IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Genetic Screening Of Fragile X Syndrome -A Hospital Based Study

Ketha Keren Beulah Samuel [1], Sunil Kumar Polipalli [2], D. Udaya Kumar [3], Sheena Priya Bandela[4], and Hancy Ujwala Thadi [5]

[1,3,4,5] Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh.
[2] Genetic and Genome Sequencing Laboratory, Department of Medical Genetics, MAMC and Associated Lok Nayak Hospital, New Delhi.

ABSTRACT

BACKGROUND: Fragile x syndrome (FXS) is an X LINKED DOMINANT genetic disorder located at chromosome Xq27.3 caused by a mutation in the fragile X messenger ribonucleoprotein 1 (FMR1) gene and known to be a leading cause of inherited intellectual disability. It is due to expansion of CGG repeat sequence in the 5'-UTR of the FMR1 gene. The FMR1 gene product, FMRP is a selective RNA binding protein that negatively regulates local protein synthesis in neuronal dendrites.

OBJECTIVES: The aim of this study is to assess the presence of FMR1gene mutations in individuals and to estimate the carrier frequency of FMR1 among individuals who attended the Genetics OPD at LN hospital, New Delhi in a North Indian cohort.

METHODS: FXS screening was offered to individuals of different age groups visiting the hospital. Genomic DNA was extracted from all the samples and were further analyzed by using the molecular techniques, polymerase chain reaction (PCR), CarrierMax™ FMR1 Reagent Kit, 3500 Genetic analyzer and results were interpreted by Gene Mapper™ Software and carrier max software.

RESULT: A total of 40 individuals were screened in this study. In this 39samples are normal and 1 individual showed positive with an expanded CGG repeats. It is high in males as they have only one x chromosome.

CONCLUSION: The findings suggest a low prevalence of FMR1gene mutations. The single positive case underscores the importance of genetic testing in diagnosing Fragile X syndrome, particularly with

intellectual disabilities

Keywords: Fragile x syndrome, Genetic screening, prenatal screening, FMRP, FMR1 gene, fragment analysis.

INTRODUCTION:

Fragile X syndrome (FXS), or Martin-Bell syndrome, is a non-Mendelian trinucleotide repeat disorder(1). Fragile x syndrome is a genetic disorder caused by a mutation in FMR1gene (fragile x mental retardation1 gene) (2), it is located on x chromosome at Xq27.3. An X-linked condition occurs when just one altered copy of the gene on the X chromosome is sufficient to result in the disorder. It is most common inherited cause of intellectual disability and a significant genetic contributor to autism spectrum disorder. The syndrome results from an expansion of CGG trinucleotide repeats in the FMR1 gene, leading to the silencing of the gene and a subsequent deficiency in the fragile x mental retardation protein (FMRP).

The number of cytosine-guanine-guanine (CGG) repeats can be measured using polymerase chain reaction (PCR). The methylation status can be detected through Southern blot analysis. There's no cure for the disease, but catching it early can make a big difference. Prompt detection and timely treatment can significantly enhance the well-being of patients and ease the burden on their families. It also helps them make more informed decisions about the future, including family planning and reproductive choices (3,4) FMRP plays a crucial role in synaptic development and neuronal communication, and its absence disrupts normal brain function.

Approximately half of all cases of X-linked intellectual disability can be attributed to this condition, making it the second most common cause of mental impairment after trisomy 21(4,6). Typical physical features may include an elongated face with a prominent forehead and jawline, flexible fingers, and large, noticeable ears. After puberty, males might develop enlarged testicles. About one-third of affected children exhibit features of autism and experience delayed speech from an early age. Hyperactivity and seizures are quite common as well (5,6).

FXS affects individual across genders, though males are generally more severely impacted due to their single x chromosome. Some of the other conditions for x linked includes parental carrier status, random x inactivation- in females one of the two X chromosomes is randomly inactivated in each cell. If the mutated gene is on the x chromosome, the female will be effected. The other condition includes genetic mutation. Clinical features include intellectual impairment, learning disabilities, behavioral challenges such as hyperactivity, anxiety, and social difficulties, along with distinct physical characteristics like an elongated face, long ears, and flexible joints. Females with fragile x syndrome can show mild symptoms as they have second unaffected x chromosome.

