Surveillance and prevalence of fragile X syndrome in Indonesia

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SUMMARY Fragile X syndrome (FXS) is the most prevalent inherited cause of intellectual disability (ID) and autism spectrum disorder (ASD). Many studies have been conducted over the years, however, in Indonesia there is relatively less knowledge on the prevalence of FXS. We reviewed all studies involving FXS screening and cascade testing of the high-risk population in Indonesia for two decades, to elucidate the prevalence, as well as explore the presence of genetic clusters of FXS in Indonesia. The prevalence of FXS in the ID population of Indonesia ranged between 0.9-1.9%, while in the ASD population, the percentage was higher (6.15%). A screening and cascade testing conducted in a small village on Java Island showed a high prevalence of 45% in the ID population, suggesting a genetic cluster. The common ancestry of all affected individuals was suggestive of a founder effect in the region. Routine screening and subsequent cascade testing are essential, especially in cases of ID and ASD of unknown etiology in Indonesia.

Keywords fragile X syndrome, intellectual disability, genetic screening, cascade testing

1. Introduction

Fragile X syndrome (FXS) is an X-linked inherited condition that causes developmental problems, including intellectual disability (ID). FXS is caused by the expansion of the cytosine-guanine-guanine (CGG) trinucleotide repeat in the 5' untranslated region (UTR) of the fragile X mental retardation (FMR1) gene (OMIM 309550). It has a prevalence of 0.5 to 3 percent in different populations with intellectual disability (ID) and autism spectrum disorder (ASD) (1). FXS is characterized by ID and emotional and behavioral disorders, including a short attention span, hyperactivity, tactile defensiveness, and poor eye contact (2). Dysmorphic clinical features of FXS include large and prominent ears, macroorchidism during and after puberty, single palmar crease, and hyperextensible joints (3). About 50 to 60% of male patients with FXS also have features of autism spectrum disorder (ASD), and FXS is considered to be the most common single gene cause of ASD (4). The expansion of CGG trinucleotide repeats is unstable within a specific threshold, with variable length in the normal population. The range of repeats in a normal individual is 5 to 44 repeats. Individuals with 45-54 repeats have variable expansion characteristics and this allele is called an intermediate or 'gray zone' allele, while individuals with 55-200 repeats are classified as premutation carriers. The phenotypes in FXS are associated with more than 200 CGG repeats and methylation, and this range is called the full mutation. Expansion instability usually results from maternal transmission, however, 1 or 2 AGG anchors after every 10 CGG repeats can lead to less frequent expansion to the full mutation when passed on by a mother to the next generation (5).

The first cytogenetic analysis of FXS identified the fragile site on the long arm of chromosome X located at Xq27.3, whereby the syndrome was named (6). Further molecular analysis for diagnosis of FXS, including a polymerase chain reaction (PCR) based method was introduced after the FMR1 gene molecular structure was identified in 1991 (7). There have been many PCR protocols developed to measure the size of CGG repeats, and PCR is one of the most inexpensive and convenient methods for diagnosis. However, the DNA fragment of expanded repeats in the mid-high premutation range does not amplify well in PCR, so full mutation alleles cannot be detected. Consequently, other methods are
added to differentiate the methylated or unmethylated full mutation alleles (7,8). Southern blot analysis using a methylation-sensitive enzyme (e.g., BstUI, EagI, Nrul, BssHIII) and a non-methylation-sensitive enzyme (e.g., EcoRI or HindIII) have been used to detect mid to high premutation and full mutation alleles. Fully expanded alleles are seen as a band significantly larger than 5.2 kb, due to the inability of the enzymes to cut the allele (9).

With regard to the accuracy of FXS diagnosis, Southern blotting was established as a gold standard procedure. This method is still considered laborious, time-consuming, and requiring larger amounts of DNA. Novel PCR approaches were studied, and a triplet repeat primed PCR (TP-PCR) was introduced to amplify the CGG-repeat more efficiently. As a result, various kits to identify repeat expansion, as well as to quantify the CGG repeat in the FMR1 gene are currently employed (10,11).

