	37	F	288/288	30/65	18	38	< 50	< 50	67.94	15	16	NA
12	25	F	30/282	30/65	11	27	62	65	88	21	21	NA
13	7	F	31/99	30/65	NA	NA	NA	NA	NA	NA	NA	NA
14	46	M	52	NA	16	47	< 50	< 50	66.2	24	24	NA

CGG, cytosine-guanine-guanine trinucleotide; MDI, mental development index; PDI, psychomotor development index; SQ, social maturity quotient; L-R, language-receptive; L-E, language-expressive; CARS, Childhood Autism Rating Scale; NA, not available.

^aAge (months) at initial visit; ^bAge (months) of walking alone; ^cAge (months) at psychological testing; ^dL-R, L-E as developmental age (months).

findings in some children at their initial visit. The girl with only FM alleles (288 and 288 repeats) (patient 11) presented with FXS features including a prominent forehead, large ears, and significant GDD with inattentive, hyperactive behavior. She began to walk independently at approximately 18 months of age. The other two girls who presented with one FM and one normal allele (patients 10 and 12) did not show any physical features of FXS. These girls reached gross motor developmental milestones on time. They began to walk independently at approximately 12 months. However, their language development was delayed, but the degree of the delay in language development was less severe than that of the boys with FM.

The girl with PM (31 and 99 repeats) (patient 13) did not show any developmental delays. She was tested at 7 months because her two older sisters both presented with an FM allele. The boy with an IM allele (52 repeats) (patient 14) demonstrated a prominent forehead, large ears, and significant GDD. His clinical presentation was indistinguishable from that of the boys with FM. However, this patient was lost to follow-up and therefore we could not evaluate other factors contributing to his development.

The nine boys with FM and the girl with only FM alleles all had significant GDD with an MDI of < 70, PDI of < 70, SQ of < 70, and markedly delayed language development. Among the 12 children, there was one boy with a CARS score > 30. Increased severity of autistic behavior, as measured by CARS, was strongly associated with more severe delays across all domains, particularly in the communication and fine motor domains [13]. Even after 2 years of age, the boys with FM could only say two to three meaningful words at best. They were inattentive and hyperactive, had difficulty remaining still, and were unaware of danger. They were highly dependent on adults and anxious about being separated from their parents. Even after 3 years of age, they communicated using single words or simple phrases, and toilet training had not been completely established. The girls with one FM allele (patients 10 and 12) showed delayed language development, but the degree of the delay was less severe than that of the boys with FM.

Discussion

FXS is found in many populations across the world; however, there is wide variation in its prevalence based on differences in the study subjects and diagnostic methods. This makes global prevalence estimates difficult. The prevalence of FXS is estimated to be ~1/4,000 to 1/7,000 in males, and ~1/6,000 to 1/11,000 in females. In addition, approximately 1/500 to 1/800 males and 1/200 to 1/300 females are PM carriers [14]. Similarly, the exact prevalence of FXS and PM carriers in the Korean population has

not been well established. Based on the results of studies on pre-conception or pregnant women, the prevalence of PM carriers is estimated to be around 1/700 to 1/800 Korean women, which is lower than that of Western populations and higher than that of other Asian populations [4,15,16]. Several studies in Korea have shown that approximately 1% to 3% of all children with GDD/ID or ASD are found to have FXS [17-21]. Hong et al. [17] reported a 1.6% prevalence of FXS in children with pervasive developmental disorders. Han et al. [21] reported a 3% prevalence of FXS in children with GDD/ID. However, there are no studies reporting the prevalence of FXS in children under 5 years of age in Korea.

In this study, there were 14 children (10 boys and four girls) with abnormal FX test results over a 13-year evaluation period. The mean age of the nine boys with FM at the initial visit was 24.8 ± 9.7 months (range, 11 to 45). Only one boy visited our clinic before the age of 12 months. All the boys with FM and the girl with only FM alleles presented with significant GDD involving difficulties with attention and inhibition control. It has been reported that boys with FXS are more inattentive, overactive, and impulsive than boys with other types of ID [22]. In boys with FXS, delayed motor and language development manifests in the first several years of life. A longitudinal study of boys with FXS (range, 24 to 72) reported that their overall development was significantly delayed, and that their rate of development was approximately half that of age-matched peers. Deficits in nonverbal communication and cognition have also been reported [23].