The prevalence of FXS is estimated to be about 1 in 4000 males and 1 in 8000 females globally (7), although many cases remain underdiagnosed or misdiagnosed due to the variability in clinical presentation. Advances in genetic testing have enhanced the ability to diagnose FXS, allowing for early intervention strategies that can improve developmental and behavioral outcomes. Women who carry genes for X-linked dominant conditions have a 50% likelihood of transmitting the altered gene to their offspring. As it is seen mostly in males due to single x chromosome and it cannot pass to generations.

Figure1: FRAGILE X SYNDROME (8)

About **FMR1 gene**- FMR refers to fragile x mental retardation 1 gene located on x chromosome and plays a crucial role in brain development and this gene provides instructions for making a protein called FMRP which is essential for the normal development of synapses, the connection between he nerve cells in the brain. Fmr1gene contains a CGG repeat expansion (repetition of DNA sequence cytosine -guanine-guanine). The sequence repeats 5 to 44 times but in fragile x it takes 200 copies and it leads to methylation, stops expression of fmr1 gene hence insufficient FMRP leads to brain disruption. It leads to anticipation; it is passed down through generation and can increase the CGG repeats in successive generation.

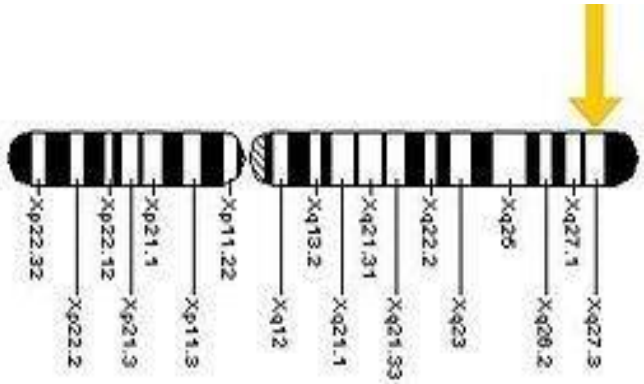


Figure 2: LOCATION OF THE FMR 1 GENE (9)

In fragile X syndrome, the expansion of repeats happens within the CGG segment of the FMR1 gene.

NORMAL: 5 TO 44 CGG REPEATS

INTERMEDIATE: 45 TO 54 REPEATS also known as gray zone FULL MUTATION: MORE THAN 200

REPEATS.

PREMUTATION: specific type of genetic variation that is associated with an increased risk of expanding to a full mutation. In FMR women experienced with fragile x associated primary ovarian insufficiency (FXPOI) leading to early menopause and fragile x associated premutation experience FXPOI. One significant risk factor is the size of the premutation repeat. Women carrying premutation alleles within the range of 80–100 CGG repeats are most vulnerable to ovarian dysfunction, whereas those with alleles surpassing 100 repeats do not face the highest risk. A male with the Fragile X premutation will pass his premutation (as a premutation, not a full mutation) on to all of his daughters and none of his sons (10).

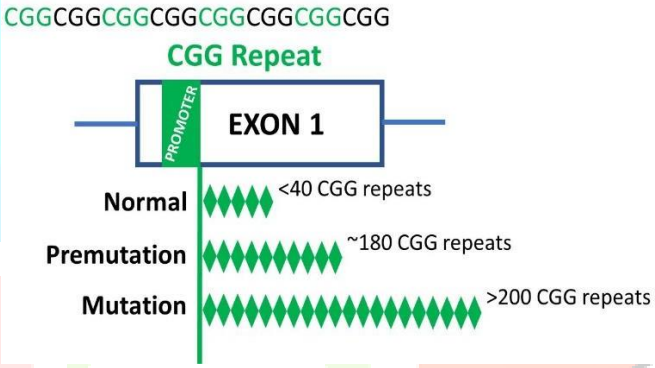


Figure 3: CGG REPEAT EXPANSION (11)

GENETIC SCREENING:

Genetic screening typically involves testing large groups of individuals to detect gene products or resulting metabolites, with the goal of identifying disorders caused by mutant genes Genetic screening aims to find changes in our genes to help identify potential health risks. By doing this, it allows us to take preventive actions and explore early treatment options to keep us healthier for longer (12).Carrier screening identifies altered genes associated with certain disorders and diseases. Among these are cystic fibrosis, a condition impacting breathing and digestion, and fragile X syndrome, a leading cause of inherited intellectual disability (12).Prenatal testing can be offered to expectant mothers to assess whether their unborn child may have a genetic condition or birth defect. This testing can help in considering various options for the pregnancy or in planning specific management strategies for the pregnancy and delivery, aiming to enhance the baby's overall outlook (13).There are several screening tests like amniocentesis, chorionic villus sampling.