In Indonesia, studies on FXS have been conducted since the early 1990s. The first case of FXS was reported in 1995, when intellectually disabled males attending a special school were screened, identifying the first Javanese family with two affected brothers. Both brothers with FXS had ID with the typical phenotype (e.g., long face, long and prominent ears, and macroorchidism) (12). Following a clinical and genetic screening of individuals with ID, other individuals with FXS were found in other institutions or special schools, mostly on Java Island. Cytogenetic analysis and conventional PCR-based screening combined with the fragile X syndrome checklist are still being done for routine FXS diagnosis because of fewer molecular analysis facilities in Indonesia. Southern blot analysis is done only to confirm inconclusive results. We aim to describe the prevalence of FXS in Indonesia, as well as the results of periodic screening and cascade testing in the high-risk population for FXS in Indonesia.

2. Data collection

The data was collected from the previous screening programs from special schools/ institutions, including our recent screening. The previous data of three screenings in 1999, 2012, and 2013 were collected and included from Winarni et al. (13). The recent screening from institutions and referred patients from the clinic during 2014-2019 were collected for routine cytogenetic analysis, including fragile site detection at chromosome Xq27.3 using G banding technique. The FMR1 gene was analyzed using PCR-based methods to determine CGG repeat length as previously described (14,15). To confirm the diagnosis of FXS, Southern blot analysis was performed (7,16). For the most recent screening from an institution, individuals were subjected to FMR1 molecular analysis using three FastFrax FMR1 Identification, Sizing, and Methylation Status Kits (The Biofactory Pte Ltd, Singapore) as reported previously (17). Other screenings in a remote area of East Indonesia were performed using the chimeric-CGG-primer-based PCR screening method from blood spots samples (18). Finally, cascade testing was performed on the family of individuals who were molecularly confirmed as FXS. Informed consent was obtained from all cases, and all studies have been approved by the ethical committee.

3. Prevalence of FXS in Indonesia

In total, six studies have been performed involving screening of high-risk populations, including ID and ASD in Indonesia (Figure 1). The first study conducted by Faradz and colleagues in 1999, yielded 5 out of 262 individuals from an ID population in a special school (1.9%) (19,20). The study done by Mundhofir and colleagues in 2012 found nine individuals (1.7%) with FXS from 527 males and females with ID in special schools and institutions in the Central Java province (16). The study in an autism population resulted in 4 out of 65 (6.15%) children with FXS, in accordance with a larger prevalence of FXS among individuals with autism (21). Blood spot screening in a population of individuals with ID in a remote area of Flores island, East Indonesia found 2 full mutation males and 1 premutation male out of 130 males and 81 females (0.9%), using dried blood spot testing (18).

Our latest FXS screening conducted using triplet repeat primed polymerase chain reaction (TP-PCR) in individuals with ID from Central Java province, yielded a prevalence of 1.83% (2 out of 109 individuals) (17). A full mutation was found in a male with an IQ of 50 and mild characteristic features of FXS such as, long face, prominent ears, macroorchidism, high arched palate and hyperextensible joints (Figure 2), and a female with mild ID, IQ of 64 without any FXS physical and behavioral characteristics (17). Both individuals were not suspected of FXS on physical examination and yielded a lower than threshold score on the Hagerman checklist (2). Both affected individuals had siblings with ID, however, follow-up by molecular testing could not be performed, because the parents did not agree on subsequent cascade testing. Thus, other family members were diagnosed based on physical examination and pedigree analysis. Altogether, the prevalence of each screening study is shown in Figure 1.

Upon finding positive results in these studies, a cascade testing was conducted on some individuals. A list of cascade testing results is described in Table 1. Aside from the screening program, some patients were referred to the Diponegoro National Hospital with clinical suspicion of FXS, combined with a family history of ID. Up until 2019, four families were diagnosed molecularly, as shown in Table 2.

Diagnosis of FXS in Indonesia is mostly conducted under university-based studies, done by physicians who lead a research-project and have research-interests in ID through clinical, cytogenetic, and molecular testing.
These studies showed a similar FXS prevalence of 1.7-1.9% of populations with ID of unknown etiology. In addition to our studies, during 2014-2019 there were only four families referred to our research center (Center for Biomedical Research/CEBIOR) by physicians for FMR1 mutation analysis (Table 2), this might be due to a lack of awareness of the need for genetic testing in those with ID or ASD. The prevalence of FXS is in agreement with the known general prevalence of FXS in diverse populations (22), although it is lower than some Asian countries. For example, the prevalence of FXS among boys with ID in Thailand was about 7% (16 of 237 individuals) (23), while in Iran, full mutation of FMR1 was found in 32 of 508 (6.3%) families studied (24). These discrepancies are mainly due to the population studied with or without cascade testing, molecular techniques being used, consanguinity, inclusion criteria, and the number of samples.