In this study, there were two girls with an FM allele and a normal allele, who did not show any typical features of FXS or delayed gross motor development. However, they could not reach language developmental milestones on time. There is considerable variability in FXS symptoms. Individuals producing higher levels of FMRP are typically less affected. Females with FM have a higher degree of variability in their FMRP production due to compensation by the second X chromosome with a healthy *FMRI* gene, individual differences in inactivation of the fragile X chromosome, or the presence of somatic mosaicism. Individuals presenting with mosaicism are likely to produce more FMRP. Both repeat-size mosaicism (e.g., FM/PM, FM/IM) and methylation mosaicism have been described [3]. Therefore, confirmation of methylation status should be complemented with analysis of the repeat number of CGG of the *FMRI* gene.

There was an unusual case of a 3-year-old girl with typical FXS features, as a result of homozygosity for the FM allele of the *FMRI* gene (288/288 CGG repeats), resulting from maternal UPD of the entire X chromosome. Her mother, who was found to be a heterozygous carrier of PM (30/65 CGG repeats), had no clinical phenotype. The mother's PM was transmitted to her offspring with

expansion to FM. UPD is a genetic condition in which two homologous chromosomes or chromosomal regions/segments are inherited from only one parent, and not from both parents [24]. Thus, UPD should be considered a possible cause in female patients with unexplained severe manifestations of GDD/ID.

Those with the PM allele do not have the phenotype of ID, but often have adult-onset phenotypes, including fragile X-associated primary ovarian insufficiency before age 40, as seen in approximately 20% of female carriers. In addition, fragile X-associated tremor/ataxia syndrome is observed in approximately 40% of male and 16% of female cases with the PM allele.

This is related to excessive *FMR1* gene activity and the deleterious consequences of elevated production of *FMR1* mRNA, leading to anxiety, attention deficit-hyperactivity, and social deficits. Approximately 10% of males with the PM allele meet the diagnostic criteria for ASD, which is usually recognized in childhood [3].

Despite delayed attainment of developmental milestones, the absence of malformations, significant medical problems, or characteristic physical features has traditionally caused FXS to be diagnosed later in childhood. The average age of diagnosis for FXS has been reported to be around 36 months of age for boys and rather later for girls [7]. FXS has a significant impact on individuals and their families; however, the diagnosis is difficult due to its subtle clinical features.

Despite the advent of CMA, next-generation sequencing testing expands the understanding of genetic causes of GDD/ID or ASD, and we therefore suggest that physicians should consider the FXS PCR test as a part of first-line testing in children with unexplained GDD/ID or ASD, despite a low diagnostic yield, to prevent underdiagnosis of this disorder, even in the absence of a family history. If there is a family history of FXS, FX testing should be screened in all family members, especially on the maternal side.

This study has several limitations. First, we did not perform FX testing on all the children who presented with GDD. Therefore, our study may have underdiagnosed the number of FXS children as we excluded those without clinical suspicion. Second, our abnormal FX genetic test results included a patient with IM alleles who could not be assessed for other diseases that could cause GDD, due to follow-up loss. Third, this was a retrospective chart review study, increasing the possibility of research bias in these results. Although our study is limited by size and researcher bias, our findings will be useful for the early diagnosis of FXS.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

ORCID

So-Hee Lee, <https://orcid.org/0000-0003-4455-1552>

Jin-Kyung Kim, <https://orcid.org/0000-0003-0018-851X>

Author contribution

Conceptualization: JKK. Data curation: SHL and JEJ. Formal analysis: SHL, JEJ, and YYJ. Methodology: YYJ. Writing-original draft: SHL. Writing-review & editing: JKK.