By chance of women had a history of particular disease then women regardless of age can be tested during pregnancy. Carrier screening is a genetic test that helps you determine if you carry genes for specific genetic disorders. By doing this test before or during pregnancy, you can understand the likelihood of passing on a genetic disorder to your child (14).Carrier screening for a specific condition typically needs to be conducted only once in a person's lifetime, and the results should be recorded in the patient's health records (15).

MODE OF INHERITANCE:

IT IS AN X LINKED. X-linked inheritance pertains to the transmission of genes located on the X chromosome. An X-linked condition arises when a pathogenic variant in a gene on the X chromosome results in disease. Females possess two X chromosomes (XX), whereas males have one X and one Y chromosome (XY). This distinction is crucial when considering X-linked inheritance. Since males have only one copy of each X chromosome gene, carrying the variant means they will exhibit the condition. Fathers will always pass their X chromosome to daughters and their Y chromosome to sons. Females, with their two X chromosomes, can be carriers of the pathogenic variant along with a second non-pathogenic (or 'wild type') gene copy. Therefore, female carriers are typically not affected by X-linked recessive conditions or are affected only mildly. They can pass either of their X chromosomes to their children (16).

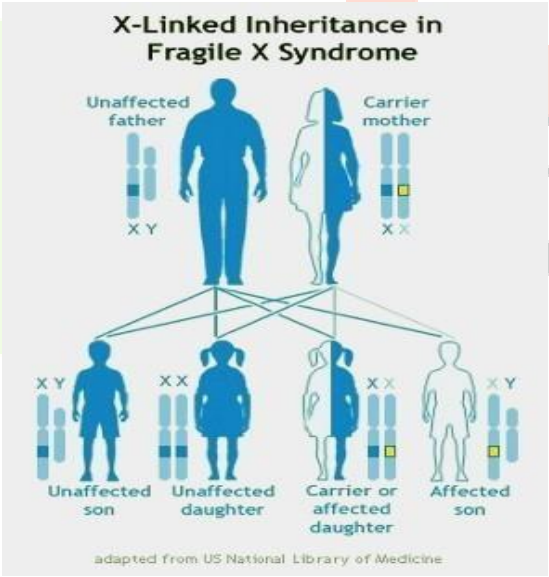


Figure 4: Pattern of x linked inheritance (17)

INHERITANCE OF FRAGILE X SYNDROME:

FXS is caused by a variant in fmr1 gene which is responsible for FMRP protein. This protein helps in brain development. Genes are present on chromosomes, each person has 23 pairs of chromosomes and from each parent mother and father 23 chromosomes are passed to the child. 23rd pair chromosomes are sex chromosomes. These chromosomes decide whether the child is male (XY) or female (XX) (18). FXS is an X-linked dominant condition. Men carry a single copy of the FMR1 gene, as they possess one X chromosome and one Y chromosome. Women, however, have two copies of the FMR1 gene, with one located on each of their X chromosomes.