4. Genetic cluster of FXS in Indonesia

A high rate of FXS cases were found from the first screening in a special school and nearby community of Semin district, of Gunung Kidul regency, province of Yogyakarta in 2000 (20). Screening from a special school revealed 21 out of 47 students (45%) were identified and affected with FXS. After cascade testing was performed, there were 16 nuclear families with 25 affected males, These studies showed a similar FXS prevalence of 1.7-1.9% of populations with ID of unknown etiology. In addition to our studies, during 2014-2019 there were only four families referred to our research center (Center for Biomedical Research/CEBIOR) by physicians for FMR1 mutation analysis (Table 2), this might be due to a lack of awareness of the need for genetic testing in those with ID or ASD. The prevalence of FXS is in agreement with the known general prevalence of FXS in diverse populations (22), although it is lower than some Asian countries. For example, the prevalence of FXS among boys with ID in Thailand was about 7% (16 of 237 individuals) (23), while in Iran, full mutation of FMR1 was found in 32 of 508 (6.3%) families studied (24). These discrepancies are mainly due to the population studied with or without cascade testing, molecular techniques being used, consanguinity, inclusion criteria, and the number of samples.

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Table 1. Cascade testing results on 15 cases from 5 studies

| Case no. | No. of nuclear families | Total affected individuals | Total affected males | Total affected females | Total carrier females | Total carrier males | Cascade testing taken from study population |
|----------|-------------------------|---------------------------|---------------------|-----------------------|----------------------|-------------------|---------------------------------------------|
| 1        | 1                       | 2                         | 2                   | 0                     | 7                    | 3                 | Faradz et al., 1999                        |
| 2        | 5                       | 5                         | 2                   | 3                     | 6                    | 2                 | Faradz et al., 1999                        |
| 3        | 2                       | 4                         | 4                   | 0                     | 9                    | 3                 | Faradz et al., 1999                        |
| 4        | 2                       | 3                         | 2                   | 1                     | 0                    | 0                 | Faradz et al., 1999                        |
| 5        | 1                       | 1                         | 1                   | 0                     | 1                    | 0                 | Winarni et al., 2013                       |
| 6        | 1                       | 3                         | 2                   | 1                     | 2                    | 1                 | Winarni et al., 2013                       |
| 7        | 2                       | 3                         | 2                   | 1                     | 1                    | 0                 | Mundhofir et al., 2012                     |
| 8        | 3                       | 3                         | 3                   | 0                     | 0                    | 0                 | Mundhofir et al., 2012                     |
| 9        | 1                       | 1                         | 1                   | 0                     | 0                    | 0                 | Mundhofir et al., 2012                     |
| 10       | 2                       | 2                         | 1                   | 1                     | 1                    | 2                 | Winarni et al., 2013                       |
| 11       | 1                       | 3                         | 2                   | 1                     | 1                    | 0                 | Simboming et al., 2020                     |
| 12       | 1                       | 3                         | 2                   | 1                     | 1                    | 0                 | Utari et al., 2020                         |
| 13       | 1                       | 1                         | 0                   | 0                     | 0                    | 1                 | Utari et al., 2020                         |
| 14       | 1                       | 2                         | 1                   | 1                     | 1                    | 0                 | Utari et al., 2020                         |
| 15       | 8                       | 12                        | 5                   | 7                     | 8                    | 1                 | Utari et al., 2020                         |
| Total    | 32                      | 47                        | 30                  | 17                    | 38                   | 13                |                                             |

Table 2. Patients referred from the clinic from year 2004 to 2019

| Case no. | No. of nuclear families | Total affected individuals | Total affected males | Total affected females | Total carrier females | Total carrier males |
|----------|-------------------------|---------------------------|---------------------|-----------------------|----------------------|-------------------|
| A        | 2                       | 1                         | 1                   | 0                     | 3                    | 0                 |
| B        | 1                       | 3                         | 2                   | 1                     | 1                    | 0                 |
| C        | 1                       | 3                         | 2                   | 1                     | 1                    | 0                 |
| D        | 2                       | 5                         | 5                   | 0                     | 2                    | 2                 |
| Total    | 6                       | 12                        | 10                  | 2                     | 7                    | 2                 |
17 affected females, 27 premutation female carriers, and 6 premutation male carriers, from one large, multi-generational pedigree of the same ancestor, suggesting this founder family was the cause of this high prevalence (14,20). The next follow-up in 2005 revealed six additional nuclear families with five affected males, five affected females, five premutation females, and five premutation males (16). Our latest follow-up in this region in 2019 revealed no new cases were found using the Hagerman fragile X checklist (2).