References

1. Moeschler JB, Shevell M; Committee on Genetics. Comprehensive evaluation of the child with intellectual disability or global developmental delays. *Pediatrics* 2014;134:e903-18.
2. Lozano R, Azarang A, Wilaisakditipakorn T, Hagerman RJ. Fragile X syndrome: a review of clinical management. *Intractable Rare Dis Res* 2016;5:145-57.
3. Lozano R, Rosero CA, Hagerman RJ. Fragile X spectrum disorders. *Intractable Rare Dis Res* 2014;3:134-46.
4. Kang MK, Sung SR, Park JE, Shin YJ, Park SH, Chin MU, et al. Carrier screening for (CGG)_n repeat expansion of *FMR1* gene in Korean women. *J Genet Med* 2016;13:14-9.
5. Yrigollen CM, Durbin-Johnson B, Gane L, Nelson DL, Hagerman R, Hagerman PJ, et al. AGG interruptions within the maternal *FMR1* gene reduce the risk of offspring with fragile X syndrome. *Genet Med* 2012;14:729-36.
6. Nolin SL, Glicksman A, Ding X, Ersalesi N, Brown WT, Sherman SL, et al. Fragile X analysis of 1112 prenatal samples from 1991 to 2010. *Prenat Diagn* 2011;31:925-31.
7. 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.
8. Borch LA, Parboosingh J, Thomas MA, Veale P. Re-evaluating the first-tier status of fragile X testing in neurodevelopmental disorders. *Genet Med* 2020;22:1036-9.
9. Bayley N. Bayley scales of infant development: manual. 2nd ed. San Antonio: Psychological Co; 1993.
10. Kim SK, Kim OK. Social maturity scale. 5th ed. Seoul: Chung Ang Aptitude Publishing Co.; 1995. p. 1-122.
11. Kim YT, Kim KH, Yoon HR, Kim HS. Sequenced language scale for infants. Seoul: Special Education Publisher; 2003.
12. Kim TR, Park RG. Korean version of childhood autism rating scale. 3rd ed. Seoul: Special Education Publisher; 2001.
13. Roberts JE, Mankowski JB, Sideris J, Goldman BD, Hatton DD, Mirrett PL, et al. Trajectories and predictors of the development

- of very young boys with fragile X syndrome. *J Pediatr Psychol* 2009;34:827-36.
14. Sherman SL, Kidd SA, Riley C, Berry-Kravis E, Andrews HF, Miller RM, et al. FORWARD: a registry and longitudinal clinical database to study fragile X syndrome. *Pediatrics* 2017;139(Suppl 3):S183-93.
 15. Kim MJ, Kim DJ, Kim SY, Yang JH, Kim MH, Lee SW, et al. Fragile X carrier screening in Korean women of reproductive age. *J Med Screen* 2013;20:15-20.
 16. Jang JH, Lee K, Cho EH, Lee EH, Kim JW, Ki CS. Frequency of FMR1 premutation carriers and rate of expansion to full mutation in a retrospective diagnostic FMR1 Korean sample. *Clin Genet* 2014;85:441-5.
 17. Hong SD, Lee S, Oh MR, Jin DK. DNA testing for fragile X syndrome in school for emotionally severely handicapped children in Korea. *J Genet Med* 1998;2:83-6.
 18. Kang KM, Kwak DI, Lee MS. Molecular genetic study for FMR-1 gene in autistic children. *J Korean Neuropsychiatr Assoc* 1999;38:1479-87.
 19. Choi YM, Hwang DY, Jun JK, Choe J, Park SH, Noh MK, et al. Incidence of fragile X syndrome in Korean patients with mental retardation. *Korean J Obstet Gynecol* 1999;42:2458-64.
 20. Kwon SH, Lee KS, Hyun MC, Song KE, Kim JK. Molecular screening for fragile X syndrome in mentally handicapped children in Korea. *J Korean Med Sci* 2001;16:271-5.
 21. Han JY, Jang W, Park J, Kim M, Kim Y, Lee IG. Diagnostic approach with genetic tests for global developmental delay and/or intellectual disability: single tertiary center experience. *Ann Hum Genet* 2019;83:115-23.
 22. Kau AS, Reider EE, Payne L, Meyer WA, Freund L. Early behavior signs of psychiatric phenotypes in fragile X syndrome. *Am J Ment Retard* 2000;105:286-99.
 23. Roberts JE, Mirrett P, Anderson K, Burchinal M, Neebe E. Early communication, symbolic behavior, and social profiles of young males with fragile X syndrome. *Am J Speech Lang Pathol* 2002;11:295-304.
 24. Liehr T. Cytogenetic contribution to uniparental disomy (UPD). *Mol Cytogenet* 2010;3:8.