A women carrying the FMR1 gene has an equal probability, or 50% of passing it on to her children, regardless of their gender. This is due to the fact that she transfers one copy of the x chromosome with each pregnancy. A man with FMR1 full mutations will pass it to all of his daughters but not to his sons, because girls get his X chromosome and boys get his Y chromosome. People who have an intermediate number of repeats do not have FXS and are not at risk for having children with FXS. However, they might have a slightly higher chance of having some symptoms related to other fragile X-associated disorders and may pass the slightly higher chance of having these disorders to their children. The inheritance pattern is complicated in premutation cases. People who have 55 to 200 repeats do not have FXS but they might have, or may later develop, other fragile X-associated disorders (19).To know the inheritance, in the *FMR1* gene where the DNA pattern of CGG (cytosine guanine guanine) is repeated. Different people have different numbers of these CGG repeats, normal people have fewer than 45 repeats. People with FXS nearly always have more than 200 repeats. Having more than 200 repeats causes the *FMR1* gene to be silenced, so it can't make FMRP (the protein made by the *FMR1* gene). Hence no production of FMRP protein the person has FXS. A diagnosis of a premutation carrier is confirmed when there are between 55-200 repeats (“premutation”), in which the gene produces protein, however the precursors to the protein, called mRNA, are produced in excess. The excess of mRNA can cause medical and psychological symptoms in premutation carriers. Premutation carriers may transmit either the full mutation or the premutation to their children. A diagnosis of intermediate/grey-zone occurs when there is 45-55 CGG repeats and the gene is less stable. Due to males having one X and one Y chromosome, and females having two X-chromosomes, females tend to be less affected with Fragile X due to the presence of the second X-chromosome, which is often able to continue to produce some FMRP protein (20).

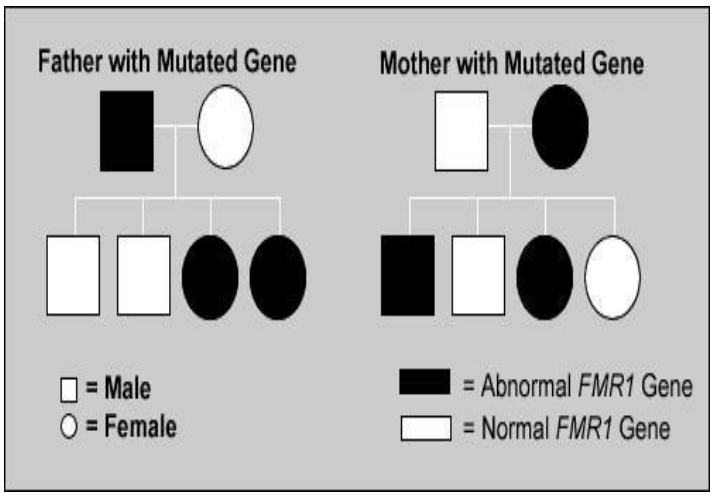


Figure 5: pedigree showing inheritance of FMR (20)

Mosaicism

Mosaicism refers to cases where individuals have both full mutation and premutation copies. Mosaicism can result from instability in the CGG repeats, and affected individuals may show classic symptoms, although some evidence suggests higher intellectual abilities compared to those with a full mutation (21).

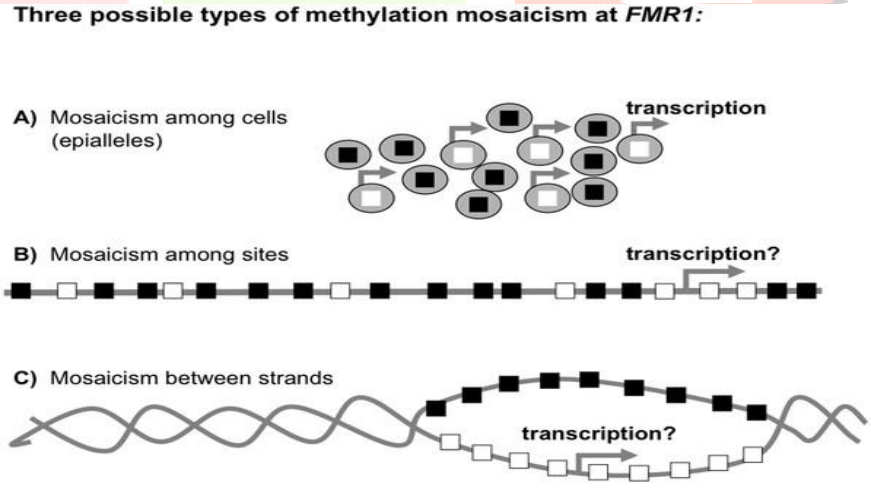


Figure 6: showing 3 possible types of methylation (22)

OBJECTIVES:

To assess the presence of FMR1gene mutations in individuals and to estimate the carrier frequency of FMR1 among individuals who attended the Genetics OPD at LN hospital, New Delhi in a North Indian cohort. By using fragment analysis we diagnose the carrier frequency of fragile x syndrome.