From approximately 55,000 inhabitants, the estimated number of people with ID in this region was around 400 cases. Out of 42 individuals identified to have a full mutation of \( FMR1 \), roughly more than 10 percent of cases with ID were FXS, significantly higher than the global prevalence (20). A genetic cluster of FXS has been reported in Ricaurte, a district in Colombia, where 1:19 men and 1:46 women carry a full mutation of \( FMR1 \), while 1:85 men and 1:25 women carry a premutation (25). The high prevalence of FXS, the limited geographical area, and the large ancestral pedigree, strongly indicate that Semin district also represents a genetic cluster of FXS.

5. Management of FXS in Indonesia

Management of individuals with FXS-associated disorders comprises long-term health supervision, starting from a young age through adulthood. In addition, to identifying those with \( FMR1 \) premutation-associated disorders such as Fragile-X associated tremor/ataxia syndrome (FXTAS) at a later age, Fragile-X associated primary ovarian insufficiency (FXPOI) among female carriers and the risk of having fragile X-associated neuropsychiatric disorders (FXAND) at all ages (26,27). To date, in Indonesia, there are no specific clinical guidelines or recommendations regarding individuals with FXS, premutation disorders or ID in general. Diagnostic workup for FXS is limited, because our center is the only center that performs FXS testing in Indonesia (16). Moreover, the cost of diagnostic testing, a multidisciplinary approach for treatment, and long term follow up in order to reach optimum developmental outcomes, besides the need for health care facilities related to comorbidities, are not fully covered by the National Health Insurance (Jaminan Kesehatan Nasional/ JKN) plan. These conditions become a challenge for individuals and families who are dealing with FXS.

Genetic counseling is recommended for all family members who: (i) have a positive result from genetic testing and who may be affected with the full mutation or premutation disorders, (ii) are at risk of having a FXS child, and (iii) are at risk of developing premutation-associated disorders. It is important to provide information about the inheritance pattern, risk of having more affected children with FXS or carriers with the premutation. Some women may have the full mutation and these women may not have an ID, but perhaps emotional or learning problems and they need an improved understanding of their health condition (26). Cascade testing has revealed that many family members have a full mutation or premutation alleles from each FXS case. Consequently, clinicians or genetic counselors have to improve their understanding of FXS and premutation-associated disorders, the clinical consequences, and the risk of transmitting the disease. If a mother of a child with FXS has a premutation or full mutation allele, she will be able to pass a full mutation to the next offspring, while a carrier father will pass only the premutation allele to all of his daughters (28). Moreover, careful consideration should be taken in order to keep the balance of family dynamics, for example on deciding the right time to test and give results to the parents or other family members. There are some cultural aspects to recognize, such as the concern for potential mistreatment of the marital relationship due to carrier status (28), or even forced marriage of individuals with FXS due to the social obligation to reproduce offspring (29). In Indonesia, aside from cultural belief, a religious aspect also contributes significantly to the attitudes towards illness and decision-making regarding healthcare. Some people would avoid genetic testing and screening due to the belief that the condition is a destiny from God or other superstition related to the carrier who "brings a bad gene" (30). The latest follow-up in the area with a high rate of FXS is in the Semin district with no new cases of FXS is suggestive of the acceptance/understanding of genetic counseling, and our long-term evaluation and follow-up may have an impact on better family planning in this population.