MATERIALS AND METHODS

A total of 40 patients from department of medical genetics in the Lok Nayak Hospital, New Delhi were enrolled in the study. The samples were collected in the genetic lab. Peripheral blood samples are collected from the patients and genomic DNA was extracted from the white blood cells and followed by PCR (polymerase chain reaction) and Fragment Analysis.

MOLECULAR STUDIES: Genomic DNA isolation from human whole blood samples (salting out method). The DNA was isolated from the samples of all the patients and control subjects by the Salting out method or perchlorate chloroform method. Quantification is done by the instrument Qubit® dsDNA HS (High Sensitivity) Assay Kit. The Qubit® dsDNA HS (High Sensitivity) Assay Kits make DNA quantitation easy and accurate. The kits include concentrated assay reagent, dilution buffer, and prediluted DNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume from 1–20 µL is acceptable), then read the concentration using the Qubit® Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is accurate for initial sample concentrations from 10 mg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (23).

For fragment analysis using the 3500/3500xL or SeqStudio™ Genetic Analyzer, PCR products are diluted appropriately—1:40 (full-length) and 1:15 (repeat primer) for the 3500/3500xL, and 1:25 (full-length) and 1:5 (repeat primer) for SeqStudio™—before capillary electrophoresis. A mixture of Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard is prepared, vortexed, and centrifuged. Each reaction well receives 9 µL of this mix and 1 µL of diluted PCR product before sealing with adhesive film, vortexing, and brief centrifugation to remove air bubbles. DNA fragments are then denatured by heating to 95°C for 3 minutes and cooling on ice for 3 minutes. The reaction plate is covered with a septa, secured with a retainer and base, and loaded onto the genetic analyzer for capillary electrophoresis according to instrument guidelines.

RESULTS & DISCUSSION

The present study comprised of 40 patients of which 10 female and 30 male individuals of all age groups. More number of males are observed in the sample studied. The prevalence is very low as 1 individual predicted as positive and 2 individuals as intermediate and 1 with pre mutation.

TABLE:1

Individuals list and the result of the sample by using fragment analysis

S.no	Age	Sex	Result
1	4 yrs	Male	Normal
2	3 yrs	Male	Normal
3	4 yrs	Male	Normal
4	10 yrs	Male	Normal
5	12 yrs	Male	Normal
6	6 yrs	Male	Normal
7	8 yrs	Male	Normal
8	5 yrs	Male	Normal
9	11 yrs	Male	Normal
10	5 yrs	Male	Normal
11	7 yrs	Male	Normal
12	10 yrs	Male	Normal
13	6 yrs	Male	Normal
14	28 yrs	Male	Normal
15	12 yrs	Male	Normal
16	9 yrs	Male	Normal
17	21 yrs	Male	Normal
18	24 yrs	Male	Normal
19	5 yrs	Male	Normal
20	2 yrs	Male	Normal

21	12 yrs	Male	Normal
22	9 yrs	MALE	POSITIVE
23	34 yrs	Female	Normal
24	14 yrs	Female	Normal
25	43 yrs	Female	Normal
26	17 yrs	Female	Normal
27	43 yrs	Female	Normal
28	17 yrs	Female	Normal
29	31 yrs	Female	Normal
30	13 yrs	Male	Normal
31	33 yrs	Female	Normal
32	14 yrs	Male	Normal
33	12 yrs	FEMALE	PREMUTATION
34	9 yrs	Male	Normal
35	3 yrs	Male	Normal
36	7 yrs	Male	Normal
37	11 yrs	MALE	INTERMEDIATE
38	8 yrs	male	Normal
39	12 yrs	male	Normal
40	7 yrs	MALE	PREMUTATION

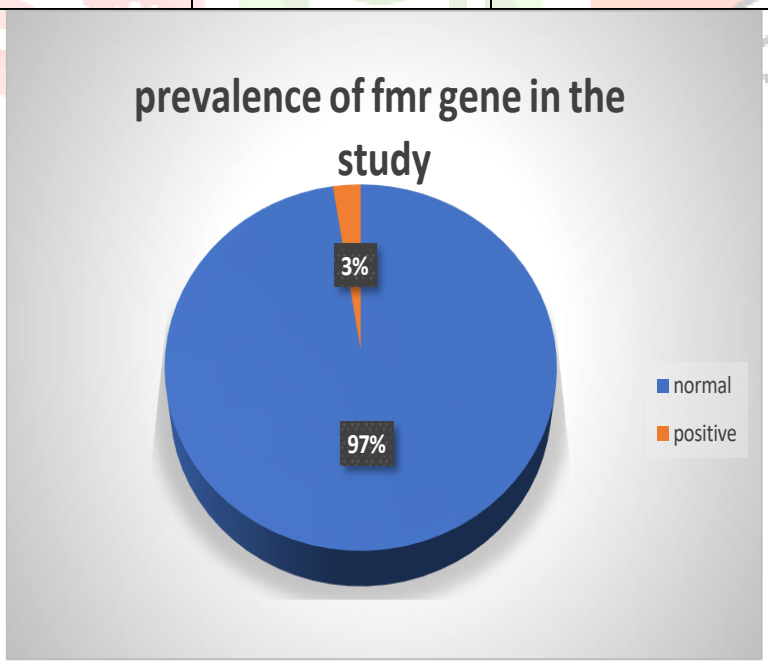
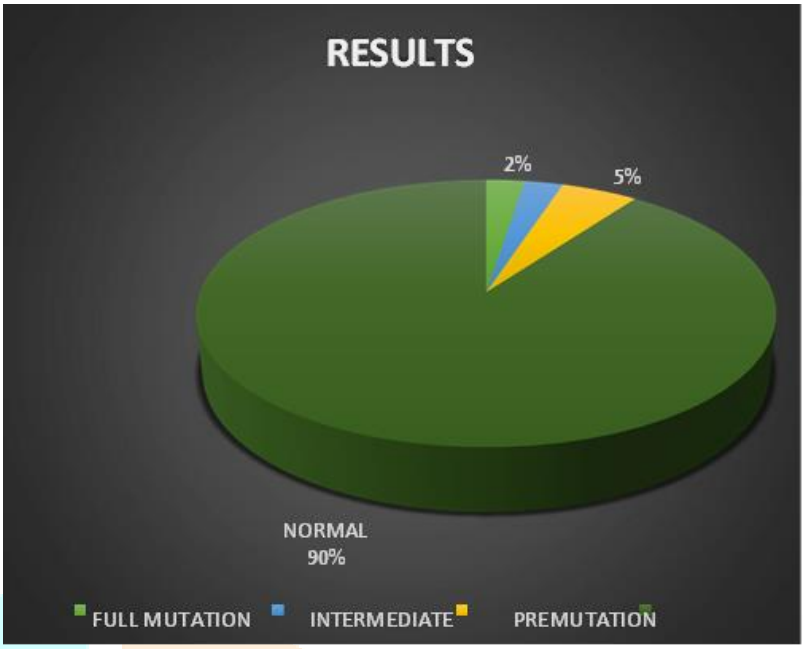


Figure 7: chart showing the prevalence of the study

Figure 8: Final results



In this study we have used Fragment analysis to detect the FMR1 mutation by observing CGG repeats. We included all age groups and we perform carrier screening.

The current distribution of FMR1 alleles indicates that most common alleles in this study are 29 and 30 CGG repeats. Previous studies have indicated that the most common alleles vary of few repeats in different populations including 28 CGG repeats (24), 29 CGG repeats in Taiwan (25), 30 CGG (26) repeats, 29 and 30 CGG repeats in Spain (27) and 29 CGG repeats in Chinese population (28). However it should be noted that small differences among studies (1-2 CGG repeats) may be a consequence of experimental errors in various labs in the absence of single repeat precision and sequenced CGG standards.

The important outcome in this study is that the frequency of premutation alleles is 2:40, the frequency of intermediate alleles is 1:40 and the frequency of full mutation alleles is 1:40. Males having more chance of getting mutation compared to females and the frequency of males is 30:40.