The ongoing research on targeted treatment for FXS has shown some medications that are currently available for clinical use, such as metformin, minocycline, and sertraline. Some promising targeted treatments are still in clinical trials, including cannabidiol, ganaxolone, gaboxadol, arbaclofen, and mavoglurant, among others (31,32). Research conducted on children with FXS treated with metformin showed improvements in language development and behavior, such as mood instability and aggressive behavior in most patients (33). The availability of screening for FXS in Indonesia, such as in our center will eventually provide access to medication for the patient and family, and will be beneficial for alleviating symptoms, such as anxiety, irritability, and mood disorders, as well as improving language, social communication, and motor skills. Clinical trials remain a challenge in our center due to limited human capacity, the weak regulatory and administrative system, and lack of laboratory facilities for diagnosis, baseline and follow up measurements. However, physicians can take advantage of targeted treatments and prescribe a medication based on the available evidence and a careful consideration of potential risks and benefits for patients.
6. Conclusion

This is the first comprehensive review of FXS in Indonesia. *FMR1* screening is necessary to identify new cases and perform genetic counseling to help families avoid having a recurrence of children with FXS. The authors have no conflicts of interest.

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References

1. Willemsen MH, Kleefstra T. Making headway with genetic diagnostics of intellectual disabilities. Clin Genet. 2014; 85:101-110.
2. Hagerman RJ, Amiri K, Cronister A. Fragile X checklist. Am J Med Genet. 1991; 38:283-287.
3. Butler MG, Mangrum T, Gupta R, Singh DN. A 15-item checklist for screening mentally retarded males for the fragile X syndrome. Clin Genet. 1991; 39:347-354.
4. Harris SW, Hessl D, Goodlin-Jones B, Ferranti J, Bacalman S, Barbato I, Tassone F, Hagerman PJ, Herman K, Hagerman RJ. Autism profiles of males with fragile X syndrome. Am J Ment Retard. 2008; 113:427-438.
5. Latham GJ, Copppinger J, Hadd AG, Nolin SL. The role of AGG interruptions in fragile X repeat expansions: a twenty-year perspective. Front Genet. 2014; 5:244.
6. Chudley AE, Hagerman RJ. Fragile X syndrome. J Pediatr. 1987; 110:821-831.
7. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG, Warren ST. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell. 1991; 67:1047-1058.
8. Tassone F. Advanced technologies for the molecular diagnosis of fragile X syndrome. Expert Rev Mol Diagn. 2015; 15:1465-1473.
9. Macpherson J, Sharif A. Practice guidelines for molecular diagnosis of Fragile X Syndrome. Association for Clinical Genetic Science; 2014.
10. Lim GX, Loo YL, Mundhofir FE, Cayami FK, Faradz SM, Rajan-Babu IS, Chong SS, Koh YY, Guan M. Validation of a commercially available screening tool for the rapid identification of CGG trinucleotide repeat expansions in *FMR1*. J Mol Diagn. 2015;17:302-314.
11. Lim GX, Yeo M, Koh YY, Winarni TI, Rajan-Babu IS, Chong SS, Faradz SM, Guan M. Validation of a commercially available test that enables the quantification of the numbers of CGG trinucleotide repeat expansion in *FMR1* gene. PLoS One. 2017; 12:e0173279.
12. Sultana S, Soemantri A, Lam-Po-Tang PRL, Wright F, Lindeman R, Purvis-Smith S. Fragile X mental retardation in an Indonesian family. Medical Journal of Indonesia. 1995; 4:17-23.
13. Winarni TI, Mundhofir FE, Faradz SM. A cohort study of intellectual disability focusing on Fragile X syndrome in Indonesia. Journal of Biomedicine and Translational Research. 2016; 1:2-10.
14. Winarni TI, Mundhofir FE, Ediati A, Belladona M, Nillesen WM, Yntema HG, Hamel BC, Faradz SM, Hagerman RJ. The fragile X-associated tremor ataxia syndrome (FXTAS) in Indonesia. Clin Genet. 2015; 83:263-268.
15. Faradz SM, Pattiiha MZ, Leigh DA, Jenkins M, Leggo J, Buckley MF, Holden JJ. Genetic diversity at the *FMR1* locus in the Indonesian population. Ann Hum Genet. 2000; 64:329-339.
16. Mundhofir FE, Winarni TI, Nillesen W, van Bon BW, Schepens M, Ruiterkamp-Versteeg M, Hamel BC, Yntema HG, Faradz SM. Prevalence of fragile X syndrome in males and females in Indonesia. World J Med Genet. 2012; 2:15-22.
17. Sihombing NRB, Cai S, Wong DPW, Guan M, Chong SS, Faradz SMH, Winarni TI. Repeat expansion and methylation-sensitive triplet-primed polymerease chain reaction for fragile X mental retardation 1 gene screening in institutionalised intellectually disabled individuals. Singapore Med J. 2020; doi: 10.11622/smedj.2020009.
18. Utari A, Basuta K, Winarni TI, Lo J, Morales GM, Faradz SM, Tassone F. Robust screening and cascade testing for fragile X expansions in a large multigenerational family identify many affected individuals: an experience in the remote area of Indonesia. Journal of Intellectual Disability - Diagnosis and Treatment. 2020; 8:9-15.
19. Faradz SM, Buckley M, Lam-Po-Tang, Leigh D, Holden JJ. Molecular screening for fragile X syndrome among Indonesian children with developmental disability. Am J Med Genet. 1999; 83:350-351.
20. Faradz SM, Winarni TI. Focal areas of a high rate of fragile X in Indonesia: a long term follow up. Journal of Biomedicine and Translational Research. 2019; 5:67-68.
21. Winarni T, Utari A, Mundhofir FE, Hagerman RJ, Faradz SM. Fragile X syndrome: clinical, cytogenetic and molecular screening among autism spectrum disorder children in Indonesia. Clin Genet. 2013; 84:577-580.
22. Peprah E. Fragile X syndrome: the *FMR1* CGG repeat distribution among world populations. Ann Hum Genet. 2012; 76:178-191.
23. Limprasert P, Ruangdaraganon N, Sura T, Vaskinananote P, Jinorose U. Molecular screening for fragile X syndrome in Thailand. Southeast Asian J Trop Med Public Health. 1999; 30 Suppl 2:114-118.
24. Payou AR, Abedini SS, Mansoorian N, Behjati F, Nikzat N, Mohseni M, Nieh SE, Abbasi Moheb L, Darvish H, Monajemi GB, Banhashemi S, Kahrizi K, Ropers HH.
Najmabadi H. Fragile X syndrome screening of families with consanguineous and non-consanguineous parents in the Iranian population. Eur J Med Genet. 2009; 52:170-173.