In the current study the repeat expansion for premutation is more than 75 for one male child and one female child, one child with full mutation having more than 200 repeat expansion. this leads to loss of protein and have mental retardation autistic behaviours etc.

Genetic screening helps in early detection of the fragile X syndrome this screening would provide parents the opportunity to learn about their child's fragile X status and their own reproductive risk, in addition to other likely benefits provided by accessing early intervention programs, which have been shown to positively influence child development and provide support to families of children with fragile X syndrome.

Conclusion:

Fragile x syndrome (FXS) is a genetic disorder caused by a mutation in the FMR1 gene on the X chromosome. As the most common inherited cause of intellectual disability and a notable contributor to autism spectrum disorders, FXS has profound implications on affected individuals. The expansion of CGG trinucleotide repeats in the FMR1 gene leads to its silencing and results in a deficiency of the fragile X mental retardation protein (FMRP), which is crucial for normal brain function. The variability in clinical presentation, including intellectual impairment, learning disabilities, behavioral challenges, and distinct physical characteristics, underscores the importance of early diagnosis and intervention. In conclusion, the present study contributes to our understanding of fragile x syndrome, particularly the fmr1 gene in the hospital. The study findings shed light on the complex interplay of genetic, and clinical factors in FMR1 Gene susceptibility and underscore the importance of comprehensive approaches in elucidating the disease's etiology and progression.

The observed age distribution within the FXS cohort, with a notable concentration of affected individuals in the 2 to 12 years age group, highlights the relevance of age-specific considerations in disease epidemiology and risk assessment. The prevalence of FXS is more in males compared to females. As there is no cure for this disease early diagnosis can be done so that we can prevent. Genetic screening for Fragile X syndrome is a critical tool in the early diagnosis and management of this condition. By facilitating early intervention, informed decision-making, and ongoing research, genetic screening can significantly improve outcomes for individuals with FXS and their families. A comprehensive approach, including awareness, education, and ethical considerations, is essential to effectively address the challenges posed by FXS.

Early intervention programs, such as speech therapy, occupational therapy, and specialized educational plans, can address specific needs and help mitigate some of the challenges associated with FXS. Furthermore, genetic screening enables families to make informed decisions about family planning and reproductive choices, thereby reducing the risk of passing the mutated gene to future generations.

REFERENCES:

1. Stone WL, Basit H, Shah M, Los E. Fragile X Syndrome. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Jan 18]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK459243/

2. About Fragile X Syndrome [Internet]. [cited 2025 Feb 4]. Available from: https://www.genome.gov/Genetic-Disorders/Fragile-X-Syndrome

3. Liang Q, Liu Y, Liu Y, Duan R, Meng W, Zhan J, et al. Comprehensive Analysis of Fragile X Syndrome: Full Characterization of the FMR1 Locus by Long-Read Sequencing. Clin Chem. 2022 Dec 6;68(12):1529–40.

4. Hersh JH, Saul RA, Committee on Genetics. Health supervision for children with fragile X syndrome. Pediatrics. 2011 May;127(5):994–1006.

5. Raspa M, Wheeler A, Okoniewski KC, Edwards A, Scott S. Research Gaps in Fragile X Syndrome: An Updated Literature Review to Inform Clinical and Public Health Practice. J Dev Behav Pediatr JDBP. 2023 Jan 1;44(1):e56–65.

6. Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB, Moine H, Kooy RF, et al. Fragile X syndrome. Nat Rev Dis Primer. 2017 Sep 29;3:17065.

7. Fragile X syndrome: MedlinePlus Genetics [Internet]. [cited 2025 Feb 4]. Available from: https://medlineplus.gov/genetics/condition/fragile-x-syndrome/

8. Syndromes: Fragile X syndrome | RCNi [Internet]. [cited 2025 Feb 7]. Available from: https://rcni.com/learning-disability-practice/evidence-and-practice/clinical/syndromes-fragile-x-syndrome-135806

9. FMR1. In: Wikipedia [Internet]. 2025 [cited 2025 Apr 30]. Available from: https://en.wikipedia.org/w/index.php?title=FMR1&oldid=1284705397

10. Whiting D. Premutation Carriers | NFXF [Internet]. National Fragile X Foundation. [cited 2025 Jan 5]. Available from: https://fragilex.org/understanding-fragile-x/fragile-x-101/premutation-carriers/

11. Persico T, Tranquillo ML, Seracchioli R, Zuccarello D, Sorrentino U. PGT-M for Premature Ovarian Failure Related to CGG Repeat Expansion of the FMR1 Gene. Genes. 2023 Dec 19;15(1):6.