25. Saldarriaga W, Forero-Forero JV, González-Teshima LY, Fandiño-Losada A, Isaza C, Tovar-Cuevas JR, Silva M, Choudhary NS, Tang HT, Aguilar-Gaxiola S, Hagerman RJ, Tassone F. Genetic cluster of fragile X syndrome in a Colombian district. J Hum Genet. 2018; 63:509-516.

26. Hersh JH, Saul RA; Committee on Genetics. Health supervision for children with fragile X syndrome. Pediatrics. 2011; 127:994-1006.

27. Winarni TI, Sumekar TA, Wibowo S, Hagerman RJ, Faradz SM. Premutation allele combined with caregiver distress factor increase the risk of depression in fragile X carriers: Indonesia setting. Journal of Intellectual Disability - Diagnosis and Treatment. 2019; 7:200-208.

28. Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB Jr, Moine H, Koooy RF, Tassone F, Gantois I, Sonenberg N, Mandel JL, Hagerman PJ. Fragile X syndrome. Nat Rev Dis Prim. 2017; 3:17065.

29. Pan L, Ye J. Sexuality and marriage of women with intellectual disability in male-squeezed rural China. Sex Disabil. 2012; 30:149-160.

30. Shankar S, Winarni TI, Chonchaiya W, Hoem G, Dy ABC, Sumpaico-Tanchanco L, Gowdra A, Salcedo-Arellano MJ, Saldarriaga W. International perspective on identification and treatment of Fragile X Syndrome and associated disorders. In: Fragile X Syndrome and the Premutation: New Developments and Treatments. Mac Keith Press; 2020, pp. 135-146.

31. Tassanakijpanich N, Cabal-Herrera AM, Salcedo-Arellano MJ, Hagerman RJ. Fragile X syndrome and targeted treatments. Journal of Biomedicine and Translational Research. 2020; 6:23-33.

32. Protic D, Salcedo-Arellano MJ, Dy JB, Potter LA, Hagerman RJ. New targeted treatments for fragile X syndrome. Curr Pediatr Rev. 2019; 15:251-258.

33. Biag HMB, Potter LA, Wilkins V, Azfal S, Rosvall A, Salcedo-Arellano MJ, Rajaratnam A, Manzano-Nunez R, Schneider A, Tassone F, Rivera SM, Hagerman RJ. Metformin treatment in young children with fragile X syndrome. Mol Genet Genomic Med. 2019; 7:e956.

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