12. Levy HL. Genetic Screening. In: Harris H, Hirschhorn K, editors. Advances in Human Genetics [Internet]. Boston, MA: Springer US; 1973 [cited 2025 Feb 4]. p. 1–104. Available from: https://doi.org/10.1007/978-1-4615-8261-8_1

13. Alliance G, ScreeningServices TNYMAC for G and N. PRENATAL SCREENING AND TESTING. In: Understanding Genetics: A New York, Mid-Atlantic Guide for Patients and Health Professionals [Internet]. Genetic Alliance; 2009 [cited 2025 Feb 4]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK115544/

14. Carrier Screening | ACOG [Internet]. [cited 2025 Apr 30]. Available from: https://www.acog.org/womens-health/faqs/carrier-screening

15. Carrier Screening for Genetic Conditions [Internet]. [cited 2025 Feb 4]. Available from: https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-for-genetic-conditions

16. Frost DA. GeNotes. [cited 2025 Feb 4]. X-linked inheritance — Knowledge Hub. Available from: https://www.genomicseducation.hee.nhs.uk/genotes/knowledge-hub/x-linked-recessive-inheritance/

17. Fragile x syndrome: Symptoms, causes, diagnosis and treatments [Internet]. [cited 2025 Feb 7]. Available from: https://www.msn.com/en-xl/health/condition/in-fragile-x-syndrome/in-Fragile%20X%20Syndrome

18. CDC. Fragile X Syndrome (FXS). 2024 [cited 2025 Jan 6]. How Fragile X Syndrome Is Inherited. Available from: https://www.cdc.gov/fragile-x-syndrome/about/how-fragile-x-syndrome-is-inherited.html

19. CDC. Fragile X Syndrome (FXS). 2024 [cited 2025 Feb 4]. How Fragile X Syndrome Is Inherited. Available from: https://www.cdc.gov/fragile-x-syndrome/about/how-fragile-x-syndrome-is-inherited.html

20. Fragile X Gene [Internet]. [cited 2025 Apr 30]. Available from: https://medschool.cuanschutz.edu/fragilex/fragile-x-gene

21. Hippocampus | Neuroscience Journal | Wiley Online Library [Internet]. [cited 2025 Feb 7]. Available from: https://onlinelibrary.wiley.com/doi/10.1002/hipo.10005

22. Testing the FMR1 Promoter for Mosaicism in DNA Methylation among CpG Sites, Strands, and Cells in FMR1-Expressing Males with Fragile X Syndrome | PLOS ONE [Internet]. [cited 2025 Feb 7]. Available from: https://journals.plos.org/plosone/article/figures?id=10.1371/journal.pone.0023648

23. Qubit Assays - IN [Internet]. [cited 2025 Apr 30]. Available from: https://www.thermofisher.com/in/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit/qubit-assays.html

24. Dawson AJ, Chodirker BN, Chudley AE. Frequency of FMR1 premutations in a consecutive newborn population by PCR screening of Guthrie blood spots. Biochem Mol Med. 1995 Oct 1;56(1):63–9.

25. Wang YC, Li C, Lin ML, Lin WH, Li SY. Molecular diagnosis of fragile X syndrome and distribution of CGG repeats in the FMR-1 gene in Taiwanese. J Formos Med Assoc Taiwan Yi Zhi. 2000 May 1;99(5):402–7.

26. Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN. Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. Am J Hum Genet. 1993 Dec 1;53(6):1217–28.

27. Milà M, Kruyer H, Glover G, Sánchez A, Carbonell P, Castellví-Bell S, et al. Molecular analysis of the (CGG)n expansion in the FMR-1 gene in 59 Spanish fragile X syndrome families. Hum Genet. 1994 Oct 1;94(4):395–400.

28. Fu YH, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. Cell. 1991 Dec 20;67(6):1047–58.